

Case Report

Floppy Infant Caused by *MTM1* Mutation: A First Genetically-Confirmed X-Linked Myotubular Myopathy Patient in Thailand

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*Floppy infant syndrome (FIS) refers to a condition wherein an infant manifests generalized hypotonia since birth or in early life. It is heterogeneous and can be caused by various central nervous system disorders, neuromuscular diseases and genetic disorders. X-linked myotubular myopathy (XMTM) is a progressive congenital myopathy morphologically characterized by the presence of centrally placed nuclei in numerous muscle fibers without any other particular pathological abnormalities. Patients are frequently born with floppiness and respiratory distress. The vast majority of patients carry a truncating or missense mutation in *MTM1*. The authors report here a full term male baby with clinicopathological features of XMTM. The diagnosis is validated by the finding of a c.141-144delAGAA mutation of *MTM1*. To the best of the authors' knowledge, the present case is the first genetically confirmed XMTM in Thailand. A brief review of various neuromuscular disorders causing floppy infant syndrome is also included.*

Keywords: X-linked myotubular myopathy, Centronuclear myopathy, Congenital myopathy, Floppy infant, *MTM1*, Myotubularin, Muscle biopsy

J Med Assoc Thai 2006; 89 (1): 99-105

Full text. e-Journal: <http://www.medassocthai.org/journal>

The floppy infant syndrome (FIS) is a common, well-recognized entity for pediatricians and neurologists. The condition refers to an infant with generalized hypotonia presenting at birth or in early life. Floppiness mainly stems from disorders of the central nervous system (CNS). However, various genetic and neuromuscular disorders could also be responsible for the abnormalities in a number of these patients⁽¹⁾. Owing to a wide variety of putative etiologies, the accurate diagnosis is important because clinical outcome and genetic counseling are different and in some cases,

specific treatment may be possible. With the advance in molecular genetics, certain neuromuscular disorders causing floppiness can now be diagnosed by DNA analysis of the peripheral blood lymphocytes such as congenital myotonic dystrophy (CDM) and spinal muscular atrophy (SMA). However, for the most part, combination of electrodiagnostic studies and muscle pathology remain essential for the diagnosis of FIS.

X-linked myotubular myopathy (XMTM) is considered a severe infantile form of congenital myopathy (CMP). CMP refers to a group of disorders causing infantile or childhood onset muscle weakness and/or hypotonia and pathologically characterized by the presence of a particular sarcoplasmic inclusion, a core structure, an abnormal localization of the nuclei or a

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disproportion of fiber type. Disorders in this category include nemaline body myopathy, central core disease (CCD), multimincore disease (MmD), centronuclear myopathy (CNM), X-linked myotubular myopathy (XMTM), desmin-related myopathy, hyaline body myopathy, reducing body myopathy and congenital fiber type disproportion (CFTD). Clinically, CMPs share several findings including generalized muscle weakness with facial muscle involvement and high-arched palate, hypotonia and normal serum creatinine kinase (CK) level. Basically, each congenital myopathy exhibits three clinical phenotypes including severe infantile, benign congenital and late-onset forms⁽²⁾. Although many causative genes for these disorders have been identified⁽¹⁾, the diagnosis of each remains

exclusively based on pathological features. According to the authors' database⁽³⁾, CMPs account for 3% of overall muscle biopsy samples and this includes 2 cases of XMTM, 2 cases of autosomal recessive (AR) CNM, 2 cases of nemaline body myopathy and a case of CCD. Herein, the authors have analyzed *MTM1* mutation in the presented 2 cases of XMTM and found a causative mutation in one patient. The authors report a genetically confirmed XMTM Thai male baby who presented with severe floppiness and profound respiratory failure. The summary of various neuromuscular disorders causing FIS is also reviewed in Table 1.

