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X linked myotubular myopathy (MTM1) maps between DXS304 and DXS305, closely linked to the DXS455 VNTR and a new, highly informative microsatellite marker (DXS1684)

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Abstract

The locus for X linked recessive myotubular myopathy (MTM1) has previously been mapped to Xq28 by linkage analysis. We report two new families that show recombination between MTM1 and either DXS304 or DXS52. These families and a third previously described recombinant family were analysed with two highly polymorphic markers in the DXS304-DXS52 interval, the DXS455 VNTR and a newly characterised microsatellite, DXS1684 (82% heterozygosity). These markers did not recombine with MTM1 in the three families. Together with the recent mapping of an interstitial X chromosome deletion in a female patient with moderate signs of myotubular myopathy, our data suggest the following order of loci in Xq28: cen-DXS304-(DXS455, MTM1)-DXS1684-DXS305-DXS52-tel. This considerably refined localisation of the MTM1 locus should facilitate positional cloning of the gene. The availability of highly polymorphic and very closely linked markers will markedly improve carrier and prenatal diagnosis of MTM1.

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X linked myotubular myopathy (MTM1, MIM 31040) is characterised by congenital hypotonia, muscle weakness, and associated respiratory insufficiency. Muscle biopsy shows many small, rounded fibres resembling myotubes, with centrally located nuclei. Stillbirth and perinatal death is common and affected boys rarely survive the first year of life.

Previous linkage studies have shown evidence for linkage between MTM1 and several Xq28 marker loci, including DXS52, DXS15, and F8C.[1-6] One meiosis recombinant for DXS52 but not for DXS304 suggested that MTM1 is proximal to DXS52, but odds were only 9:1 versus MTM1 being distal.[3] Another recombination event reported between MTM1 and DXS52 was not analysed at DXS304.[6] Because of the very high neonatal mortality and the low incidence of this disease, informative families are very rare. We recently reported preliminary analysis of a cytogenetically detectable interstitial deletion in a young female patient who presented with a milder form of myotubular myopathy.[7] This suggested that MTM1 is located proximal to DXS305, a marker within the DXS304-DXS52 interval; however, other interpretations of the data are possible. In order to improve the genetic mapping of the MTM1 locus, and specifically to refine the analysis of critical recombinant families reported at the recently created International MTM Consortium,[8] we decided to develop microsatellite markers for the DXS304-DXS305 interval, a region of approximately 2 Mb[9] currently devoid of such markers. We report the isolation of a highly polymorphic microsatellite (DXS1684) that was analysed, together with the DXS455 VNTR[10] in three recombinant MTM1 families. The cosegregation of MTM1 and both DXS455 and DXS1684 in these three families indicates that MTM1 is localised within the DXS304-DXS52 interval which overlaps with the distal end of the region deleted in the female patient, thereby validating the latter case for mapping the MTM1 locus. Taken together, the linkage data and the analysis of the deletion patient[7] (Dahl *et al*, in preparation) considerably refine the localisation of MTM1 to a region of less than 1 Mb.

Material and methods

CHARACTERISATION OF MICROSATELLITES

Restriction digests of cosmid contigs from the Xq28 region were screened with a random primed CA:TG repeat polynucleotide (Pharmacia). Positive cosmids assigned to contigs in the DXS304-DXS305 interval (Gong *et al*, in preparation) were restriction digested with *Sau*3A and shot gun cloned in pBluescript, and subclones were rescreened for CA repeats. Selected subclones were sequenced using cycle sequencing with fluorescent dideoxynucleotides and analysis on an Applied Biosystems sequencer. Three different CA repeats were characterised and flanking primers were synthesised. Only one of them (St71.1, DXS1684) was found to be polymorphic.

FAMILY STUDIES

DNA from available family members was analysed by Southern blotting for DXS304 (probe U6.2, *Taq*I digest), DXS455 (probe 346.72, *Bst*YI digest), DXS52, and F8C (F814, *Bcl*I digest).[10-12] Polymerase chain reaction was per-

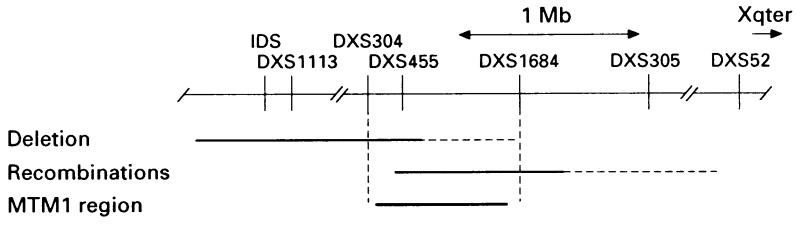

Figure 1 Map of the Xq28 region. Relative order of markers and distances are derived from Poustka et al[9] and Rogner et al (in preparation). Extent of the MTM1 female deletion[7] (Dahl et al, in preparation) and the interval between recombination events are indicated (dotted segments correspond to the regions that contain the distal deletion breakpoint or the recombination events).

