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ORIGINAL INVESTIGATION

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Skewed X-inactivation in a manifesting carrier of X-linked myotubular myopathy and in her non-manifesting carrier mother

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Abstract X-linked recessive myotubular myopathy (XLMTM) is a muscle disorder usually affecting newborn males. In the majority of cases, muscle weakness and hypotonia lead to a rapid demise at neonatal age. The responsible *MTM1* gene is located in proximal Xq28. Heterozygous carriers are described as being asymptomatic but, in a few cases, mild facial weakness has been reported. We report a family in which a 39-year old female showed severe progressive muscle weakness. XLMTM was initially diagnosed in the male offspring of one of the patient's sisters. The patient, one of her sisters, and their mother were heterozygous carriers for a common *MTM1* gene mutation. We found an extremely skewed X-inactivation pattern in the patient and, in the opposite direction, in her non-manifesting carrier mother, thus explaining her normal phenotype and indicating a possible inheritance of skewed X-inactivation. Linkage analysis excluded a possible involvement of the *XIST* locus at Xq13.

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Introduction

X-linked recessive myotubular myopathy (XLMTM; MIM no. 310400) is a severe congenital disorder characterized by generalized muscle weakness and hypotonia. The majority of patients usually survive only for a few months (Wallgren-Pettersson et al. 1995; Wallgren-Pettersson 1997). The gene responsible, *MTM1*, lies in proximal Xq28 and has recently been isolated (Laporte et al. 1996). It encodes a 603-amino-acid protein named myotubularin. This protein contains a conserved protein tyrosine phosphatase domain, putatively involved in signal transduction pathways regulating terminal differentiation of secondary myotubes into functional muscle fibers (Laporte et al. 1997, 1998; Cui et al. 1998). A detailed functional role for this protein in the pathophysiology of XLMTM remains to be ascertained. However, the identification of more than 100 different pathogenetic *MTM1* mutations (e.g., Laporte et al. 1996, 1997; de Gouyon et al. 1997; Tanner et al. in press) underlines a primary role for this gene in the etiology of this disorder.

Female patients suffering from XLMTM are rare in the literature (Helliwell et al. 1997, 1998), and only Dahl and colleagues (1995) have reported a manifesting girl with an established *MTM1* gene involvement. Her moderate phenotype was caused by a large deletion of Xq27-q28 containing the *MTM1* locus and a skewed X-inactivation pattern. In agreement with a recessive inheritance of XLMTM, heterozygous carriers of *MTM1* gene mutations are usually asymptomatic, although mild facial weakness has been reported (Heckmatt et al. 1985; Wallgren-Pettersson et al. 1995).

We report a 39-year-old female (Fig. 1, II-3) with a histological and clinical phenotype consistent with XLMTM. One sister (II-2) gave birth to at least two boys with established histopathological features of XLMTM. The patient was shown to be a carrier of the most common *MTM1* gene mutation 420FIQ. An extremely skewed X-inactivation pattern favoring the mutated X-chromosome could explain the phenotype in this carrier. However, an extremely skewed X-inactivation pattern in the opposite direction was unexpectedly found in her healthy carrier mother.