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A 4-month-old male infant was born to a

Table 1. Summary of key clinicopathological distinctions of neuromuscular disorders underlying floppy infant syndrome (FIS)

Disorders	Key clinical manifestations	CK	EMG	Muscle biopsy
CMP (severe infantile form)	Facial muscle weakness and high-arched palate; MH in CCD or MmD	N/+	MP	Structural or nuclear location abnormality; fiber type disproportion; common type 1 predominance
MDC				
1) With CNS lesion	Brain or eye abnormalities; muscle and tongue hypertrophy	++	MP	Active N/R; faint or absent α -DG staining
2) Without CNS lesion	Distal joint hyperlaxity in Ullrich disease or spinal rigidity in RSMD 1	N/+	MP	Mild N/R instead of marked fibrosis
Myotonia congenita	Myotonia with mild weakness; no hypotonia or MR	N/+	MT	Many fibers with centrally-placed nuclei
CDM	Clinically indistinguishable from XMTM except myotonic discharge in maternal EMG; frequent MR	N/+	MP	Morphologically indistinguishable from XMTM; DMPK analysis showing CTG expansion
SMA type I	Tongue fasciculation	N/+	NP	Fascicular atrophy and fiber type grouping
CHM	Distal dominant weakness & sensory loss	N/+	NP	Hypomyelinated nerve & many type 2C fibers
Infantile AVM	Clinically indistinguishable from CMP	N/+	MP	AVSF with sarcolemmal MAC staining & multilayered vacuolar membrane
Glycogenesis and lipid storage myopathy	Cardiomegaly and/or hepatomegaly	N/+	MP	Polyglucosan body in type IV and PAS-positive vacuoles in type II and III glycogenesis; lipid accumulation in lipid storage myopathy

AVM = autophagic vacuolar myopathy; AVSF = autophagic vacuoles with sarcolemmal features; CCD = central core disease; CDM = congenital myotonic dystrophy; CHM = congenital hypomyelination; CMP = congenital myopathy; CNS = central nervous system; α -DG = α -dystroglycan ; EMG = electromyography; MAC = membrane-attack complex ; MDC = congenital muscular dystrophy; MmD = mutimincore disease; MP = myopathic changes; MR = mental retardation; MT = myotonic changes; NP = neuropathic changes; N/R = necrotic and regenerating fibers; RSMD = rigid spine muscular dystrophy; SMA = spinal muscular atrophy; N/+ = normal or slightly elevated; ++ = markedly elevated