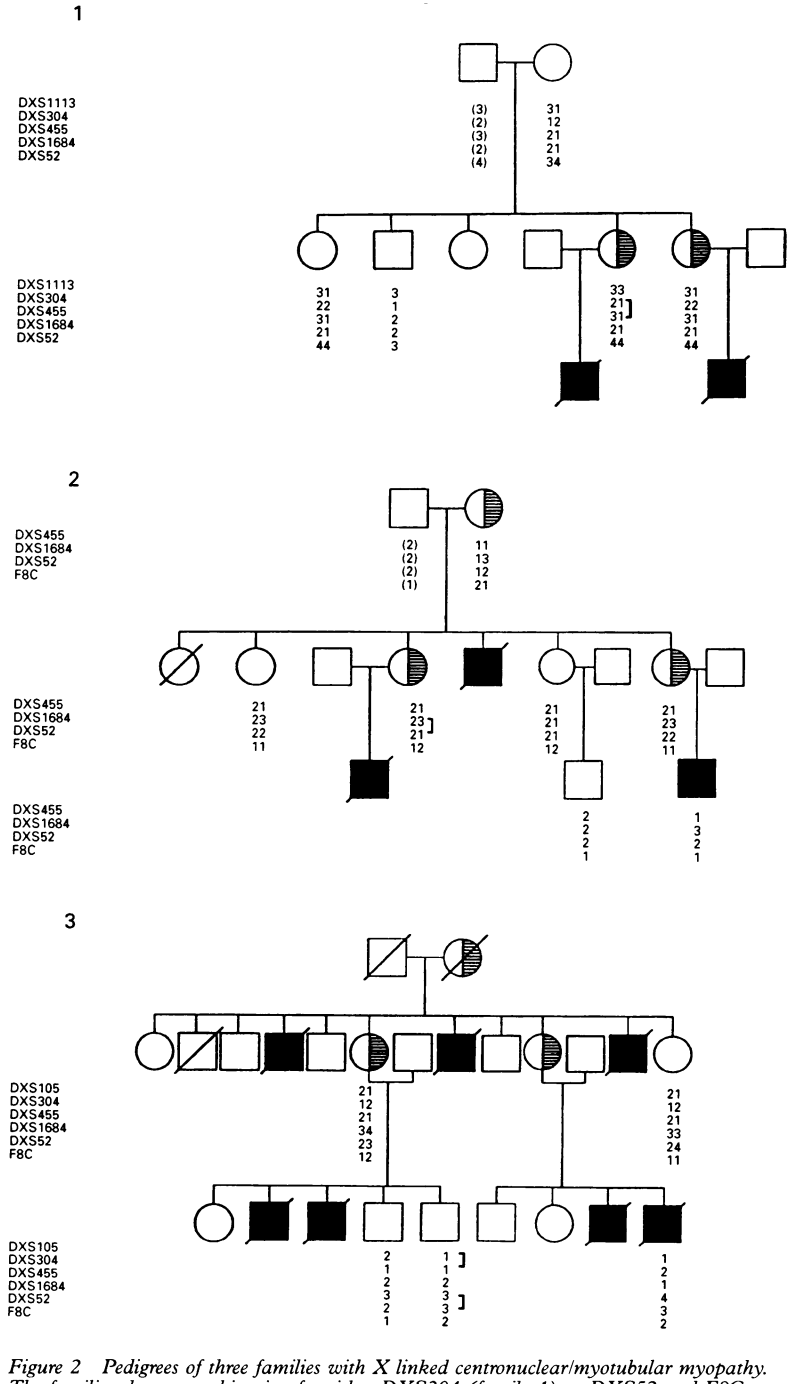

Figure 2 Pedigrees of three families with X linked centronuclear/myotubular myopathy. The families show recombination for either DXS304 (family 1) or DXS52 and F8C (families 2 and 3) versus the MTM1 locus. Haplotypes shown are those that minimise recombination. Genotypes in parentheses are deduced. Family 1 (from France) was obtained through FS, family 2 (from USA) through GH, and family 3 (from Finland[3]) through NT.

formed for DXS1113, a marker very close to the IDS locus[13] and for the St71.1 microsatellite (DXS1684). Primers for DXS1684 were 5′-AGCACCCAGTAAGAGACTGAAC and 5′-CCTCAGTGGCAACCACTCAAG. One primer was end labelled with γP32 ATP and polynucleotide kinase. Four pmol of each primer, including 0·3 pmol of the kinased primer, were used in 25 μl reactions with 100 ng DNA.[13] Thirty cycles of PCR were performed; 94°C for one minute, 65°C for one minute, and 72°C for two minutes, with a final elongation step of 10 minutes. Alleles (128–148 bp) were separated on a 6% denaturing polyacrylamide gel.

