Affected female carriers of *MTM1* mutations display a wide spectrum of clinical and pathological involvement: delineating diagnostic clues

Valérie Biancalana1,2,3,4,5 · Sophie Scheidecker1 · Marguerite Miguët1 · Annie Laquerrière6 · Norma B. Romero7,8 · Tanya Stojkovic8 · Osorio Abath Neto9 · Sandra Mercier10,11,12 · Nicol Voermans13 · Laura Tanner14 · Curtis Rogers15 · Elisabeth Ollagnon-Roman16 · Helen Roper17 · Célia Boutte18 · Shay Ben-Shachar19 · Laurens Pasquier24 · Pascale Marcorelle25,26 · Armelle Magot12 · Benno Küsters27 · Nathalie Streichenberger28 · Christine Tranchant29 · Nicolas Donadain1 · Raphael Schneider23,4,5,30 · Claire Gasnier1 · Nadège Calmels1 · Valérie Kremer31 · Karine Nguyen32 · Julie Perrier12 · Erik Jan Kamsteeg33 · Pierre Carlier34 · Robert-Yves Carlier35 · Julie Thompson30 · Anne Boland36 · Jean-François Deleuze36 · Michel Fardeau7,8 · Edmar Zanoteli9 · Bruno Eymard21 · Jocelyn Laporte23,4,5

Received: 9 May 2017 / Revised: 24 June 2017 / Accepted: 2 July 2017 © Springer-Verlag GmbH Germany 2017

Abstract X-linked myotubular myopathy (XLMTM), a severe congenital myopathy, is caused by mutations in the *MTM1* gene located on the X chromosome. A majority of affected males die in the early postnatal period, whereas female carriers are believed to be usually asymptomatic. Nevertheless, several affected females have been reported. To assess the phenotypic and pathological spectra of carrier females and to delineate diagnostic clues, we characterized 17 new unrelated affected females and performed a detailed comparison with previously reported cases at the clinical, muscle imaging, histological, ultrastructural and molecular levels. Taken together, the analysis of this large cohort of 43 cases highlights a wide spectrum of clinical severity ranging from severe neonatal and generalized weakness, similar to XLMTM male, to milder adult forms. Several females show a decline in respiratory function. Asymmetric weakness is a noteworthy frequent specific feature potentially correlated to an increased prevalence of highly skewed X inactivation. Asymmetry of growth was also noted. Other diagnostic clues include facial weakness,
ptosis and ophthalmoplegia, skeletal and joint abnormalities, and histopathological signs that are hallmarks of centronuclear myopathy such as centralized nuclei and necklace fibers. The histopathological findings also demonstrate a general disorganization of muscle structure in addition to these specific hallmarks. Thus, MTM1 mutations in carrier females define a specific myopathy, which may be independent of the presence of an XLMTM male in the family. As several of the reported affected females carry large heterozygous MTM1 deletions not detectable by Sanger sequencing, and as milder phenotypes present as adult-onset limb-girdle myopathy, the prevalence of this myopathy is likely to be greatly underestimated. This report should aid diagnosis and thus the clinical management and genetic counseling of MTM1 carrier females. Furthermore, the clinical and pathological history of this cohort may be useful for therapeutic projects in males with XLMTM, as it illustrates the spectrum of possible evolution of the disease in patients surviving long term.

Keywords  MTM1 · X-linked myotubular myopathy · Centronuclear myopathy · Congenital myopathy · X inactivation

Introduction

Myotubular myopathy (XLMTM, MIM #310400) is a severe congenital myopathy linked to the X chromosome. While affected males display severe hypotonia and weakness at birth and have a very short life expectancy even with interventional care and ventilatory support, female carriers were reported to be either asymptomatic or affected through a wide spectrum of severity. The disease in females is much less characterized to date. Moreover, as affected females reach adulthood, they could potentially reveal additional symptoms that are not described in XLMTM in males. In this study, we thus aimed to better define the symptoms, the genotype–phenotype correlation and the disease progression in females with XLMTM through a thorough characterization of a large cohort of novel cases and comparison with the literature.