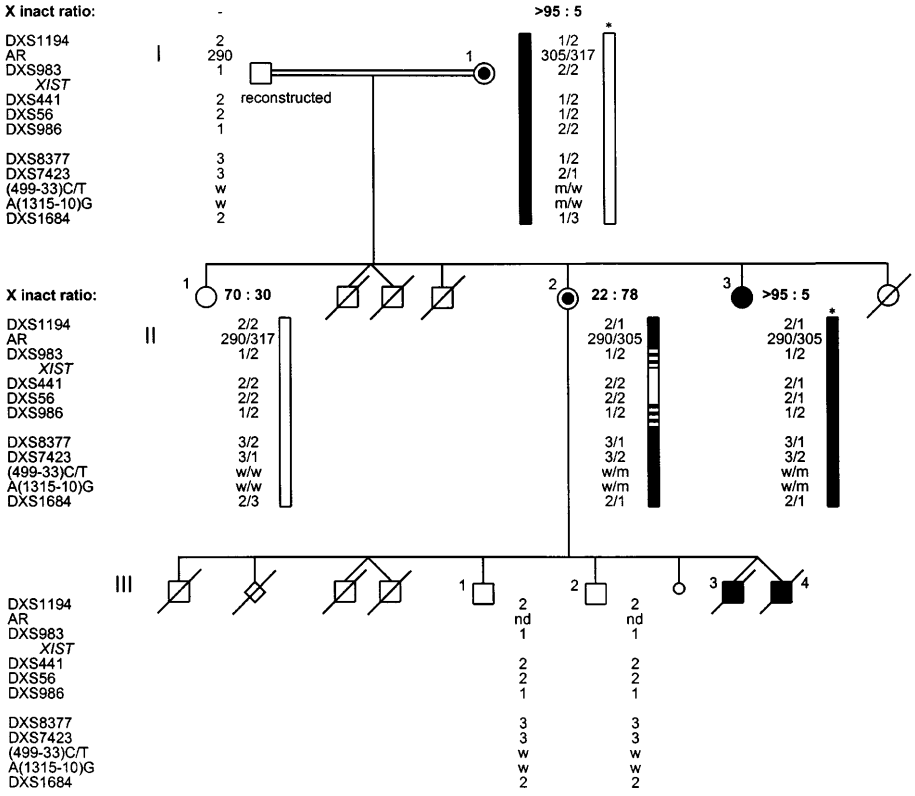

Fig. 1 Pedigree with X-inactivation and haplotype data. The microsatellite markers, the gene loci, the *MTM1* polymorphism (499–33)C/T, and the *MTM1* mutation A(1315–10)G are placed on the X-chromosome in Xq11.2-q21.1 (DXS1194-DXS986) and in Xq28 (DXS8377-DXS1684) according to Willard et al. (1994), Hu et al. (1996), and Laporte et al. (1996). *Filled bars MTM1*-mutation carrying X-chromosome, *open bars* normal X-chromosome, *striped regions* possible extensions of a double recombination in II-2, *asterisk* the active X-chromosome in I-1 and II-3, *open symbols* non-affected individuals, *filled symbols* XLMTM-affected individuals, *dot-filled circles* non-manifesting mutation carriers, *slashed symbols* deceased family members, *w* wild-type sequence, *m* mutated sequence, *nd* not done. Note that the haplotype of the father was reconstructed

Subjects and methods

Case report

This 39-year-old female (Fig. 1, II-3) of Yemenite origin was the product of a normal pregnancy and delivery. She developed normally until the age of 5 years when gait difficulty was first noticed. She showed weakness first in the lower and then in the upper extremities and underwent corrective surgery for deformity of the ankles. The patient has a normal intellectual capacity and is still ambulant.

Physical examination revealed an elongated face, prognathism, and crowded teeth. Her speech was dysarthric with a nasal quality. She had marked kyphoscoliosis and bilateral pes equinovarus. There was moderate weakness of her facial muscles and neck flexors and winging of the right scapula. The proximal upper limb muscles and the distal hand muscles were weak and wasted, whereas the forearm muscles showed almost normal strength. In the lower leg, the pattern of weakness was similar with severe pelvic girdle and distal weakness. Serum creatine kinase was normal (104 µg/l) and electromyography showed a myopathic pattern with no fibrillations or other spontaneous activity. Her brain magnetic resonance imaging was normal. Muscle biopsy demonstrated variability in fiber size, numerous internally located nuclei, mild fatty replacement, and minimal fibrous proliferation. Fiber type distribution appeared normal (Fig. 2).

