

Short reports

Extensive germinal mosaicism in a family with X linked myotubular myopathy simulates genetic heterogeneity

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Abstract

A family with two male cousins affected with myotubular myopathy (MTM) was referred to us for genetic counselling. Linkage analysis appeared to exclude the Xq28 region. As a gene for X linked MTM was recently identified in Xq28, we screened the obligatory carrier mothers for mutation. We found a 4 bp deletion in exon 4 of the MTM1 gene, which originated from the grandfather of the affected children and which was transmitted to three daughters. This illustrates the importance of mutation detection to avoid pitfalls in linkage analysis that may be caused by such cases of germinal mosaicism.

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Myotubular myopathy is a congenital disease usually inherited as an X linked trait, characterised by severe generalised hypotonia and muscle weakness associated with respiratory failure. Polyhydramnios and reduced fetal movements are often present prenatally. Examination of muscle biopsy shows a central

area devoid of myofibrils and centrally positioned nuclei in most muscle fibres (OMIM 310400¹). The MTM1 gene was localised in Xq28 by linkage analysis.^{2,3} We recently reported the identification of the MTM1 gene, which codes for a putative tyrosine phosphatase and was found to be mutated in many patients with X linked myotubular myopathy.^{16,17}

We report on a family with typical myotubular myopathy and evidence of X linkage, but in which the disease appeared to be unlinked to Xq28. Further study showed, however, a frameshift mutation in the MTM1 gene that originated from the grandfather of the affected children and that was transmitted to three daughters. This illustrates the importance of mutation detection to avoid pitfalls in linkage analysis that may be caused by such cases of germinal mosaicism.

In family D, two male cousins (III.2 and III.5, fig 1) died in the neonatal period from X linked myotubular myopathy. The family was referred to us for genetic counselling.

III.2 was born in 1985 after a pregnancy complicated by polyhydramnios and weak fetal movements. The boy was severely hypotonic and needed artificial ventilation. Reaction to

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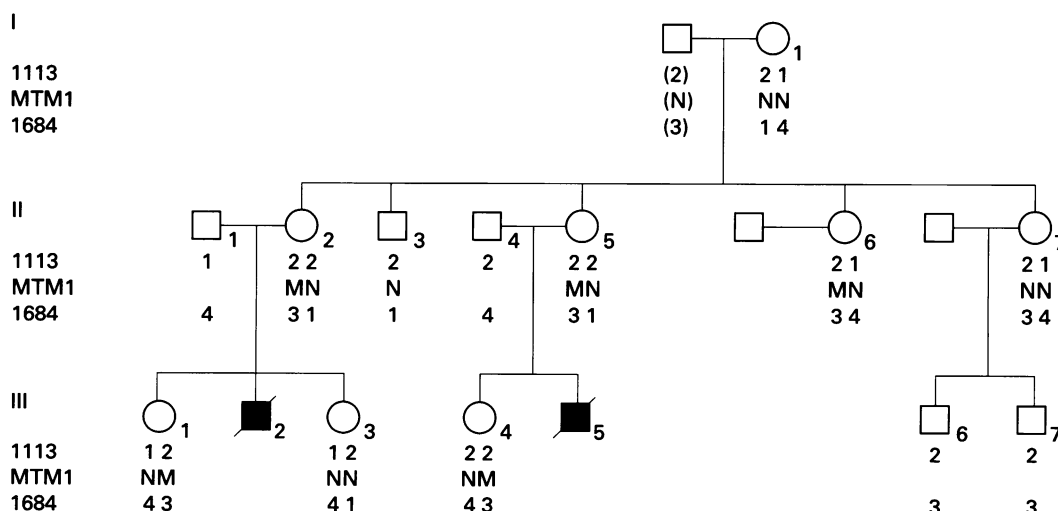


Figure 1 Pedigree of family D. Genotypes are indicated for microsatellites DXS1113 and DXS1684, which flank the MTM1 gene. At the latter locus, the normal allele and mutated allele are labelled N and M respectively. Allele M in II.2, 5, 6 and III.1, 4 corresponds to a deletion (266 del ATTT) in exon 4 of the MTM1 gene.

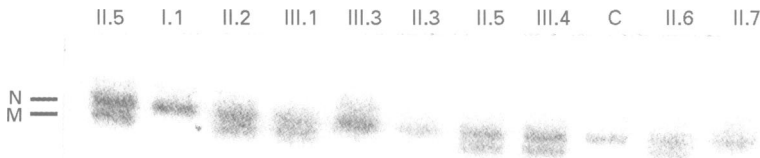


Figure 2 SSCP analysis of family D. Analysis was performed as described⁴ using fast migration for five hours at 25 W at 4°C. Primers for exon 4 will be described elsewhere (J Laporte, in preparation). The SSCP pattern shows two bands in female carriers (II.2, 5, 6 and III.1, 4). C=control DNA.

painful stimuli, deep tendon reflexes, and sucking were all weak. He was unable to swallow. EMG showed important myopathic changes. Serum creatine kinase level was slightly raised (330 U/l). The child was weaned off the ventilator at 3 weeks of age and died two days later.