XLMTM is caused by mutations in the MTM1 gene (MIM 300415) encoding the ubiquitous phosphoinositide phosphatase myotubularin (MTM1) [38]. This is the most severe form of centronuclear myopathies, a group of myopathies caused by different genes and modes of inheritance whose hallmark histological abnormalities are centrally located nuclei [2, 8, 11, 12, 15, 38, 44, 48, 52, 65], without dystrophic features. Around 500 males with XLMTM have been reported and they usually present at birth with severe
hypotonia, muscle weakness and respiratory insufficiency [19, 36, 45, 57, 62]. XLMTM is non-progressive and those affected males who survive several years may show additional non-muscle clinical features, the most common being liver peliosis [27, 30, 43, 59]. Atypical forms have been reported in some boys and adult men with a clinical classification based on respiratory and milestone features [30, 36, 42]. Mutations are found in all 15 exons of MTM1 and encompass point mutations (missense, nonsense, splice site mutations), insertions, small and large deletions and duplications [4, 45, 61]. The majority of mutations are clustered in exons 4, 8, 9, 11 and 12. Duplication has been described in some cases and a deletion of exon(s) or of the whole MTM1 gene was reported in 7% of patients. Large deletions including part or the entire neighboring MAML1 gene cause a contiguous gene syndrome with hypospadias and myotubular myopathy in males [6, 24, 32, 37].

Female carriers are usually asymptomatic but a total of 26 affected females aged 5–71 years have been reported presenting with severe neonatal form [33, 51] or moderate to mild forms [7, 18, 20, 22, 23, 25, 28, 46, 50, 55, 56]. Truncating mutations as well as missense mutations were observed scattered through the gene. Skewed X inactivation was reported in some as a potential explanation for their affected status compared to non-affected carriers, while others did not show skewing.

In this study, we reviewed the clinical, histological, imaging and molecular data from 43 affected females, including 17 new cases. We have identified consistent asymmetry in muscle involvement and have better defined the spectrum and progression of the disease, the frequency of clinical, investigation and histological findings, and discuss the clinical correlation with the type of mutation and X inactivation.

Patients and methods

Patient cohort

Data from the 43 females affected with XLMTM are summarized in Supplementary Tables 1–3. The 17 new unrelated females originated from Brazil, Finland, France, Israel, The Netherlands, UK and USA. Fifteen were from new families, while F8 is the sister of an affected male previously reported as patient HC19 [10] and patient F1 is the sister of an affected female previously reported as patient 1 by Bevilacqua and colleagues [7]. Clinical data were collected from the clinicians who referred patients for a genetic diagnosis (25 patients) or from the literature (18 patients).

Morphological studies

Muscle biopsies were performed on 14 out of the 17 new patients (Supplementary Table 1). Muscle sections were stained with hematoxylin–eosin (H&E), modified Gomori trichrome, periodic acid Schiff (PAS) and Sudan red and black. Histochemistry was performed using routine histochemical methods [21]. Ultrastructural study was carried out according to standardized protocols. Briefly, tissue samples were fixed in a 2% glutaraldehyde fixative solution, post-fixed with osmium tetroxide and embedded in resin epoxy. Semi-thin sections were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

Molecular analysis

DNA from the 17 new patients and relatives was extracted from blood or muscle samples by standard methods.

Sequence analysis of the MTM1 gene was performed by bi-directional sequencing of the 15 exons with flanking intronic sequences (primer sequences and PCR conditions are available on request). A search for copy number variations (deletion or duplication of exon or whole gene) was performed by MLPA analysis (multiplex ligation-dependent probe amplification) using the P309-A1-MTM1 kit (MRC Holland) for patients F2, F3 and F4. Patient F11 was included in MTM1 Sanger analysis due to the detection on Western blot [60] of a decreased level of myotubulin on protein extracts from lymphoblasts and muscle biopsies (manuscript in preparation). Patients F15 and F12 were diagnosed by exome sequencing in the scope of a French consortium research project (MYOCAPTURE). Exome analysis was performed using SureSelect Human all Exon 50 Mb capture library v4 (Agilent, Santa Clara, USA) and paired-end sequenced on Illumina HiSeq 2500 sequencer at the French National Center of Genotypage, Paris, France. Patient F17 was diagnosed by exome sequencing using SureSelectXT Human all Exon 50 Mb capture library (Agilent, Santa Clara, USA) on IlluminaHiSeq at BGI-EUROPE in Denmark. Patient F13 was diagnosed using a multi gene panel, MGZ laboratory, Munich, Germany. Patient F16 was diagnosed using targeted exons sequencing of a custom panel of 210 genes linked to neuromuscular diseases (SureSelect, Agilent, Santa Clara, CA) (MYOCapHYS panel, Strasbourg, France, manuscript in preparation).

Affymetrix Cytogenetics Whole-Genome 2.7M Array was used to delineate deletion breakpoints for patient F3. DNA quantity was insufficient to perform this study for patients F2 and F4.

