X linked neonatal centronuclear/myotubular myopathy: evidence for linkage to Xq28 DNA marker loci

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Abstract

We have studied the inheritance of several polymorphic Xq27/28 DNA marker loci in two three generation families with the X linked neonatal lethal form of centronuclear/myotubular myopathy (XL MTM). We found complete linkage of *XLMTM* to all four informative Xq28 markers analysed, with *GCP/RCP* ($Z=3.876$, $\theta=0.00$), with *DXS15* ($Z=3.737$, $\theta=0.00$), with *DXS52* ($Z=2.709$, $\theta=0.00$), and with *F8C* ($Z=1.020$, $\theta=0.00$). In the absence of any observable recombination, we are unable to sublocalise the *XLMTM* locus further within the Xq28 region. This evidence for an Xq28 localisation may allow us to carry out useful genetic counselling within such families.

Myotubular myopathy (MTM) was first described in 1966 by Spiro *et al*¹ in an isolated male infant. Subsequently, a severe neonatal form of this condition has been well documented that is usually X linked.²⁻⁴ There is severe generalised hypotonia and muscle weakness, with consequent respiratory embarrassment; affected males often die in utero, in the neonatal period, or within the first three years of life. Muscle biopsy shows hypotrophic fibres characterised by a central area devoid of myofibrils but containing

mitochondria, glycogen, and large nuclei, singly or in chains. This central area is similar in appearance to the myotubes seen in fetal muscle. Central nuclei are seen in other neonatal myopathies, including congenital myotonic dystrophy, but do not usually persist in these conditions, either in tissue culture or in muscle biopsies taken at a later stage. In contrast, the myotubular appearance does persist in the severe neonatal MTM, being found after tissue culture for several weeks,⁵ and on repeat muscle biopsy after many months.⁶ This supports the concept of this form of MTM displaying a maturational arrest of muscle ultrastructural development, and so the pathogenetic term 'myotubular' is preferred by some to the purely descriptive 'centronuclear', although neither word encompasses all the pathological characteristics of the disorder.⁴

There are some features of MTM that suggest a neurogenic element in its aetiology. Thus, degeneration and regeneration have been described in axons of the sciatic nerve of a male infant who died at 9 months.⁶ The fact that this boy survived for so long might indicate, however, that the diagnosis in his case could be distinct from the X linked severe neonatal form considered in this paper. In another study, electromyography indicated fibrillation potentials in several cases, indicative of incomplete innervation despite partial or complete innervation dependent histochemical differentiation.^{2 6 7} The pathological neuromuscular interaction in MTM is poorly understood.

In X linked MTM, a number of obligate female carriers have been shown to have abnormal muscle biopsies. Two of three such women biopsied in one family showed scattered abnormal fibres, indicating myotubes,² as did four of five carriers in a second family.³ Increased variability of fibre size without myotubes has been found in three definite female carriers.⁴ However, abnormalities have occasionally been found in the fathers of sporadic cases and this prevents a simple interpretation of a biopsy from the mother of such affected males.⁴ Another obligate female carrier has been found to have a normal muscle biopsy,⁸ and so a likelihood ratio of 2:1 for a female

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carrier to have abnormal muscle pathology may be appropriate in familial cases. Caution must still be applied in using this figure in counselling relatives of an isolated affected male.

Because of the difficulties of carrier detection in this disorder, and hence of genetic counselling, we have undertaken a genetic linkage study using a panel

of polymorphic DNA markers from the X chromosome. These have been applied to two families which show typical clinical and pathological characteristics of X linked MTM. The objective is to localise the X linked MTM locus for the purposes of genetic counselling, and to permit the future isolation and cloning of the disease gene itself.

Pedigrees and DNA probe results.

Summary of linkage data. Lod scores for linkage between X linked MTM and marker loci at different values of θ .

Probe	Marker locus	θ						
		0.00	0.01	0.05	0.10	0.20	0.30	0.40
DX13	<i>DXS15</i>	3.737	3.676	3.424	3.092	2.364	1.543	0.664
St14	<i>DXS52</i>	2.709	2.666	2.487	2.252	1.742	1.175	0.569
F8C	<i>F8</i>	1.028	1.011	0.938	0.841	0.624	0.360	0.136
hs7	<i>CBD/CBP</i>	3.876	3.819	3.576	3.244	2.491	1.632	0.718

Methods

FAMILY STUDIES

Both families were clinically examined by us, and all the affected subjects, who were male, expressed the severe neonatal form of the disorder. Muscle biopsies were performed on all affected males and on many of the obligate or possible carrier females.

