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Linkage analysis of two cloned DNA sequences flanking the Duchenne muscular dystrophy locus on the short arm of the human X chromosome

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ABSTRACT The inheritance of two restriction fragment length polymorphisms (RFLPs) on the short arm of the human X chromosome has been studied relative to Duchenne muscular dystrophy. This provides a partial genetic map of the short arm of the human X chromosome between Xp110 and Xp223. The data were derived from the segregation between a RFLP located at Xp21-Xp223, the DMD locus, and a RFLP located at Xp110-Xp113. The genetic distance from Xp110 to Xp223 was found to be approximately 40 centimorgans (cM). This provides experimental confirmation that 1cM corresponds to approximately 1,000 kilobase pairs of DNA for this region of the human X chromosome. Our data confirm that the DMD mutation lies between Xp223 and Xp110. The availability of flanking probes surrounding the DMD locus will assist in the ordering of further DNA sequences relative to the mutation.

INTRODUCTION

More than one hundred genes have been assigned to the human X chromosome from the study of sex-linked diseases and using rodent/human somatic cell hybrids containing the human X chromosome. In spite of this, the chromosome has not been extensively mapped genetically, and the regional localisation of most of these genes is not known (1). Two main linkage groups have been defined: one near the end of the short arm of the chromosome including the genes for Xg blood group and steroid sulphatase, and the other near the end of the long arm of the chromosome including the genes for haemophilia, HGPRT, G6PD and colour blindness. The recombination frequency between the two linkage groups is too high to demonstrate linkage, and

nothing is known about the frequency of recombination of intervening X-chromosome segments because of the paucity of phenotypic markers.

Recombination in the male occurs about 50 times during each meiosis if it is assumed that every chiasmata corresponds to a point at which cross-over occurs (2). Detailed studies of human male meiosis have suggested that the distribution of chiasmata is not random along a chromosome, and that chiasmata are observed more frequently towards chromosome ends than near the centromere (3). These meiotic observations are supported by data showing high recombination between gene markers on the distal part of the short arm of chromosome 1 (4) and the long arm of the X chromosome (5). Although it has been suggested that the female meiotic length for autosomes may be as much as twice the male meiotic length (4, 5, 6), there are apparent differences in the relative male and female recombination frequencies in different chromosomal regions.

DNA restriction fragment length polymorphisms (RFLPs) are detected as a result of DNA base changes or small insertions or deletions of DNA sequence which remove, insert or rearrange restriction enzyme sites (7, 8). Variations in DNA sequence of this type have been shown to occur in the normal population once in every 100-200 base pairs around the β-globin locus (9), and may be visualised using random DNA sequence probes (10). The Mendelian inheritance of these RFLPs demonstrates their usefulness as genetic markers (11, 12, 13). A collection of approximately 1600 random, or 600 chromosome-specific, DNA sequence polymorphisms would be required to map the whole human genome with a probe every 20cM along its length (7, 8); these

estimates are based on an assumption of an even distribution of recombination along a chromosome.

We are particularly interested in the construction of a genetic linkage map of the short arm of the human X chromosome. We have already established loose linkage (θ = approximately 0.15) between the locus for DMD and a DNA sequence polymorphism located between Xp21 and Xp223 (13). This is compatible with the prior assignment of DMD to the region at band Xp21, based on a small number of apparently balanced translocations in females giving rise to a Duchenne phenotype in females (14, 15). In this paper we describe a three-point linkage which defines the position of the DMD locus more accurately. An extension of the map for this region of the short arm of the X chromosome will simplify the assignment or exclusion of other X-linked phenotypes.

MATERIALS AND METHODS

Origin of hybridisation probes

Two X-chromosome single copy probes were derived as follows. Phage λRC8 was obtained from an human X chromosome-specific genomic library prepared by Davies et al (16). It was isolated after prescreening of the library plaques with total [32]P-labelled human DNA. Plasmid L1.28 was selected as a single-copy probe from a library of random human genomic DNA cloned in pBR322 (Pearson, Wieacker et al, submitted for publication).

Preparation of human DNA

Human DNA was prepared from whole blood as described by Kunkel et al (17). Families in which Duchenne muscular

dystrophy had occurred in at least two cases were identified from a genetic register for the disorder in Wales supplemented by families from other centres; obligatory carriers were used for these studies in addition to affected and unaffected males (13). Restriction enzyme digestions, agarose gel electrophoresis, the blotting of gels, hybridisation and autoradiography were carried out as described previously (16). Southern blots on Schleicher and Schuell BA85 nitrocellulose filters (18) were reused after elution of radioactive probes with 0.2N NaOH at room temperature for 15 min. The filters were washed extensively with 2xSSC and prehybridised before reuse.