X-chromosome inactivation (XCI) analysis was performed using HpaII predigestion of DNA followed by PCR of the highly polymorphic CAG repeat of the AR gene
(HUMARA) [3] and the CGG repeat within the \textit{FMR1} gene [13]. XCI with an allele ratio &lt;80:20 was considered a random pattern, a ratio equal or greater than 80:20 was considered to be skewed and a ratio greater than 90:10 was considered to be highly skewed.

**Results**

Clinical, imaging, histological, ultrastructural and molecular data are summarized in Supplementary Tables 1–5 for the 43 females including 17 novel cases and 26 previously reported cases, resulting in the largest XLMTM female cohort characterized so far. The main clinical and histological findings representing the new cases are presented (Figs. 4, 5, 6, 7).

**Females with XLMTM demonstrate an independently recognizable myopathy**

To assess if females with XLMTM signs define a disease entity, we analyzed the familial history for XLMTM or undefined muscle disorder. The female patient was the first diagnosed case of XLMTM in 28 out of the 36 families, with 20 being sporadic cases. The diagnosis of XLMTM was previously established or suspected (based on clinical and histopathological data) in a male relative in only 8 of the 36 families (Fig. 1a; Supplementary Table 2). In four of the seven families including several affected females, there was no affected male (Fig. 1b; Supplementary Table 2). The mutation occurred de novo for the older affected females in 13 of the 19 families in which samples from parents were available for further analysis. Altogether, these findings indicate that females with XLMTM define a specific myopathy cohort, independently of the presence of male XLMTM.

**Females with XLMTM are usually less severely affected than males, with overlapping clinical hallmarks**

Most males with XLMTM are severely affected from birth with weakness and respiratory distress, needing respiratory and feeding support. To assess the distinctive features between male and female XLMTM, we analyzed the age of onset and severity in 43 females with XLMTM to compare with affected males.

The clinical phenotype is highly variable in age of onset and severity, in particular regarding weakness and respiratory muscle involvement (Fig. 2; Supplementary Table 3). The age of onset ranged from birth or fetal life to adulthood. Only eight of 42 females were reported to be hypotonic and have respiratory or feeding problem at birth. The pregnancy for two of these eight patients was associated with reduced fetal movements and/or polyhydramnios and was normal for two others (no data available for four patients). Most of the females affected from birth displayed generalized muscle weakness. For 30 out of 42 female patients, weakness was clinically manifest during childhood (2–14 years) with the first impairment being proximal in the lower limbs in 17 patients (delayed motor milestones, gait difficulties, difficulty with climbing stairs or raising from a squatting position) and in the upper limbs in five.

The most severely affected female in our cohort displayed similar clinical symptoms and course as an XLMTM male (F2). She was affected with severe neonatal form and died aged 18 months old. There was no family history of neuromuscular conditions. She was born eutrophic at 41 weeks of gestation, and Apgar scores were 9 and 10 at 1 and 5 min, respectively. Thirty minutes after birth, she presented with severe hypotonia and respiratory distress. Clinical examination revealed severe left facial weakness with left ptosis, stridor due to laryngomalacia and swallowing difficulties. At the age of 1.5 months, she demonstrated fluctuating and moderate axial hypotonia and a Moebius
syndrome diagnosis was initially considered. From the age of 2.5 months, she had recurrent episodes of respiratory distress and a congenital myasthenic syndrome was considered with the findings of axial hypotonia, absent deep tendon reflexes and worsening of the facial weakness. Quadriceps wasting was noted at age 15 months and a muscle biopsy was performed showing multiple anomalies (see below). The child was subsequently treated with anticholinesterase drugs (pyridostigmine) with a very transient improvement. She started walking with support at the age of 18 months, but died from an acute bronchopneumonia shortly after.

In all other cases, there was a slowly progressive course, with limb weakness being the major sign, sometimes predominating in the pelvic girdle and spreading to distal muscles. Several of the 43 patients showed significant difficulties in standing from a seated position and/or in climbing stairs and/or running. Eight patients were never able to walk ([18, 33], F2) or run ([23, 28, 50, 51], F16): seven had lost the ability to walk independently, with three patients needing a cane at ages 40, 50 and 77 years (F11 patient from [46], F10) and four needing a wheelchair at ages 13, 32, 53 and 66 years (F4, F3, F17, the mother in [29]). Pelvic floor weakness with urinary incontinence was reported in seven cases (at the ages of 6, 27, 35, 35, 52, 55 and 79 years, respectively). Neck flexor weakness was reported in 12 cases.