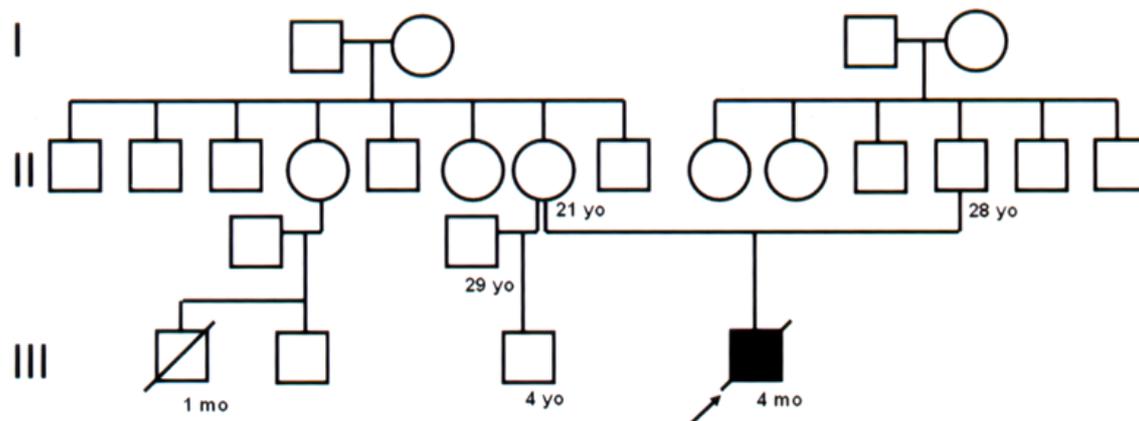


Fig. 1 Pedigree

G₂P₁A₀, 21-year-old healthy mother at the gestational age of 37 weeks with the birth weight of 2610 grams. Based on regular antenatal care, no abnormal findings were reported until birth when the Apgar scores at 1 and 5 minutes were 4 and 7, respectively. Endotracheal intubation was required immediately. Later on, the patient developed neonatal sepsis due to prolonged premature rupture of membranes. Although the infection was ameliorated by the administration of intravenous Penicillin G sodium and Gentamycin, the ventilator support remained mandatory. Neither relevant family history nor parental consanguinity was reported. However, one of his cousins died of unknown cause at the age of 1 month with questionable impairment of sucking reflex (Fig. 1). Careful physical examination further revealed generalized hypotonia and weakness with complete absence of deep tendon reflex, but primary reflexes were preserved. The myopathic facies and a high-arched palate was seen. Computed tomography (CT) of the brain depicted only mildly low density of bilateral periventricular white matter around the anterior horn. *SMN* analysis displayed no mutation suggestive of spinal muscular atrophy (SMA).

Based on the suspicion of a neuromuscular disorder, muscle biopsy from biceps brachii at the age of 1 month was performed following the protocol previously reported⁽³⁾. On hematoxylin and eosin staining, the biopsy showed mild variation in fiber size ranging from 10 to 30 μm in diameter (Fig. 2). All fibers were round in shape and 40% contain a single, centrally-placed nucleus. Neither necrotic nor regenerating fibers were seen. No interstitial fibrosis was observed. Modified Gomori trichrome revealed no sarcoplasmic inclusions, rimmed vacuoles or ragged-red fibers. Oxidative enzyme histochemistry, succinic dehydro-

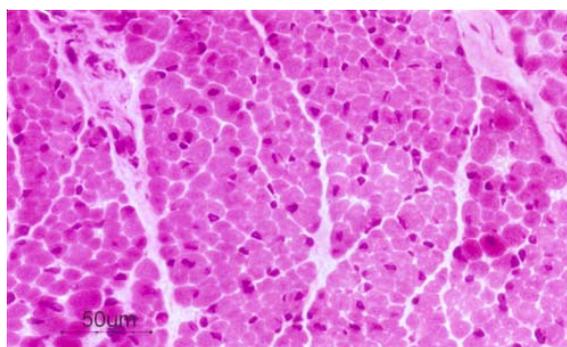


Fig. 2 Small, round muscle fibers with centrally placed nuclei (H&E 400x)

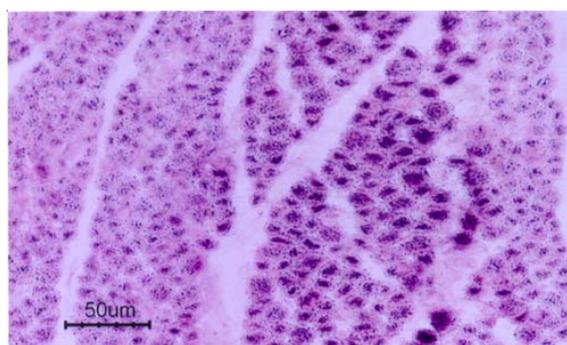


Fig. 3 Intense oxidative enzyme reactivity due to mitochondrial aggregates (SDH 400x)

genase (SDH) portrayed strong oxidative reactivity reflecting mitochondrial aggregates around myonuclei. (Fig. 3). On myosin ATPase staining, there was no fiber type disproportion or fiber type grouping. Type 1 fibers were rather atrophic and type 1 fiber predominance was seen (Fig. 4). No type 2C fibers were seen. All findings were compatible with XMTM. Electron-

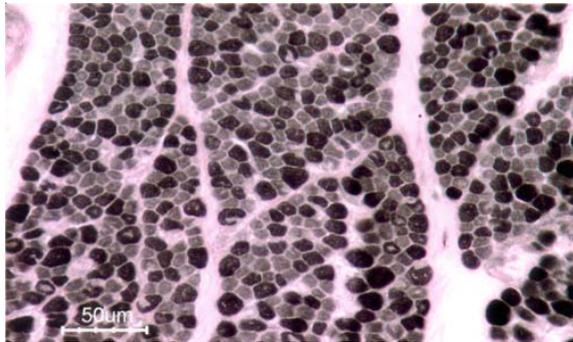


Fig. 4 Type 1 fibers (pale staining) are smaller than type 2 fibers. Central nuclei are not reactive creating halo (myosin ATPase 9.7 400x)

