Genetic mapping of the human X chromosome by using restriction fragment length polymorphisms

(arbitrary marker loci/linkage families)

DENNIS DRAYNA*, KAY DAVIES†, DAVID HARTLEY†, JEAN-LUIS MANDEL‡, GIOVANNA CAMERINO‡§, ROBERT WILLIAMSON†, AND RAY WHITE*

*Howard Hughes Medical Institute and Department of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City, UT 84132; †Department of Biochemistry, St. Mary's Hospital Medical School, Paddington, W2 1PG, London, England; and ‡Laboratoire de Génétique Moléculaire des Eucaryotes, Centre National de la Recherche Scientifique, Faculte de Medicine, 67085 Strasbourg, France

ABSTRACT   Using a human X chromosome-specific DNA library, we have found arbitrary single-copy DNA sequences that reveal useful restriction fragment length polymorphisms. The inheritance of these and other available polymorphic DNA markers has been studied in a series of unrelated three-generation families with large sibships. These families reveal parental phase and allow determination of recombination frequencies by counting recombinant and nonrecombinant chromosomes. The resulting genetic map indicates that the minimal distance from Xp22 to Xqter is 215 recombination units. The spacing of the marker loci is such that the majority of the loci on the X chromosome, including disease loci, will lie within 20 centimorgans of at least one of these loci.

Restriction fragment length polymorphisms (RFLPs) are powerful tools for the construction of linkage maps and for linkage studies with human genetic diseases (1, 2). They can also be used to study other fundamental genetic questions, including the role of chromosomal changes in tumorigenesis, the distribution of meiotic and mitotic exchanges, as well as genome evolution (3, 4). A number of diseases have already been linked to one or more RFLPs, and RFLP-to-RFLP linkage studies have successfully mapped short regions of several chromosomes (5–7).

DNA libraries specific for the human X chromosome have made possible the development of a number of arbitrary RFLP markers for this chromosome (8, 9). Because of the genetic interest and clinical importance of the many genetic diseases due to mutations located on the X chromosome, we have characterized a series of X-specific RFLP markers and used them, together with other available markers, to develop a large-scale genetic linkage map of this chromosome.

MATERIALS AND METHODS

Derivation of Polymorphic Marker Probes. Arbitrary DNA sequences that reveal polymorphism were derived from an X chromosome-specific library cloned in phage λ gt*WES*·λ B (8). Phages were prescreened by filter hybridization to ^{32}P-labeled total human DNA, and the resulting midrepeat-free clones were chosen for study. DNAs from phages free of repetitious sequences were tested for ability to reveal polymorphism directly. DNA from other phages, carrying small amounts of repetitive sequences, as well as DNA from other unscreened phages, was digested with EcoRI and HindIII, and the resulting fragments were resolved by electrophoresis in 1% agarose gels. The DNA was then blotted onto Zetapor membrane (AMF-Cuno, Meriden, CT) (10) and hybridized to ^{32}P-labeled total human DNA to reveal single-copy fragments. Then 0.1 to 0.5 μg of the single-copy fragments great-

er than 0.5 kilobase in length were electroeluted from a preparative agarose gel onto a NA45 membrane (Schleicher & Schuell) and were eluted in the presence of 0.05 M arginine/0.1 M NaCl at 65°C for 3–5 hr (11). The fragments were labeled with ^{32}P by nick-translation (12) and hybridized to a panel of DNAs from eight unrelated women that had been digested with restriction enzyme, electrophoresed, and blotted onto Zetapor membranes. Sequences that revealed polymorphism were then hybridized to DNAs from members of a family to assure their X-linked inheritance. Such fragments were subcloned in pBR322 for all subsequent experiments.

Genotypic Determinations, Other Markers, and Linkage Families. DNA isolation, electrophoresis, blotting, hybridization, and autoradiography were performed as described (13). Other markers used in this study have been described elsewhere: λ RC8 (14), L1.28 (5), DXYS1 (15), HPRT (16), and factor IX (17). The families used to determine linkage relationships are large three-generation nuclear families previously described (7).

RESULTS

Phage from an X chromosome library were screened for single-copy sequences. Those clones and associated restriction enzymes that reveal useful polymorphism are listed in Table 1. Allele frequencies and physical locations (unpublished data) are also indicated. It should be noted that all three markers are physically located on the long arm of the X chromosome. Fig. 1 illustrates the X-linked inheritance of each of these markers. More extensive studies have established this mode of inheritance for each marker. Table 1 also indicates the restriction enzymes, allele frequencies, and physical locations of the previously described marker loci that were also used in this study.