The frequency of additional clinical signs in this cohort of 43 females is summarized in Fig. 3. Bulbar or pseudo bulbar symptoms were reported for 24 patients including facial weakness, limitation of extra-ocular movements, ophthalmoparesis, uni- or bilateral ptosis and dysarthria, whereas no such involvement was noted for ten patients. Respiratory muscle involvement was noted for 14 out of 33 patients, with a decrease in forced vital capacity in nine patients (at 37–83% of age and sex-matched predicted values). Severe restrictive respiratory dysfunctions with a hemidiaphragmatic paresis led to death of the oldest patient [46] at 84 years of age. Serum CK (creatine kinase) levels were normal in 21 patients and slightly elevated in 10 patients, supporting the finding that disease is not dystrophic. Skeletal and joint abnormalities were observed in 17 out of 28 cases, including kyphoscoliosis, scoliosis, joint hyperlaxity, joint contractures of the lower extremities, foot deformities and hand and/or facial contractures.

Five of the patients were reported to be overweight.

Overall, while a few females presented with similar onset to the male XLMTM (with fetal involvement and/or neonatal generalized weakness) with one case with an identical course (F2), the vast majority of females with XLMTM had later onset and were less severely affected than males with XLMTM. Affected females with XLMTM display similar facial signs as males with XLMTM, namely facial weakness, ophthalmoparesis and ptosis, and these appear to be important clues for diagnosis.
Females with XLMTM frequently show a pattern of asymmetric involvement

At all ages, a consistent and striking feature was asymmetric symptoms, seen in 29 out of 35 patients. These included muscle weakness and wasting, especially of the arm, leg, and calf (Fig. 4). Hemidiaphragm elevation and asymmetric scapular winging were also noted.

There was also consistent asymmetry of facial involvement, notably asymmetric ptosis and facial weakness; examples are given for four patients of different ages in Fig. 4a–d. These asymmetries were mainly on a left–right axis.

Asymmetric involvement has been previously reported in several cases (Supplementary Table 1 and 3) and Drouet et al. reported two females with unilateral weakness [20]. In addition to asymmetric weakness, skeletal asymmetry was noted in some patients. For example, patient F5 has a right hand smaller than her left hand (Fig. 4d) and patient F13 has a lower limb length discrepancy. Grogan et al. reported females of two unrelated families with asymmetric weakness, hemidiaphragm elevation, and arms and fingers of different sizes when comparing the right and left sides [25].

Both our large cohort of novel cases and the descriptions of those in the literature highlight the frequency of asymmetric muscle involvement in females with XLMTM and suggest skeletal defects are a primary symptom of the disorder.

Imaging investigations highlight general and asymmetric muscle involvement

We next assessed whether imaging confirms the clinical symptoms, including asymmetry, and whether this yields additional diagnostic clues. Magnetic resonance imaging (MRI) findings for six newly described patients and nine previously reported patients [7, 20, 33, 46, 50, 51] are summarized in Supplementary Table 4 and representative images are depicted in Fig. 5 for several of the novel cases, including whole body MRI.

For patient F14, whole body MRI demonstrated asymmetric fatty involvement of the muscles, which predominates in the left side of the upper and lower limbs. In the lower limbs, the fatty involvement involves the anterior and posterior part of the legs: the soleus is near normal in the right side, whereas the left soleus muscle showed a moderate fatty infiltration (Fig. 5a). In the thigh and the pelvis, the asymmetric distribution of the fatty infiltrates is obvious, with gracilis, sartorius, biceps femoris, quadriceps and glutei muscles (minimus, medius and maximus) demonstrating almost complete fatty involvement (Fig. 5b, c). In the upper limbs, the fatty involvement predominates in the deltoid muscles, infra and supraspinatus and subscapularis muscles, with the left side showing the most evident fatty infiltration (Fig. 5e). Asymmetric fatty involvement is also noted in the neck extensors, while facial muscles are spared.
Several of these features are found in other patients (Fig. 5 and Supplementary Table 4). For patient F17, there is severe atrophy and fatty infiltration of paraspinal, gluteal and psoas muscles and the upper leg muscles, while the lower leg muscles are less severely affected (Fig. 5g–n). The more proximal muscles (obturatorius, piriformis and transverse abdominal) are relatively spared. For patient F13, prominent fatty infiltration of the muscles of the lower limbs was noted, both at the thighs and legs (Fig. 5o–q). All the muscles of the thighs were affected, but the right semimembranosus and the left vastus intermedius were slightly less affected. The anterior compartments of the legs were more affected, while the gastrocnemius and soleus were nearly spared. For patient F16, muscle involvement with clear asymmetry was noted mainly for the distal muscles, including involvement of the left tibialis anterior and the right peroneus (Fig. 5s–u). In the thighs, the anterior and posterior