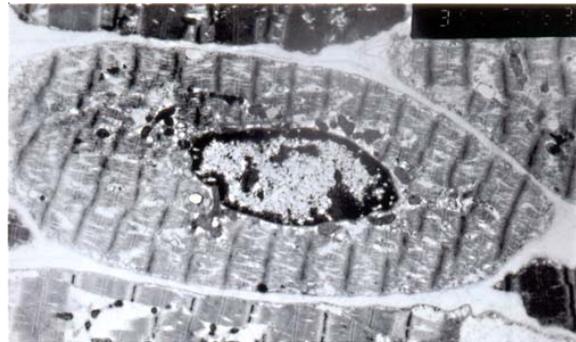


Fig. 5 Electronmicrograph of muscle fibers. Central nuclei and myofibril free space packed with mitochondria (7700x)

microscopic study demonstrated central nuclei surrounded by mitochondria and glycogen (Fig. 5).

During his hospitalization, there were repeated attacks of lower respiratory tract infections, which mainly resulted from *Klebsiella pneumoniae*. The patient eventually died of profound respiratory failure and sepsis at the age of 4 months. Postmortem examination revealed mild hypoxic-ischemic encephalopathy and left ventricular hypertrophy. Peripheral nerve was well-myelinated. Spinal cord showed no remarkable changes. Muscle necropsy taken from biceps brachii and diaphragm exhibited the similar findings to antemortem sample. Using genomic DNA extracted from muscle necropsy specimen, we screened for the possible mutation of myotubularin encoding gene, *MTM1*, using the primer set previously reported⁽⁴⁾. The authors found a four-nucleotide deletion in exon 4 (c.141_144delAGAA) (Fig. 6), which led to premature

termination of translation at the 24th codon from the deleted site. This finding certainly validated the diagnosis of XMTM.

Discussion

Myotubular and centronuclear myopathies belong to a genetically heterogeneous nosological group of congenital myopathy with clinical variability ranging from fatal disorder to mild weakness. Pathologically, both diseases share the identical portrait of numerous muscle fibers with centrally-located nuclei and lacking of any other inclusions or myofibrillar rearrangement forming core structures⁽²⁾. These myofibers partly replicate the characteristics of myotube, a transitional stage of development between myoblasts and mature myofibers. The term “XMTM” specifically refers to a rather uniform severe infantile phenotype with X-linked recessive inheritance, while CNM covers a variety of milder phenotypes with later age of onset usually with autosomal inheritance. XMTM has been proven to be caused by *MTM1* mutations. For autosomal forms, recently Guicheney P et al has reported a mutation of dynamin 2 (*DNM2*) in patients with autosomal dominant centronuclear myopathy (ADCNM)⁽⁵⁾. Virtually all ADCNM patients develop the first symptom in their adolescent or early adulthood with relatively slow progressive clinical course and frequent association of ptosis and ophthalmoplegia⁽⁶⁾. Although the genetic basis of AR CNM has not been elucidated, the canine model of this disorder has been linked to the mutation in *PTPLA*⁽⁷⁾.

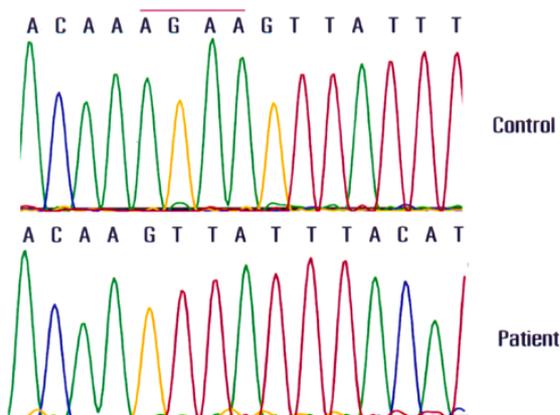


Fig. 6 Direct sequence of genomic DNA PCR. Electropherogram shows c141-144delAGAA in exon 4 of *MTM1* gene (red bar indicates deleted fragment)