Results

ISOLATION OF A NEW MICROSATELLITE

As part of the systematic mapping of Xq28, YAC and cosmid contigs have been constructed in Heidelberg[14,15] (Gong *et al* and Rogner *et al*, in preparation). Cosmids assigned to the DXS304-DXS305 interval were screened for CA repeats and appropriate subclones were sequenced. Three different CA repeats were identified (with 16, 18, and 22 CA dinucleotides respectively). Further PCR analysis showed that only one of them (St71.1, DXS1684) was polymorphic. Seven alleles have been detected, and a heterozygosity of 82% was calculated from analysis of 36 independent X chromosomes. Two CEPH families (35 and 884) that show recombination between DXS304 and DXS52 or F8C were analysed, confirming the localisation of the polymorphism distal to DXS304. DXS1684 was further mapped at about 600 kb proximal to DXS305 on a YAC contig from the region (Rogner *et al*, in preparation) (fig 1). Single copy subclones, derived from the same cosmid as St71.1, were used as probes for Southern blot analysis of five independent somatic cell hybrids retaining the deleted X chromosome from the female patient with moderate myotubular myopathy[7] (Dahl *et al*, in preparation). This showed that DXS1684 is located distal to the telomeric deletion breakpoint.

ANALYSIS OF RECOMBINANT FAMILIES

Three families showing recombination between MTM1 and the Xq28 markers DXS304 or DXS52 (fig 2) were analysed with the new DXS1684 microsatellite, and with the DXS455 VNTR previously localised in the DXS296-DXS305/DXS374 interval.[10,16] In family 1, although no DNA was available from the two affected males, their obligate carrier mothers carried different maternal alleles at DXS1113 (near IDS) and at DXS304. The DXS1684 microsatellite, DXS455, and DXS52 were all informative and segregated concordantly in the two carriers. This provides evidence for the MTM1 locus being located telomeric to DXS304. (Segregation in other family members is consistent with the grandmother being a carrier, but it cannot be formally excluded that the mutation originated by germinal mosaicism from the grandfather, as was described by Ar-

veiler *et al*[17] in a family with Wiskott-Aldrich syndrome.) In family 2, an obligate carrier showed recombination between MTM1 and both DXS52 and F8C, but was non-recombinant with DXS1684 (DXS455 and DXS304 were not informative). In family 3, a normal male was shown previously to be recombinant for DXS52 and F8C, but not for DXS304[3] (fig 2). DXS455 and DXS1684 were both informative and non-recombinant in this male. A more proximal recombination, between DXS105 (in proximal Xq27) and DXS304, was also detected in the same person. The presence of a double recombination is not surprising, as the interval between DXS105 and DXS52 is about 30 cM and ~20 cM between DXS105 and DXS304.[11] The recombinants in families 2 and 3 thus place MTM1 well proximal to DXS52 and close to DXS1684.

Discussion

We have isolated a new highly informative microsatellite in the DXS304-DXS305 interval, and analysed it, together with the DXS455 VNTR, in three recombinant MTM1 families. Our results indicate that MTM1 is located between DXS304 and DXS52, and appears closer to DXS455 and DXS1684, as these markers showed no recombination with the disease locus in the three families. The approximately 5 Mb DXS304-DXS52 interval[9] overlaps with the heterozygous deletion recently analysed in a female with moderate myotubular myopathy; the deletion encompasses DXS304 and DXS455, while DXS305 and DXS1684 are distal to it. The phenotypic expression is likely to be the result of a preferential inactivation of the normal X chromosome[7] (Dahl *et al*, in preparation). The overlap with the candidate region defined by linkage analysis validates the use of this deletion to map the MTM1 gene. Taking into account the marker order determined previously,[9 16 18] the following order can be proposed: cen-IDS, DXS1113-DXS304-(DXS455, MTM1)-DXS1684-DXS305/DXS374-DXS52-F8C-qter. The candidate region for MTM1 spans about 1 Mb (fig 1) and includes the highly informative marker DXS455. This considerable refinement in the localisation of MTM1 will facilitate positional cloning strategies. Our results also allow for a more reliable and efficient carrier and prenatal diagnosis. While some female carriers can be diagnosed on the basis of a muscle biopsy showing abnormal small muscle fibres with centrally located nuclei, a normal biopsy does not exclude carrier status. Linkage analysis is thus required for genetic counselling purposes. Available genetic maps indicate that the distance between the IDS, DXS1113, and DXS305 flanking markers is about 7 to 8 cM.[19 20] The flanking loci DXS1684 and DXS1113 now provide a very informative PCR test for carrier and prenatal diagnosis of MTM1 which, if consistent with the haplotypes of other family members, will give an accuracy of greater than 0·99. Other highly informative markers close to the MTM1 locus, such as DXS304 and DXS455, will increase the informativeness and accuracy of diagnosis, but they still at present require Southern blot analyses.

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