Fig. 4 Facial and general muscle weakness and asymmetric involvement. a Patient F4 presenting with facial asymmetry (left weaker), deficit in arm elevation and retractions. b Patient F16 presenting with left side ptosis, facial asymmetry, high arched palate, reduced muscle bulk and scapula winging. c Patient F17 presenting with left side ptosis, deficit in arm elevation and needing support for standing. d Patient F5 presenting with mild left ptosis. Right hand smaller than left hand.
muscles were affected, especially visible on the left in the vastus externus and adductor magnus.

Taking both the novel reported cases and previous reports (Supplementary Table 4), these data confirm a general involvement of muscles and often highlight an asymmetric pattern correlating with the asymmetric muscle weakness observed on clinical assessment.

Histological and ultrastructural features are similar in males and females with XLMTM

Biopsies were available for 38 patients, including one that was not interpretable due to massive fatty replacement. Variation in myofiber diameter with atrophic round fibers was reported in 25 cases, type 1 fiber predominance in 17
cases, and endomyosial fibrosis and/or fatty replacement in 20 cases (Fig. 6; Supplementary Table 1 and 5). There was no fiber necrosis or inflammatory change. Abnormally positioned nuclei varied from a few scattered to numerous non-peripheral nuclei in all interpretable biopsies. Six biopsies were described with internalized nuclei, 19 biopsies with central nuclei, and 12 biopsies had both. Necklace fibers and radial sarcoplasmic strands (RSS), as previously reported in adult XLMTM patients and also in patients with DNM2-related dominant centronuclear myopathy, were described in 11 and 3 out of the biopsies, respectively. Necklace fibers display a basophilic ring underneath the sarcolemma that is strongly reactive with PAS and oxidative reactions.

**Fig. 6** Histological features in females with XLMTM. Muscle biopsies from patient F2 (a–d) and F4 (e, f). a A necklace fiber displaying a basophilic ring located underneath the sarcolemma (arrow) (hematoxylin and eosin staining). b A necklace fiber strongly reacting with PAS (arrow). c Type I predominance with small type I (light) and II (dark) fibers (myosin adenosine triphosphatase preincubated at pH 9.4 technique). d A necklace fiber strongly reactive for oxidative reactions (arrow) (nicotinamide adenine dinucleotide-tetrazolium reductase technique). e Numerous centralized nuclei and fiber size heterogeneity (Gomori trichrome stain). f Necklace fibers strongly reactive for oxidative reactions (arrow) (nicotinamide adenine dinucleotide-tetrazolium reductase technique).
At the ultrastructural level, internal nuclei may display an altered shape (Fig. 7). There were a range of signs of myofibrillar disruption from focal loss of myofibrils to fibers with complete disorganization of the myofibrillar network. Z disk streaming was also common. Necklaces form a clear ring devoid of organelles under the sarcolemma and are associated with an oblique orientation of the myofibrils.

Overall, the histopathology and ultrastructural defects in females with XLMTM appear very similar to those seen in males with XLMTM, albeit necklace fibers are

---

**Fig. 7** Ultrastructural defects in females with XLMTM. Electron microscopy for muscles from patient F2 (a–d) and F11 (e–g). a Focal loss of myofibrils in a small muscle fiber (arrow) (OM ×5000). b Z disk streaming running over two sarcomeres (arrow) (OM ×6000). c Internalized nuclei in an atrophic fiber with a complete disorganization of the myofibrillar network (arrow) (OM ×4000). d Ultrastructural pattern of a necklace, forming a clear ring devoid of organelles under the sarcolemma, with an oblique arrangement of the myofibrils (arrow) (OM ×4000). e, f Muscle fibers showing internalized nuclei, cisternae (blue arrow), vacuoles (red arrow) and material accumulation (green arrow).
mainly found in adult patients and are less observed in neonatal affected males.

