Genetic linkage between Becker muscular dystrophy and a polymorphic DNA sequence on the short arm of the X chromosome

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SUMMARY A study of DNA restriction fragment polymorphisms and Becker muscular dystrophy has shown eight families informative for the cloned sequence L1.28, which is located on the short arm of the X chromosome between Xp110 and Xp113. Analysis of these families reveals linkage between the two loci, with the maximum likelihood estimate of the genetic distance being 16 centi-Morgans (95% confidence limits between 7 and 32 centiMorgans). Since a study of DNA polymorphisms in Duchenne muscular dystrophy has shown a comparable linkage distance with L1.28, our results suggest that the locus for Becker muscular dystrophy, like that for Duchenne dystrophy, is on the short arm of the X chromosome, and further that these two loci may be closely linked or possibly allelic.

An X linked muscular dystrophy more benign than the Duchenne type was first described by Becker and Kien in 1955. They suggested that these two forms of dystrophy might be produced by different mutations, possibly allelic. However, subsequent linkage studies of families with Duchenne and Becker muscular dystrophies with the Xg blood groups, colour blindness, and G6PD variants have indicated that separate loci may be responsible for the two disorders. Evidence for linkage between Becker muscular dystrophy (BMD), colour blindness, and G6PD suggested a location for the BMD gene on the long arm of the X chromosome. By contrast, measurable linkage was excluded between these marker loci and Duchenne muscular dystrophy (DMD). Reports of DMD in girls with X chromosome abnormalities have suggested a short arm location for the DMD locus at Xp21.

Recently it has become possible to use recombinant DNA technology in gene mapping. Restriction fragment length polymorphisms (RFLPs) result from DNA base changes, or small insertions or deletions of DNA sequences which remove, insert, or rearrange restriction enzyme sites. These are inherited in Mendelian fashion, and may be used in conventional linkage studies.

Studies have been undertaken in Duchenne muscular dystrophy using two X chromosome single copy probes, phage λRC8 and plasmid L1.28, located between Xp21 and Xp223 and Xp110 and Xp113, respectively. The data show that the DMD locus is linked to both of these probe loci and lies between them, at an approximate distance of 15 centiMorgans from each.

In this paper we describe the results of a genetic linkage study of Becker muscular dystrophy and the probe L1.28 and examine the linkage relationship between the BMD and DMD loci.

Materials and methods

SUBJECTS Families were studied in which there were two or more males with Becker muscular dystrophy, with affected males occurring in separate sibs to be certain of X linked inheritance. Confirmation of the diagnosis had been made in at least one affected person in each family by muscle biopsy, usually supplemented by electromyography, and all males had CK estimations performed in order to detect preclinical cases. Only females who were obligate carriers were included in the linkage analysis.

DNA EXTRACTION AND ANALYSIS Total human DNA was extracted from whole blood collected into EDTA, using the method described by
Kunkel et al. The DNA was then completely digested with TaqI restriction enzyme which recognises and cleaves the nucleotide sequence T+C+G+ A. The fragments of DNA resulting were separated by molecular weight using agarose gel electrophoresis. Southern blotting of the gels onto nitrocellulose filters, hybridisation to the DNA probe, and autoradiography were performed according to the method described by Southern.

The DNA probe used was a single copy DNA sequence derived from a library of random human genomic DNA cloned in plasmid pBR322 and designated L1.28.

Results

The DNA sequence polymorphism detected by L1.28 after digestion of human DNA with TaqI restriction enzyme shows a two allele pattern. Autoradiography following hybridisation to the probe demonstrates one band at 12·3 kb for the allele denoted C1, and the other at 9·5 kb for the rarer allele, C2.

Of the normal British female population, 47% were found to be heterozygous at the L1.28 locus, the gene frequency for the rarer allele (C2) being 0·30. Thirty female carriers of Becker muscular dystrophy from 15 families were tested. Fifteen females from

\[\theta = \text{recombination fraction (centiMorgans).}\]
\[n = \text{number of informative kindreds.}\]
eight families were heterozygous for the L1.28 variants. All available males, both affected and unaffected, from these families were tested together with maternal grandparents when possible.

Inheritance of alleles of L1.28 in a BMD pedigree is illustrated in fig 1. In generation II, the Becker gene is segregating with the C2 allele, and there is no cross-over between the BMD gene and L1.28 alleles in generation III. Variants identified by L1.28 in TaqI digested DNA in the same family are shown in fig 2.

A total of 35 informative meioses provided data for linkage analysis, although maternal grandparents could not be typed for all affected males. The probabilities of linkage at various recombination fractions were calculated using the LIPED computer programme. The lod scores derived express in logarithmic form the probability that linkage exists between the two loci and are shown in the table. The probabilities of linkage at the various recombination fractions are plotted in fig 3.

The overall probability of linkage between the BMD and L1.28 loci is 315:1. Assuming this, the likelihood of linkage at 16 centiMorgans is 962:1, with the 95% confidence limits lying between 7 and 32 centiMorgans.

Discussion

Approximately 450 genes have been assigned to particular chromosomes in man. Mapping distances between loci are defined in centiMorgans (cM), one cM being the distance apart of two loci that show 1% cross-over at meiosis (recombination fraction \( \theta = 0.01 \)). One centiMorgan corresponds to approximately \( 1 \times 10^6 \) base pairs (1000 kb). Genes may be mapped to within 5 to 10 cM of their locus by low resolution somatic cell procedures, while high resolution recombinant techniques, with restriction site and nucleotide sequence analysis, should provide mapping data at a level of single nucleotide base pairs to the 100 kb level.

The results of this present study suggest linkage between BMD and the L1.28 locus at a distance of 16 cM, which is comparable with the results obtained for DMD by Davies et al. The estimate of the recombination fraction for DMD and L1.28 is 0.17 with 95% confidence limits of 0.07 to 0.33, and an overall probability of linkage of 130:1. Linkage studies with DMD and probe \( \lambda \text{RC8} \) indicate that the two probes flank the DMD gene on the short arm of the X chromosome. We have studied \( \lambda \text{RC8} \) in our Becker families, but as yet the data are insufficient to draw firm conclusions.

This study provides the first linkage evidence that BMD and DMD may be allelic, contrary to the findings of Skinner et al and Zatz et al. The data in their linkage studies of BMD gave maximal lod scores at 25 cM of 1.25 for colour blindness and 0.094 for G6PD. The overall likelihood of linkage between BMD and colour blindness was 7:1. Our study did not include any kindreds with clear evidence of colour blindness, but the data firmly locate the BMD gene on the short arm of the X chromosome, excluding linkage with these long arm markers.

Linkage of BMD to the L1.28 locus can now be used in informative families to supplement existing methods of carrier detection. The linkage is not sufficiently close to be used in antenatal diagnosis, as cross-overs would be expected to occur in approximately 15% of cases. Now that loose linkage is established, however, other single copy sequences mapping closer to the BMD locus could be isolated and used in antenatal diagnosis if linkage is sufficiently close.

Our findings further suggest that future DNA markers showing close linkage to the DMD locus will show comparable linkage with BMD, and that their practical application within families will be similar. Finally, if, as seems possible, the two disorders are allelic, this will imply an abnormality of the same gene product, a situation which will have important consequences for our understanding of the nature and relationship of the two disorders.

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References