The linkage relationships among these marker loci were determined by recombination studies in a series of three-generation, nuclear families with large sibships, including all four grandparents. To determine which mothers were heterozygous for each marker locus and thus informative for the linkage study, their genotypes were determined first. The genotypes of the husbands, parents, and children of such heterozygous mothers were then determined and each child's maternally derived X chromosome was scored as recombinant or nonrecombinant. This was possible since our knowledge of the genotype of the maternal grandfather explicitly determined the allele distributions on (i.e., the haplotypes of) the mother's X chromosomes.

Table 2 shows the number of recombinant chromosomes over the total number of chromosomes scored as informative for each pairwise linkage test among the nine loci. Even

Table 1. Marker loci

Probe	Enzyme	Allele frequencies	Physical location
		Described in this study	
S21	Taq I	0.35/0.65	Xq213-Xq220
	Msp I	0.1/0.90*	
52A	Taq I	0.50/0.50	Xq27
DX13	Bgl I	0.45/0.55	Xqter
		Previously described	
Xga	Protein polymorphism	0.65/0.35	Xp22
RC8	Taq I	0.2/0.8	Xp21
L1.28	Taq I	0.35/0.65	Xp11-Xp13
DXYS1	Taq I	0.48/0.52	Xq13
HPRT	BamHI	0.77/0.16/0.07	Xq26
F IX	Taq I	0.7/0.3	Xq28

*This polymorphism is in linkage equilibrium with the Taq I polymorphism.

though 23 families with mothers heterozygous for one or more loci with an average of eight children each were screened (approximately 180 maternal X chromosomes in the sample set), the total number of useful chromosomes that could be scored varied from as low as 7 to as high as 40, with an average of 20. This relatively low yield of useful chromosomes is due to the low frequency of heterozygotes obtained with these primarily two-allele marker loci.

Table 2. Linkage relationships

Pairwise cross	No. of recombinants/ total no. of chromosomes	lod score
Xg-RC8	4/8	—
RC8-L1.28	7/21	0.58
L1.28-DXYS1	8/15	—
DXYS1-S21	4/32	4.4
S21-HPRT	2/7	0.29
HPRT-52A	1/20	4.3
52A-F IX	2/28	5.2
52A-DX13	12/40	1.43
F IX-DX13	6/21	0.89