RESULTS

Each of the DNA sequence polymorphisms used in this analysis was detected with the enzyme TaqI and show a simple two-allele pattern. One of the clones, λRC8, is localised between Xp21 and Xp223, as shown using DNA from a panel of rodent-human hybrid cells containing portions of the human X chromosome (13). The TaqI variants for λRC8 found in the normal population are shown in figure 1a. 22% of the British DMD carrier population (and 29% of the normal London population) are heterozygous at this locus, the gene frequency of the rarer allele (B2) being 0.13. Allele B1 shows a Southern blot pattern containing a band at 3.2 kilobase pairs (kb) and allele B2 gives a band at 5.3kb. There is also an invariant band of 6.6kb present in all individuals (13).

The second cloned sequence used in this study, denoted L1.28, is localised between Xp110 and Xp113 (Pearson, Wieacker

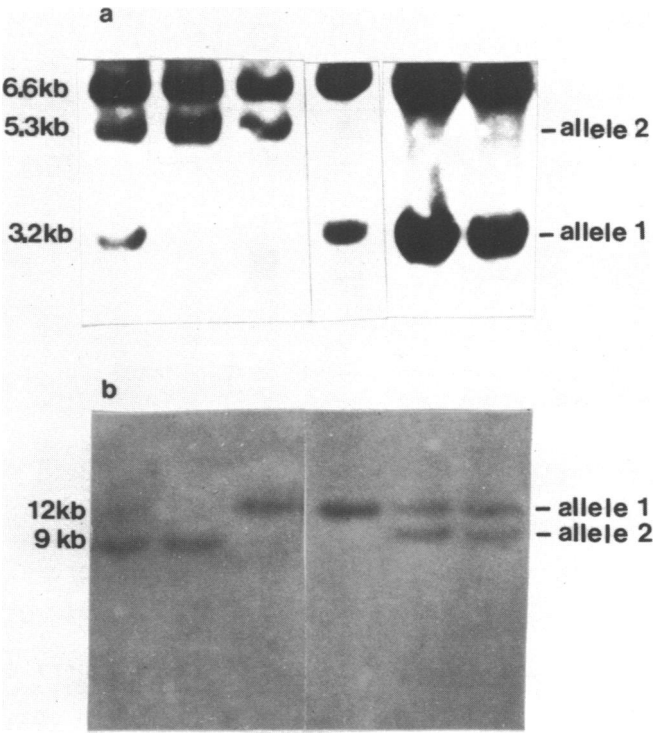

FIGURE 1 Variants identified by λRC8 (a) and L1.28 (b) in TaqI-digested total DNA from females. 10 μg DNA was analysed in each lane. Filters were washed to 1xSSC and autoradiographed for 72h.

et al, submitted for publication). 45% of the DMD carrier population shows the TaqI polymorphism shown in figure 1b. The gene frequency of the rarer allele in the normal Dutch population is 0.32. The allele denoted C1 shows a Southern blot band at 12.0kb and the rarer allele, C2, visualises a band at 9.0kb.

When DNA samples from individuals showing the TaqI polymorphisms are digested with other restriction enzymes such as BamHI or SstI, no differences are seen. The larger band is seen after partial digestion with TaqI when the smaller band

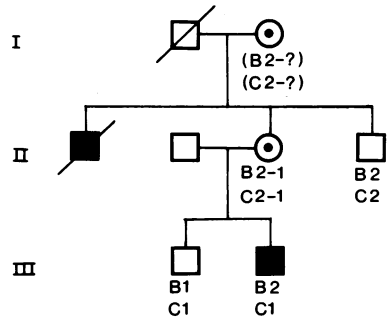

FIGURE 2 The Mendelian inheritance of TaqI alleles of λRC8 and L1.28 in a DMD pedigree.

occurs after complete digestion. Although these data suggest strongly that these polymorphisms are due to a simple base change and not to rearrangements, we have not rigorously proven their nature, as it is not relevant to the experiments described. It is only essential that they are inherited in a Mendelian fashion. All of the data described were obtained after complete digestion of genomic DNA samples with TaqI.

The inheritance of alleles of λRC8 and L1.28 in a DMD pedigree is shown in figure 2. Clearly crossovers have occurred within this pedigree; both boys in generation III have inherited C1 from their mother, and only one has DMD. Since a double crossover between λRC8 and L1.28 is very unlikely, the probes segregate independently relative to the disease. This is consistent with the location of the probes on either side of the DMD locus. The lod scores for λRC8 and L1.28 with DMD are shown in Table 1. In the case of \RC8 these include some data already published by Murray et al (13). The new lod scores include the analysis of extra members of the original families. The probabilities of linkage at various recombination fractions are plotted in figure 3.

TABLE 1. Linkage data for Duchenne muscular dystrophy, λRC8 and L1.28. Results are expressed as lod scores (log of the odds) for different values of recombination fraction (θ). The number of informative kindreds for each comparison is indicated (N).