Clinically, XMTM patients usually develop muscle weakness *in utero*, so that pregnancies are often complicated by polyhydramnios and reduced fetal movement. The history of miscarriage and neonatal deaths of male infants in the maternal line are not

unusual. The patients at birth commonly present with severe floppiness and weakness, feeding difficulties, and respiratory distress often requiring assisted ventilation. Cognitive development is normal in the absence of significant hypoxia. Generally, the long-term prognosis is extremely poor. Patients usually die from respiratory failure during infancy or within the first year of life. Many affected males fail to achieve independent respiration, so the management is mainly conservative and consists mainly of ventilatory care. Female carriers, on the other hand, are usually asymptomatic or experience only mild facial weakness, except for a few female carriers developing severe phenotype due to extremely skewed X-inactivation pattern⁽⁸⁾. Prenatal diagnosis may therefore be performed for all mothers having a child affected with XMTM.

In the present case, the clinical picture especially the pathognomonic myopathic facies is compatible with congenital myopathy, but only the presence of central nuclei is non-specific for XMTM or CNM. Generally, nuclear centralization is common in many muscle disorders leading to myofibers regeneration e.g. muscular dystrophy, chronic myopathy and chronic denervation. Mature myofibers always appear polygonal in shape and contain peripheral-located nuclei. Any cause of muscle injury activating regenerating process will accelerate the differentiation and maturation of satellite cells, a postnatal myogenic stem cell, to become myofibers with centrally-located nuclei or the so-called myotube-like fibers, and eventually to become mature muscle fibers. So other pathological features, such as the peripheral halo and the radial array of sarcoplasmic strands on oxidative enzyme stainings, type 1 fiber predominance, the involvement of both types of muscle fibers, and the high frequency of central nuclei in the absence of any other identifiable alterations may be useful in differentiating XMTM and CNM from other disorders. However, despite applying all these myopathological clues for distinction, CDM may still be difficult to differentiate from XMTM. Not only the pathological similarity, but also the clinical resemblance of both disorders is perplexing. Although recently Horinouchi H et al⁽⁹⁾ reported some morphometric differences in muscle biopsies between XMTM and CDM including a higher percentage of fibers with centrally-placed nuclei (XMTM 14.5 +/- 10.6%; CDM 4.4 +/- 6.8%), a higher proportion of type 1 fibers (XMTM 69 +/- 19%; CDM 32 +/- 19%), and fewer numbers of type 2C fibers (XMTM 7.8 +/- 8.1%; CDM 42 +/- 31%) in XMTM, these could not replace the benefit that EMG and DNA analysis of the

patient's mother can offer. In contrast to an XMTM patient, EMG study in a CDM patient's mother frequently displays myotonic discharge. Unfortunately, the authors could not obtain maternal EMG data in the present case, so mutation analyses of *MTM1* and *DMPK* genes were employed.

The *MTM1* on chromosome Xq28 was found to be mutated in the vast majority of the patients with clinicopathological diagnoses of XMTM⁽⁸⁾. This gene is highly conserved and is ubiquitously expressed with one muscle-specific transcription isoform. The *MTM1* is made up of 15 exons and encodes myotubularin protein which consists of 603 amino acids. The myotubularin protein localizes to the nucleus of mammalian cell and has significant similarities to tyrosine phosphatase. Although its function is not clearly defined, myotubularin likely plays an important role in controlling critical aspects of gene expression related to growth control and differentiation of skeletal muscle. Mutations of *MTM1* are widely distributed throughout the gene, although more have been found in exon 4, 12, 3, 8, 9 and 11, in that order, when comparing the number of mutations to the nucleotide-length ratio of each exon⁽⁸⁾. Mutations reported are missense (30%), small insertion or deletion (25%), nonsense (20%), splicing variant (20%) and large deletion (5%)⁽⁸⁾. Missense mutations are clustered between exon 8 and 12, around the functional domain of myotubularin, protein tyrosine phosphatase (PTP) motif. Generally, truncating mutations produce severe and lethal phenotype while missense mutations usually result in a milder form with prolonged survival, but there are no such definite genotype-phenotype correlation in XMTM⁽¹⁰⁾. For example, the common frameshift deletion, which are also found in the present case, c.141_144delAGAA, leading to protein truncation has been shown to cause both severe and mild phenotypes⁽⁸⁾. Although these patients usually die soon after birth, some have been reported to live to the age of 9 years⁽¹¹⁾.