**Most MTM1 mutations in females were associated with a severe phenotype in males**

Females with XLMTM are usually more mildly affected than males. We explored if there was a correlation between the type of mutation and the phenotypic severity. First, we analyzed the MTM1 gene through approaches including Sanger sequencing, targeted and exome sequencing, MLPA and DNA microarray to delineate the genetic defects. Analysis of MTM1 revealed four new heterozygous mutations in the 17 new female patients reported: an in-frame deletion encompassing exons 9 and 10, the intrinsic mutation c.1644+1G>A predicted to disrupt the donor splice site of exon 14 and two stop gain c.548G>A (p.Trp183*) and c.1601G>A (p.Trp534*) (Supplementary Table 1). Patients F3, F4 and F17 were heterozygous carriers of a large deletion of the whole MTM1 gene and the neighboring MTMRI gene. For patient F3, comparative genomic hybridization (CGH) study delineated the deletion with a minimum size of 595,302 bp (from genomic position chrX: 150,443,004–151,038,095; hg38) and a maximum size of 597,756 bp (from genomic position chrX: 150,442,220–151,039,765;hg38) encompassing MAMLD1, MTM1, MTMRI, CD99L2 and HMGB3 genes. For patient F17, exome analysis delineated a 474,391 bp deletion (from genomic position chrX: 150,512,003–150,986,191; hg38) encompassing MTMRI and CD99L2, as well as part of MAMLD1 and HMGB3.

Genotype–phenotype correlation in males has established that some mutations are more likely to be associated with a mild or moderate phenotype, probably being compatible with a residual normal function of the mutated myotubularin. Conversely, severely affected males tend to have an undetectable or a marked reduction in the protein level [39]. Twenty of the 29 mutations present in this cohort of 43 affected females have previously been found in male patients, with 16 being reported in association with a severe form of the disease, 3 predominantly with a severe form but occasionally with a mild/moderate form (c.109C>T, c.614C>T, c.1262G>A), and 1 (c.1354-1G>A) described once in a moderate case [30] and also found once in a severe case (Biancalana, unpublished observation). Among the ten mutations described only in females, nine are in-frame or out-of-frame deletions, truncations or splicing mutations predicted to be associated with a severe phenotype in males, and one is a missense mutation (c.1115T>A) which has been shown to lead to a reduced protein level. Thus, females with XLMTM in this cohort do not have specific MTM1 mutations compared with males, but rather harbor mutations usually associated with a severe phenotype in males with XLMTM, while these females display a milder phenotype.

**Enhancement of skewed X chromosome inactivation in females with XLMTM**

The difference in severity between males with XLMTM and females is not explained by the type of mutation. In a female heterozygous for an X-linked recessive mutation, half of the cells on average should have a normal level of the gene product, while the other half would express the mutated allele. However, skeletal muscle fibers are syncytia formed from the fusion of different myoblasts. It was shown at least in mice that different nuclei from single myofibers have random X-chromosome inactivation (XCI), predicting that each fiber would have about 50% expression level [66]. A skewed X-inactivation pattern favoring the mutated X chromosome has been hypothesized to be a determinant of the variability of the phenotype observed in females with XLMTM, but several reports did not confirm a skewed XCI in affected females (Supplementary Table 1). To better evaluate XCI as a determinant of the phenotype severity in females, we compared the disease onset versus the XCI pattern (random, skewed and highly skewed; see “Patients and methods”). Unfortunately, the test could not determine which X chromosome was preferentially active. XCI studies were informative in 32 out of 43 patients. Evidence of skewed XCI (80:20–90:10) or highly skewed XCI (≥90:10) was identified in 15.6 and 18.75% of patients aged <55 years, respectively, whereas in the general adult population the ratio was around 14 and 4%, respectively [5]. This enhancement in XCI among females with XLMTM is significant for the highly skewed group (p < 0.025) and is even more significant when considering that for the six patients with highly skewed XCI, the ratio was in fact >95:5 (found in only 1.7% of the normal population). In conclusion, there is an increased prevalence of highly skewed X inactivation in the cohort of females affected by XLMTM.

**Discussion**

We have described or reviewed the clinical, morphological and molecular data obtained from 43 X-linked myotubular myopathy female carriers from 36 unrelated families. To date, only 26 manifesting female carrier cases have been reported in the literature. Analyzing our data on 17 new affected females and those previously reported (see Supplementary Table 1), we were able to establish several specific observations. Females with XLMTM often present with a myopathy, independently of the presence of affected males...
in the family. This myopathy can be highly heterogeneous in
terms of onset and severity and shares several clinical fea-
tures with male XLMTM including similar facial involve-
ment. However, females with XLMTM frequently display
asymmetric involvement and specific histological features
develop with age, as necklace fibers. While they usually
have a milder phenotype than males with XLMTM, females share MTM1
mutations associated with severe cases in
males, potentially due to the fact that they can express a nor-
mal allele from the non-mutated X chromosome.