Family history

Her parents were first cousins, and she had two healthy sisters (II-1 and II-2). Her 67-year-old mother (I-1), who was considered healthy, had three sons who died in infancy and one daughter that died at a young age. The diagnosis in these children was unknown. Her second sister (II-2) gave birth to five male babies who died in early infancy. Muscle biopsy in male twins (III-3 and III-4) was diagnostic for XLMTM. Further clinical information on the female cousins was not available.

MTM1 gene analysis

DNA was isolated from peripheral blood lymphocytes by standard protocols. Mutational analysis of the complete *MTM1* gene was performed by individual exon amplification, combined single-strand conformation polymorphism and heteroduplex analysis, and subsequent DNA sequencing as described elsewhere (Tanner et al. 1998).

X-chromosome inactivation analysis

X-chromosome inactivation was studied by polymerase chain reaction (PCR) analysis of a polymorphic CAG repeat in the first exon of the androgen receptor (AR) gene on lymphocyte DNA (Allen et al. 1992). Methylation of sites close to this short tandem repeat correlates with X-chromosome inactivation. The site is methylated on the inactive X-chromosome and therefore resists cleavage by *Hpa*II, and a PCR product is obtained from the inactive X-chromosome only. The technique was modified for use on an ABI 373A automated sequencer, and the PCR products were analyzed by GeneScan software (Applied Biosystems; Pegoraro et al. 1994). The X-inactivation pattern was given as the ratio between the amount of PCR products, with the smaller allele being indicated first, and were classified as random (ratios 50:50 to <65:35), moderately skewed (ratios 65:35 to <80:20), skewed (ratios 80:20 to <95:5), and extremely skewed (ratio 95:5 or higher). All samples were analyzed in duplicate.

Haplotype analysis

Mapped microsatellite markers were chosen from the X-chromosome map of the Fifth International Workshop on Human X Chromosome Mapping 1994 (Willard et al. 1994) and other sources (Gong et al. 1994; Hu et al. 1996). DXS1194 and AR are in band Xq11; DXS983, *XIST*, DXS441, and DXS56 are in Xq13; DXS986 is in Xq21; and DXS8377, DXS7423, *MTM1*, and DXS1684 are in Xq28.

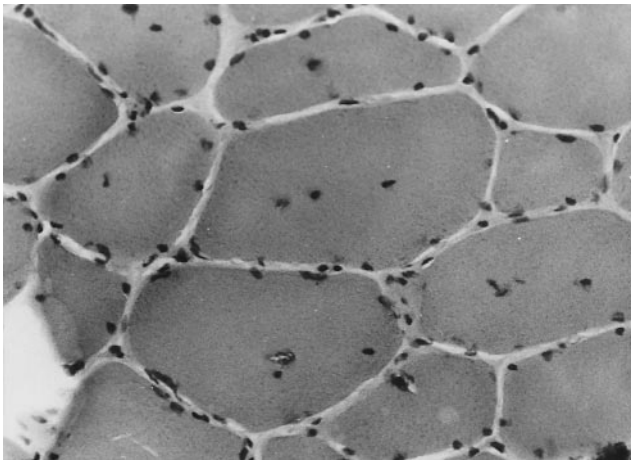

Fig. 2 Transverse cryostat section of a quadriceps biopsy from patient II-3 showing variability in fiber size and numerous internal nuclei

Results

MTM1 gene analysis

Molecular analysis of the complete *MTM1* gene in all available family members revealed two different sequence variants (Fig. 1). We detected a common *MTM1* mutation in intron 11, A(1315–10)G, which leads to the activation of a cryptic splice site, resulting in an in-frame insertion of the three amino acids FIQ after codon 420. This mutation gives a severe phenotype in males (Laporte et al. 1997; Tanner et al. 1998) and was found in a heterozygous state in all females examined except for II-1. We also found a putative polymorphism in intron 6, (499–33)C/T, cosegregating with the mutation in our family. Although we had no definitive proof, this base change probably made no clinical contribution, because of its position far upstream of the exon-intron boundary, but it was useful as an intragenic marker in this family. As expected, the two healthy sons (III-1 and III-2) of II-2 did not carry any of these nucleotide changes (Fig. 1).