About 20% of XMTM patients do not have an identified mutation. This group of patients may represent as yet unrecognized mutations within the *MTM1* locus, including its non-coding regions or mutations in another X-linked genes encompassing *MTM1* homologue, *MTMRI*. As mentioned earlier, besides this present patient, the authors do have another affected male presenting with the similar clinicopathological findings without other affected family members. By analysis of the entire coding sequence of *MTM1* gene, no mutation was found. It is possible that in such a sporadic male case, X-linked inheritance cannot

be strongly concluded on the basis of pedigree information. In fact, a girl with a severe infantile form of myotubular myopathy without mutation in *MTM1* was reported and the presence of a rare autosomal recessive severe infantile form has been suggested⁽¹²⁾. The authors' second patient may also have such an autosomal recessive disease. Since the clinical pictures of ADCNM are different from the second patient, the molecular analysis of *DNM2* has not been performed.

In conclusion, neuromuscular disorders underlie abnormalities in a number of floppy infants. Even in the age of advanced molecular genetic testing, muscle biopsy remains an essential diagnostic apparatus. The authors report the first genetically-confirmed Thai XMTM patient presenting with infantile floppiness due to the worldwide common *MTM1* gene deletion mutation, c.141_144delAGAA. Genetic counseling and intensive respiratory care remain major management strategies in XMTM patients and their families. The genetic basis of mutation-negative XMTM and a majority of AR CNM cases remain to be further elucidated.

Acknowledgements

The authors wish to thank Dr. MC Maclidan (NCNP) for critical review of the manuscript, Ms Naree Choosrichom, Ms Pimchanok Sehamo, Mr. Theeraphong Pho-Iam for laboratory assistance, and Mr. Vicha Sookpatdhee for photographs.

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รายงานผู้ป่วยตัวอ่อนปวกเปียกจากการกลายพันธุ์ของยีน MTM1 รายงานแรกของประเทศไทย

อิริน ลิวลักษณ์, นที รักษาदारณ, ชนินทร์ ลิ้มวงศ์, Ichizo Nishino, ตัมทิพย์ แสงรุจิ

กลุ่มอาการทารกตัวอ่อนปวกเปียกหมายถึงภาวะตัวอ่อนปวกเปียกของทารกแรกเกิดหรือวัยเด็กเล็ก อาจเกิดจากหลายสาเหตุ ได้แก่ ความผิดปกติของระบบประสาทส่วนกลาง ระบบประสาทส่วนปลาย ระบบกล้ามเนื้อ และโรคทางพันธุกรรม X-linked myotubular myopathy (XMTM) เป็นโรคกล้ามเนื้อลายผิดปกติแต่กำเนิด ซึ่งมีลักษณะเฉพาะทางพยาธิวิทยาคือเซลล์กล้ามเนื้อลายส่วนใหญ่มีขนาดเล็กและมีนิวเคลียสอยู่กลางเซลล์คล้ายเซลล์ระยะ Myotube ของตัวอ่อนและไม่มีความผิดปกติอื่นใด ผู้ป่วยส่วนใหญ่มีอาการตัวอ่อนปวกเปียก และหายใจลำบากตั้งแต่แรกเกิด การกลายพันธุ์ของยีน MTM1 เป็นสาเหตุหลักของโรค XMTM คณะผู้รายงานได้ทบทวนวารสาร และศึกษาผู้ป่วยทารกไทยเพศชาย ซึ่งมีอาการทางคลินิกและผลการตรวจทางพยาธิวิทยาเข้าได้กับโรคนี้ การตรวจทางอณูพันธุศาสตร์พบการกลายพันธุ์ c 141-144delAGAA ของยีน MTM1 เป็นการยืนยันการวินิจฉัยโรค คาดว่าเป็นรายงานแรกของประเทศไทยที่มีการศึกษาทางพันธุศาสตร์เพื่อวินิจฉัยโรคนี้ สาเหตุอื่นของกลุ่มอาการทารกตัวอ่อนปวกเปียกจากความผิดปกติของกล้ามเนื้อได้สรุปไว้ตอนท้ายของบทความ