DNA STUDIES

Blood was taken from relevant family members and the DNA was extracted from leucocytes and purified using a standard phenol/chloroform technique. Post-mortem liver and spleen were the source of DNA from two affected male infants in family 1.

DNA was digested with appropriate restriction enzymes and buffers using conditions recommended by the suppliers. The digested DNA was separated by electrophoresis in 0.8% agarose gels, and then transferred to nylon membranes by Southern blotting in 0.7 mol/l ammonium acetate, 0.01 mol/l sodium hydroxide. The membranes were dried and the DNA was bound to the membrane by exposure to UV light (268 nm) for 40 seconds. The membranes were prehybridised in 1 mol/l sodium chloride, 0.05 mol/l Tris (pH 7.5), 1% sodium dodecyl sulphate (SDS), and 10% dextran sulphate at 65°C for at least two hours. The DNA probes, in the form of inserts excised from their parent plasmids, were radiolabelled by the random oligonucleotide method⁹ to a specific activity of 5×10^7 to 1×10^8 dpm/ μ g. The denatured probe was added to the hybridisation mix and incubation continued at 65°C for 18 to 20 hours. Post-hybridisation washes were initially in 2 $\times$ SSC, 0.1% SDS for 2 $\times$ 5 minutes at 65°C (SSC is 0.15 mol/l sodium chloride, 0.015 mol/l sodium citrate). The stringency was increased by sequential washing of the membranes in a decreasing concentration of SSC (1 $\times$ to 0.1 $\times$) and 0.1% SDS at 65°C, depending on the strength of the radioactive signal. The washed membranes were exposed to Fuji x ray film, with Cronex intensifying screens for one to seven days at -70°C.

The panel of DNA probes used covered the major areas of the X chromosome. The DNA probe results were analysed for linkage to the disease locus using the computer package LINKAGE (version 4.7).¹⁰

Results

HISTOPATHOLOGY

The results of the detailed examination of the muscle biopsies from affected males, and from obligate and possible carrier females, will be reported separately in a paper by Braga *et al.* It is important here, however, to report the results in family 1: muscle biopsies from the obligate carrier grandmother (I.1) and all of her daughters showed myopathic changes strongly suggestive of X linked MTM. All these women, therefore, have been classified as carriers for the purposes of the DNA study. In this family, three of the six phase unknown meioses, and two of the four phase known meioses, depend upon this classification being correct.

DNA STUDY

Preliminary linkage analysis on family 1 in 1984 used a limited panel of DNA probes from Xp22 to Xq21. Of these, only D2 (*DXS43*), L1.28 (*DXS7*), DP34 (*DXYS1*), and p212 (*DXS178*) were at all informative. Probes RC8 (*DXS9*) and p754 (*DXS84*) were uninformative. No evidence of linkage to any of these loci was found.

Recent studies have used usefully polymorphic probes from the distal long arm of the X chromosome. We have linkage data using markers 114.12 (*F8C*),¹¹ St14 (*DXS52*), DX13 (*DXS15*), and hs7, the red/green pigment gene *CBD/CBP*.¹² The segregation of alleles for these four probes in the two families are illustrated in the pedigrees in the figure. Unfortunately, only the colour vision pigment gene probe was fully informative in all meioses. A summary of the linkage data is presented in the table, showing the lack of any identified recombination between *MTM* and these marker loci. The two physically linked loci, *DXS52* and *DXS15*,¹³ show high lod scores of 2.71 and 3.74 respectively at 0 cM, but the highest lod score was obtained with *CBD/CBP*: 3.876 at 0 cM. If our assignment of definite carrier status to three females in family 1 were incorrect, then this peak lod score would be 2.3 at 0 cM; however, we have every confidence in the pathological studies in this family, and hence in our linkage data.

Discussion

We have examined two families segregating for X linked MTM over three generations, and used a panel of DNA probes to search for genetic linkage between MTM and these marker loci. In a preliminary study, we have found recombination for markers on the short arm and the proximal long arm of the X chromosome, but have so far found no recombination with loci from the Xq28 region. Linkage of this devastating disease to these distal long arm markers will permit the more accurate genetic counselling of females from such families. Caution is required, however, because the question of genetic heterogeneity of XLMTM has been raised (J-L Mandel, personal communication), although not so far confirmed. Further delineation of the linkage analysis with more probes may permit us to define closely linked flanking DNA markers, and hence may indicate a route to isolating the gene itself.

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