X-inactivation analysis

The patient (II-3) had an extremely skewed X-inactivation pattern (>95:5) with the maternally inherited X-chromosome as the active X in most of her cells. This finding may explain her affected phenotype. However, her unaffected carrier mother (I-1) also had an extremely skewed pattern. The skewed X-inactivation in the mother was in the opposite direction, since the AR allele active in the mother was not the allele that she had given to her affected daughter (Fig. 3). Both the non-carrier (II-1) and the carrier sister (II-2) of the patient had a moderately skewed X-inactivation pattern, a pattern that has been found in 38% of control females (Ørstavik et al. 1998).

Haplotype analysis

We found an extremely skewed X-inactivation both in the mother and in the affected daughter. The *XIST* (X-inactivation specific transcript) gene located at the X-inactivation center (XIC) in Xq13 is involved in X-inactivation, and a rare mutation in the minimal promoter of the *XIST* gene has recently been reported in a family with familial skewed X-inactivation (Plenge et al. 1997). We therefore performed a haplotype analysis of the family to examine whether the skewed X-inactivation in the mother and her daughter was in agreement with a possible involvement of the *XIST* gene. Our results are summarized in Fig. 1.

We found a double recombination in II-2, covering at least DXS441 and DXS56 but possibly extending proximally to DXS983 and distally to DXS986. However, as the X-inactivation pattern in II-2 might be random, we did not pursue this recombination any further. More importantly, we found the same allelic AR/*XIST* region present on the X-chromosome carrying the mutation in I-1 and II-3, and as X-inactivation was in the opposite direction, this excluded *XIST* from being involved in the skewed patterns observed in I-1 and II-3.

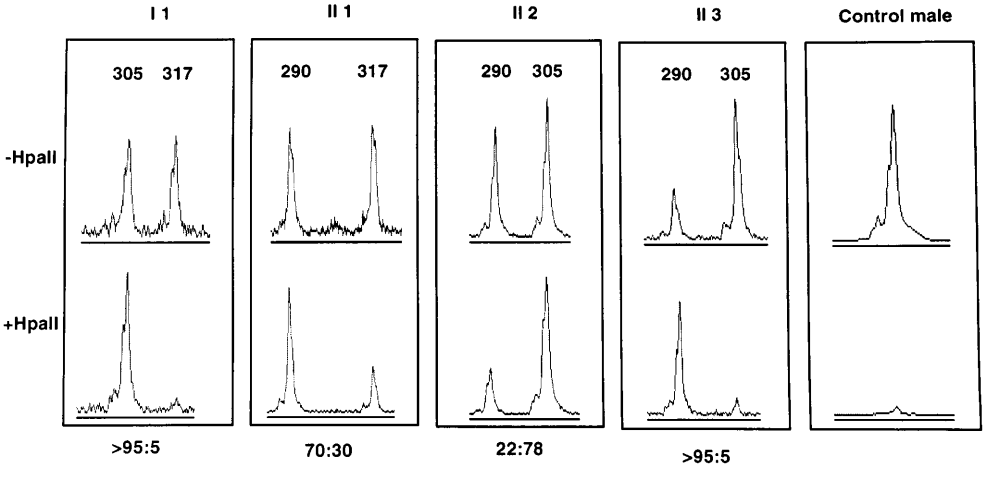

Fig. 3 X-chromosome inactivation analysis of the family. *-HpaII* Before digestion with *Hpa*II, *+HpaII* after digestion with *Hpa*II. PCR products were obtained from the inactive X-chromosome only, and a PCR product is therefore not seen in the control male after digestion with *Hpa*II. Note the extremely skewed X-inactivation pattern in the patient (*II-3*) and her mother (*I-1*) and a moderately skewed pattern in the two healthy sisters. The *numbers above* the curves indicate the number of base pairs at the AR locus