θ	DMD-λRC8 N=10	DMD-L1.28 N=17
0.01	- 1.290	- 2.361
0.05	+ 0.768	+ 1.253
0.10	+ 1.433	+ 2.304
0.11	+ 1.493	+ 2.393
0.12	+ 1.543	+ 2.470
0.13	+ 1.580	+ 2.525
0.14	+ 1.602	+ 2.558
0.15	+ 1.615	+ 2.583
0.16	+ 1.620	+ 2.595
0.17	+ 1.623	+ 2.597
0.18	+ 1.611	+ 2.588
0.19	+ 1.598	+ 2.570
0.20	+ 1.578	+ 2.545
0.25	+ 1.414	+ 2.324
0.30	+ 1.170	+ 1.985
0.35	+ 0.886	+ 1.571
0.40	+ 0.584	+ 1.094
0.45	+ 0.290	+ 0.568

DISCUSSION

Clones λRC8 and L1.28 both show linkage to DMD. In the case of λRC8 10 families were studied; 17 kindreds were analysed with L1.28. The two loci are each approximately 15cM from DMD. Analysis of the 5 families informative for both λRC8 and L1.28 shows that DMD segregates with λRC8 in one case, with L1.28 in two cases, and with both in two cases, demonstrating that DMD is located between the loci defined by the two probes. This is further supported by the higher recombination frequency between λRC8 and L1.28, 3 recombinants in 9 meioses, which is substantially higher than between either λRC8 or L1.28 and DMD.

λRC8 has been mapped to the region between Xp21 and Xp223 using rodent-human somatic cell hybrids and *in situ* hybridisation (13), and L1.28 has been mapped to between Xp110

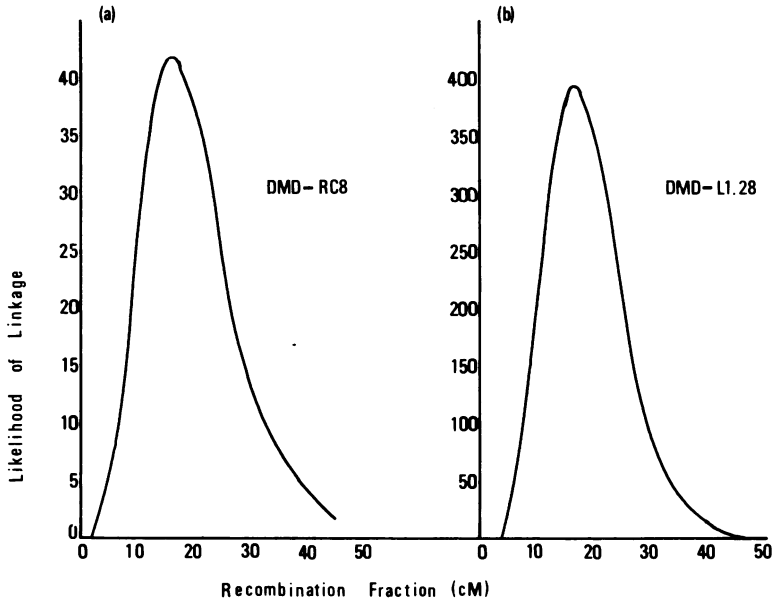

FIGURE 3 The probability of linkage at various recombination fractions between λRC8 and DMD (a) and L1.28 and DMD (b).

and Xp113 using rodent-human somatic cell hybrids and human deletion mutants. Close linkage between λRC8 and the Xg blood group has been previously shown to be unlikely (13) but data are insufficient to give an accurate direct estimate of the distance between these loci. We can nonetheless conclude that the order of genes on the short arm of the X chromosome is :-

centromere - L1.28 - DMD - λRC8 - Xg - terminus

If the data presented in this paper are combined with the estimated distance between the Xg blood group and λRC8 (at least 40cM), we can estimate genetic distances for this region of the short arm of the X chromosome. In addition to defining a genetic distance across the DMD locus, these two DNA sequence polymorphisms will be very valuable for the localisation of other X-linked phenotypes occurring on the

short arm of the X chromosome.

The human X chromosome has been estimated to contain 5.4% of the total human genomic DNA, as measured by gallocyanine-chrome alum staining of metaphase chromosomes (20). Since this is (to a first approximation) a direct measurement of DNA content, and since the total number of base pairs in the human haploid genome is $3x10^9$ (21), a single human X chromosome contains $1.5x10^8$ base pairs. The short arm of the X chromosome is approximately one-third of the total, corresponding to $5X10^7$ base pairs. Assuming that DNA condensation is uniform, the maximum possible distance between the probes (from Xp110 to Xp223) is half the total length of the short arm ($2.5x10^7$ base pairs), and we show above that the genetic length between these two sites is approximately 40 centimorgans. Therefore, one centimorgan corresponds to approximately $1x10^6$ base pairs, or 1,000 kilobases. It is of interest that this distance is very similar to that deduced from the total number of chiasmata seen for human male meiosis (22).

The availability of flanking probes surrounding the DMD locus should allow ordering of further markers relative to the DMD mutation, and will also be of value in determining carrier status of females in affected families.

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