Assessing the full clinical spectrum and frequency
of females with XLMTM

XLMTM female carriers were previously considered to be
usually non-affected or mildly affected. However, includ-
ing the novel cases characterized in this study, 43 females
from 36 unrelated families were reported to show signs of
muscle involvement, with several presenting with muscle,
respiratory or skeletal involvement significantly impacting
on their daily life. Nevertheless, this cohort is not likely to
represent accurately the spectrum of involvement, as sev-
eral recruitment biases may exist. Females with XLMTM
may be recognized either because they are severely affected
(they are the proband) or are mildly affected and evalu-
ated following the identification of males with XLMTM
in the family. Those females from the 12 “female families”
include some who are mildly affected, suggesting that an
unknown proportion of females with XLMTM may have
never come to clinical attention without a prior XLMTM
diagnosis in their relatives (i.e., patient F1). It is also likely
that some XLMTM manifesting females are not diagnosed
because the MTM1 gene is not suspected as the cause of
their symptoms, particularly in the absence of suggest-
histology. The proportion of reported manifestation
of females with XLMTM compared with all diagnosed
patients is around 6%, but this figure is not homogeneous
across countries. For example, this proportion is increased
in France with around 13% of XLMTM French patients
being female (19 females of the present cohort). This varia-
tion suggests that there is likely to be a lack of considera-
tion of the diagnosis in females and strongly suggests that
the frequency of females with XLMTM is underestimated.
Thorough characterization and comparison of females
with XLMTM in large cohorts, as attempted in this report,
should increase awareness and provide diagnostic clues to
better recognize this myopathy.

Clinical spectrum and asymmetric involvement in
females with XLMTM

Females with XLMTM present usually with a progressive
pattern of limb-girdle myopathy, possibly associated with
respiratory muscle and/or skeletal involvement. The impair-
ment may be as severe as in a severe male neonatal form
which may be responsible for death within the first months,
as observed in patient F2, ranging to a milder form occur-
ing mostly during childhood but impacting daily life sig-
nificantly. Many carriers were considered as non-affected in
childhood, but were never athletic at school. Females with
XLMTM display similar facial features as affected males,
with facial weakness, ophthalmoplegia and ptosis being
important diagnostic clues. The predominant proximal and
lower leg involvement pattern observed on MRI or CT scan
for most of the 15 female patients reviewed in this study is
similar to the only two observations reported for affected
males [7, 47].

In this analysis of a large patient cohort, we found recur-
cent asymmetric involvement that is also underlined in MRI
analyses. This asymmetry may involve limb and facial mus-
cle weakness and also skeletal development in several cases
(this study, [20, 25]). We and others found a strong bias in
the XCI in affected females, but this was not always consist-
ent with the severity of the symptoms. Such an asymmetric
weakness in manifesting carrier females is indeed described
in other X-linked diseases such as Duchenne muscular dys-
trophy [54, 58], suggesting that a distinct X-chromosome
inactivation ratio in different muscles could be responsible
for this asymmetry, leading to greater expression of the
mutated gene on one side of the body. It was not possible to
directly investigate several muscles from the same patient
for XCI, but such a study would help delineate a correla-
tion with asymmetric features. Similarly, the skeletal asym-
metry may be caused by differential expression of normal
MTM1 in the body, as MTM1 is suspected to be linked to
skeletal growth, given that males with XLMTM deficient
in myotubularin often show features of overgrowth at birth.
However, a mildly affected XLMTM male presenting with
muscle asymmetry has been described [7] and this clearly
could not be linked to XCI. Further observations are needed
to establish the prevalence of asymmetry in males.

In females, asymmetric involvement detected through
clinical and/or imaging investigations is another diagnostic
cue to suggest undertaking MTM1 genetic testing.

Muscle biopsy features which may be an indication
for MTM1 sequencing and XLMTM diagnosis

Muscle biopsy is routinely used to direct the molecular diag-
nosis of congenital myopathies. In females with XLMTM,
abnormal nuclei internalization and centralization is similar
to that observed in males with XLMTM. Necklace fibers
were observed in nearly one-third (11 out of 37) of females
with XLMTM for whom muscle biopsies were interpre-
table, confirming this is a prevalent histological marker in
milder XLMTM, particularly in manifesting carriers [7],
although they can also be present in severe cases as in patient F2 [1, 26]. However, this marker is not exclusive for XLMTM, being described in other forms of centronuclear myopathy due to mutations in DNM2 [14, 41].