Discussion

Many females affected with an X-linked disorder, such as hemophilia and Duchenne muscular dystrophy, have a skewed X-inactivation as an explanation for their phenotype (Nisen and Waber 1989; Wadelius et al. 1993; Acquila et al. 1995; Schröder et al. 1997). In our family, a skewed X-inactivation pattern has been found in the manifesting carrier as expected. To our knowledge, this is the first report of an adult and cytogenetically normal female being severely affected by XLMTM with an established *MTM1* gene mutation and skewed X-inactivation. Interestingly, the patient shows a milder phenotype with later onset than male patients with the same mutation (Laporte et al. 1997; de Gouyon et al. 1997; Tanner et al. in press). It is possible that the multinucleated muscle fibers still express some functional myotubularin, even if X-inactivation is very biased; this might compensate for the most detrimental effects in early childhood. Unfortunately, we have not had access to tissues other than lymphocyte DNA to investigate this point further. However, our findings warrant that females showing features of the autosomal recessive (MIM no. 255200) or the autosomal dominant (MIM no. 160150) forms of myotubular myopathy (Wallgren-Pettersson et al. 1995) should be screened for mutations in *MTM1* and analyzed for their X-inactivation patterns. Unexpectedly, an extremely skewed X-inactivation pattern has also been found in the non-manifesting carrier mother.

Recently, an increased frequency of skewed X-inactivation has been found in females of more than 60 years of age, possibly because of stochastic clonal loss with age (Busque et al. 1996; Gale et al. 1997). Therefore, it cannot be excluded that the skewed X-inactivation found in the 67-year-old mother (I-1) of the patient is related to her age. However, since her X-inactivation is extremely skewed, we do not find this a likely explanation.

Because of the small number of cells present at the time of X-inactivation, a skewed X-inactivation may be the result of a chance occurrence (Puck and Willard 1998). This is not a likely explanation for the findings in our family, since an extremely skewed X-inactivation pattern is a rare occurrence and has not been found in any of 148 control females (Ørstavik et al. 1998).

A possible explanation for the findings in our family is that the skewed X-inactivation pattern is a result of a genetic influence. Several families with X-linked disorders have been reported with more than one affected female (Reddy et al. 1984; Taylor et al. 1991). One family with Duchenne muscular dystrophy is similar to our family, since both an affected daughter and the non-affected carrier mother have a skewed X-inactivation pattern (Tihy et al. 1994). Furthermore, several families have been reported in which a skewed X-inactivation seems to segregate in a Mendelian manner, in both normal families and families with X-linked disorders (Hoffman and Pegoraro 1995; Naumova et al. 1996; Ørstavik et al. 1996). The *XIST* gene is expressed from the inactive X only and must be present for X-inactivation to take place (for a review, see Brockdorff and Duthie 1998). This gene is therefore a candidate gene for mutations causing a skewed X-inactivation. In our family, however, haplotype analysis has excluded a possible involvement of *XIST* as an explanation for the occurrence of a skewed X-inactivation in the two females.

A skewed X-inactivation pattern may also be the result of selection against cells in which the mutant gene is located on the active X-chromosome. This is the presumed reason for the skewed X-inactivation found in Wiskott-Aldrich syndrome (Fearon et al. 1988) and ATR-X syndrome (Gibbons et al. 1992) and is in agreement with the observation that carriers of these disorders have a completely normal phenotype. Selection against cells with the mutant *MTM1* gene on the active X-chromosome can be excluded in our family, since it would not explain the patient's phenotype.

It has recently been argued that familial skewing of X-inactivation not linked to XIC most probably results from mutations that influence cell proliferation (Migeon and Haisley-Royster 1998). A possible explanation for the findings in our family is therefore an unrecognized X-linked mutation that affects cell proliferation following random X-inactivation.

In conclusion, we suggest that the affected phenotype in our patient II-3 is the result of an inherited mutation affecting X-inactivation not linked to XIC or of an inherited mutation affecting cell proliferation. However, it cannot be excluded that the skewed X-inactivation in the two females is a chance occurrence.

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