III.5 was born prematurely at 32 weeks' gestation. During pregnancy polyhydramnios was observed, but the mother was not aware of reduced fetal movements. At birth the boy was asphyxiated. A left pneumothorax was observed and resolved spontaneously, but the child remained unable to breathe and was ventilated. Muscle tone was very decreased with absence of deep tendon reflexes. The boy was non-reactive and could not swallow. Serum creatine kinase level was normal. Despite mild improvement of the muscle weakness the child died at the age of 2½ months. A muscle biopsy obtained at 1 month of age showed classical features of myotubular myopathy (analysis by Professor Krivosic, Lille). The nuclei were round, large, and centrally located in about 20% of the fibres. Histochemical preparations showed aggregation of oxidative enzymes and decreased ATP-ase activity in the central part of the fibres.

The mother of III.2 was treated in infancy for dorsal scoliosis. At examination at the age of 40 years, slight pes cavus, slender fingers, and moderate hand muscle weakness were observed. Deep tendon reflexes were all weak. Slight anomalies were observed on the EMG. No muscle biopsy was obtained. The mother of III.5 was examined at the age of 33. She was normal except for obesity and slender fingers. EMG was normal. No muscle biopsy was obtained. The 65 year old maternal grandmother of the patients (I.1) was not examined. According to her children, she never complained of any muscle weakness. The grandfather died of silicosis at the age of 53 and no DNA was available for study. The fathers of the affected children showed no muscle weakness and had normal EMG.

Linkage analysis was performed in this family. No sample from the two affected children was available for DNA extraction. The two obligate female carriers had inherited the same maternal alleles as their unaffected brother (II.3) at the DXS1113 and DXS1684 loci flanking MTM1. Their paternal haplotype was also present in their unaffected nephews (III.6 and III.7). As DXS1113 and DXS1684 are

located at about 4-5 cM from each other,⁵ a double recombination appeared to be excluded in II.3. There was thus no evidence for linkage to Xq28 and we were then unable to counsel the other women at risk (II.6 and II.7). We thus started a linkage analysis using markers in other regions of the X chromosome on this family and another one that appeared also to exclude linkage to Xq28.⁶

Meanwhile, the MTM1 gene was cloned and we decided to search for a mutation using SSCP analysis.⁴ In exon 4,¹⁶ we found two different band patterns. Five women in the family were heterozygous for the variant band (fig 2). Sequencing of this variant showed a deletion (266 del ATTT), which generates a frameshift. This mutation was found in the two obligate carriers and in one of their sisters (II.6), who carries a different maternal haplotype around MTM1. The finding of this mutation in two daughters (III.1 and III.4) of the obligate carriers indicated that it was in fact carried on the grandpaternal haplotype. The segregation of the disease can be explained by extensive germinal mosaicism in the normal grandfather, who transmitted the new mutation to three of his daughters. On the basis of these results, accurate genetic counselling was then given to the family.

Cases of male or female germline mosaicism have been identified in several X linked diseases, including Duchenne muscular dystrophy,⁷⁻¹⁰ ornithine transcarbamylase deficiency,¹¹ Wiskott-Aldrich syndrome,¹² severe combined immunodeficiency (SCID),¹³ X linked hydrocephalus,¹⁴ and others. Similar transmission of a paternal new mutation to three daughters has been reported in at least two other cases.¹⁰⁻¹² The possibility of germline mosaicism has obvious implications for genetic counselling, since it means that there is a significant risk of recurrence of a new mutation, and since it can lead to misinterpretation in linkage analysis.¹²⁻¹⁴ Direct detection of the mutation, when possible, avoids such pitfalls.

Genetic heterogeneity is always a complicating issue for genetic counselling. In the case of myotubular myopathy, the present family and another one described previously⁶ appeared to be unlinked to Xq28. We also recently found an MTM1 mutation in the single proband of the other family. In the latter case, the presence of a small number of centrally located myotubes in muscle biopsies of several women in the family had been initially interpreted as indicating carrier status, but these women do not in fact carry the mutation.¹⁵ In conclusion, there is no more evidence for genetic heterogeneity of X linked myotubular myopathy, and this should give increased confidence in the use of indirect linkage based diagnosis in familial cases in which mutation has not yet been found.⁵

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