Fatty replacement and/or fibrosis, as described in males with XLMTM, was also observed in 20 out of 38 biopsies. Such a gradual replacement of contractile tissue with non-contractile connective tissue or fat is described in congenital myopathies, in particular severe, advanced or chronic cases. The fibrosis in XLMTM may be the result of a replacement of fibers which disappear and are not regenerated due to a defect in the number of satellite cells in the muscle of the patients [52].

Overall, the presence of centralized nuclei and/or necklace fibers is a strong diagnostic clue for XLMTM in females. Nevertheless, several females with XLMTM have non-informative muscle biopsies that show dystrophic features.

**The genotype is a suggestive, but not a reliable predictor of clinical prognosis for females with XLMTM**

Nearly all MTM1 mutations detected in females with XLMTM are described in or predicted to be associated with a severe phenotype in males. It is possible that mutations associated with a mild or moderate phenotype in males are not associated with a phenotype in females due to the MTM1 expression from the non-mutated X chromosome, but a clinical evaluation of females carrying such mutations is needed to assert this hypothesis.

A non-random XCI pattern has been proposed as an explanation for the development of symptoms in manifesting carriers without chromosomal translocations [63]. The skewed X inactivation observed in muscle for patient F2 correlated with previous reports showing a similar bias in females with a severe phenotype [33, 34]. We found here an increased prevalence of highly skewed XCI in the females with XLMTM, but there is no clear correlation between the XCI ratio and the phenotype. Thus, XCI cannot be a reliable molecular diagnostic test for clinical prognosis. Several factors prevent us from drawing correlations from XCI studies including the small number of described manifesting females, the difficulty in defining a classification of the phenotypic severity, the difficulty of defining which chromosome is predominantly active, the fact that XCI varies with age and that the XCI ratio can differ between tissues and hence the results from lymphocytes may not reflect the status in muscle. Moreover, XCI studies have not been performed in asymptomatic MTM1 carrier females to assess potential correlation with disease severity. Thus, while skewed XCI is noted in several females with XLMTM, it does not correlate with disease severity but is potentially the molecular basis of the asymmetric muscle involvement.

**Benefits of XLMTM diagnosis in affected females**

Identifying the genetic cause of the disease allows appropriate care and management. A female affected with XLMTM benefits from specific care, in particular respiratory monitoring and close follow-up during and after pregnancy (F11 and F14 were reported to worsen after a pregnancy). A study of respiratory muscle function in a cohort of ten surviving males with XLMTM has shown that the respiratory muscle function declines over time [53], suggesting that females are at risk of a gradual deterioration of respiratory function.

Genetic counseling would inform affected women of the risk of occurrence of a severely affected male and the possibility of prenatal diagnosis. When a de novo mutation arises in a female child, with no evidence of parental origin, both parents should be made aware of the risk of recurrence due to possible germinal mosaicism [64].

Females with XLMTM survive longer than most male patients. A study of affected females may thus yield crucial information on the minimal dosage of myotubularin needed to minimize symptoms and also guide future therapeutic development by documenting disease evolution and complications as targets for management. Moreover, several therapeutic proof of concepts recently reported in animal models may represent potential therapies for females with XLMTM [16, 17, 35, 40, 49].

**Conclusion**

The diagnosis of XLMTM should be suspected in a female presenting with a myopathy, despite the absence of a family history and/or the presence in a muscle biopsy of some dystrophic features, particularly if facial weakness and asymmetric involvement are noted. The diagnosis of XLMTM affected females is likely to increase with the increasing use of massively parallel sequencing in a diagnostic setting ([50], this study). The occurrence of large heterozygous deletions in 4 of the 17 newly described patients underlines the importance of searching for copy number variations in the MTM1 gene [9]. Systematic long-term clinical assessment of heterozygous female relatives of any XLMTM male would allow a better ascertainment of the clinical spectrum and frequency of involvement in XLMTM carrier females. As more than 500 males have been reported to date in the literature, around 425 of their mothers were heterozygous carriers (85% of mother of affected males are carriers) as well as their other female relatives.
Authors' contributions VB and JL directed the study; VB, SS and CREGEMES for the MYOdiagHTS Project. VB and JL wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest.

Statement of human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from patients F4, F5, F16 and F17 for whom identifying photographic information is included in this article.

References

of three female carriers in a family with no affected male. Rev Neurol (Paris) 164:169–176