However, significant linkage data were obtained. Linkage in two regions was established with significant lod scores. Linkage of *S21* to *DXYS1* was found at a distance of 12.5% recombination. A linkage group was also established over the interval from *HPRT* to *DX13*. Both the *52A* and factor IX loci are included in the interval. 5% recombination was seen between *HPRT* and *52A*, 7% recombination between *52A* and factor IX, and 28% recombination between factor IX and *DX13*. Confidence in this latter value was supported by the determination of 30% recombination between *52A* and *DX13*. Close linkage between the *HPRT* and factor IX loci was supported by finding no recombinants among six informative progeny chromosomes. Since physical mapping has placed *HPRT* at Xq26, *52A* at Xq27, factor IX at the proxi-
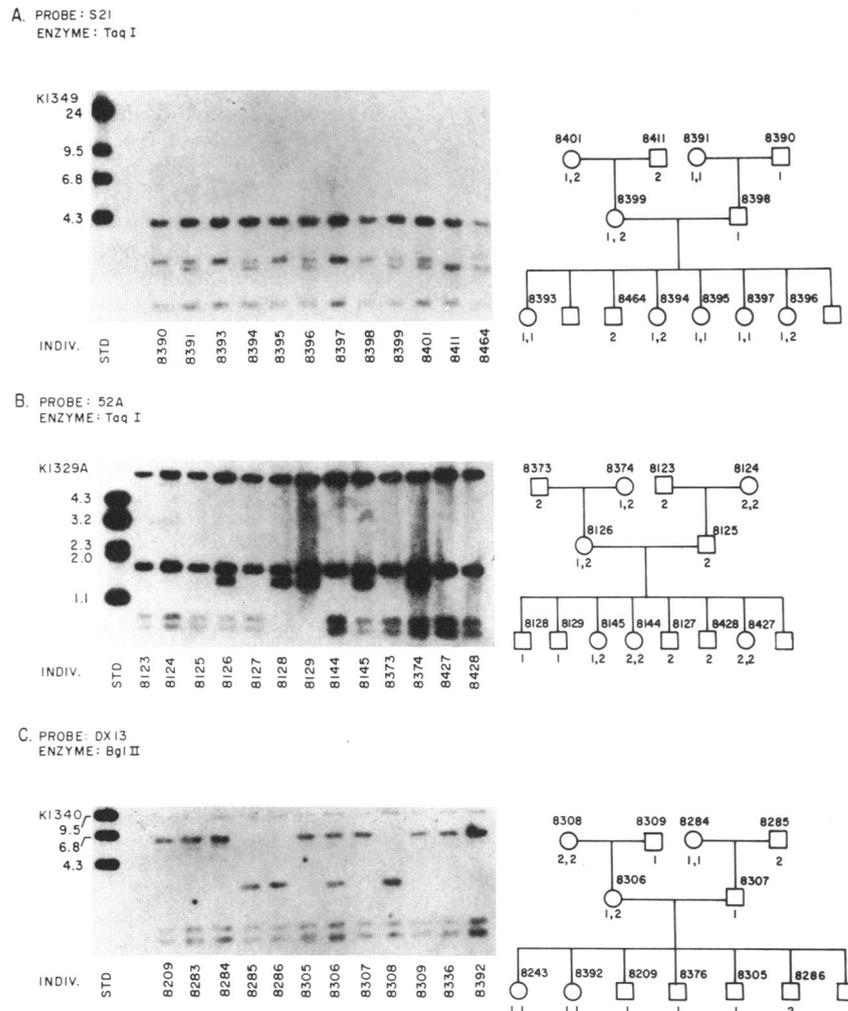
FIG. 1. X-linked inheritance of arbitrary RFLP marker loci. Genotypes are represented on pedigrees as follows: 1, slower-migrating allele; 2, faster-migrating allele. Lengths of standards are given in kilobases.
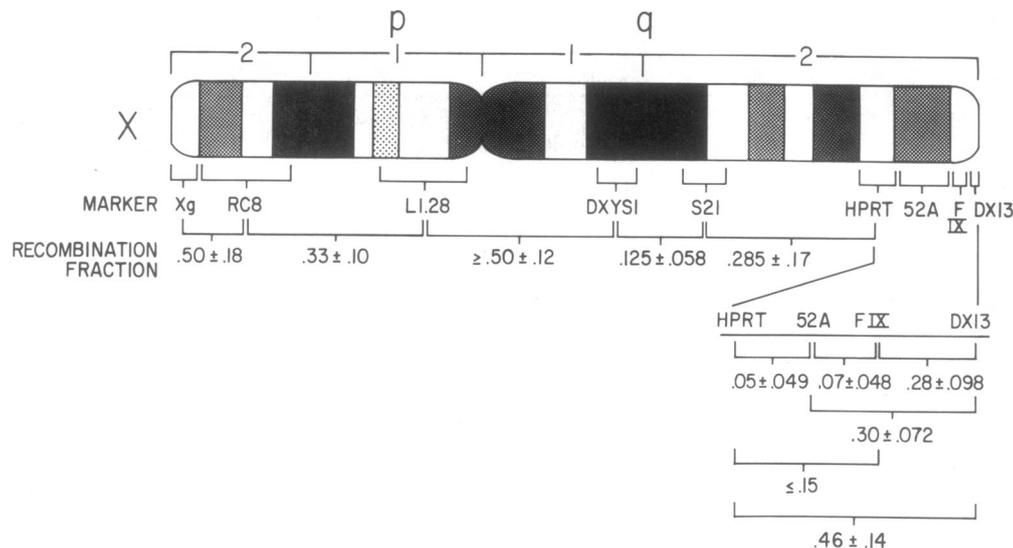
FIG. 2. Linkage map of the X chromosome. Recombination fractions are given with limits for 90% confidence.

mal side of Xq28, and *DX13* at Xqter (18, 19), these physical location data combined with the two-factor cross recombination data suggest the gene order *HPRT–52A*–factor IX–*DX13*. For example, the 30% recombination observed between *DX13* and *52A* is greater than the 28% recombination seen between *DX13* and factor IX or the 7% recombination found between factor IX and *52A*, consistent with placement of factor IX between *52A* and *DX13*.

Furthermore, the gene order *HPRT–52A–DX13* was supported by the multifactor crosses in the data set. Two families were triply informative for these three markers. Among the 13 maternally derived progeny X chromosomes, only 1 showed a crossover between *HPRT* and *52A*. This chromosome was not recombinant between *52A* and *DX13*. A total of five chromosomes showed recombination between *52A* and *DX13*, and none of these five chromosomes was recombinant between *52A* and *HPRT*. Double exchanges would be required to account for this data, given either of the other two possible gene orders.

DISCUSSION

These results provide an overall estimate of the genetic linkage map of the human X chromosome (Fig. 2). Two-factor crosses have been used to determine genetic distances, and these have been combined with three-factor cross data as well as physical data to establish marker order. Since phase is known in the three-generation families, direct counting of recombinant and nonrecombinant chromosomes was possible, thus simplifying and speeding linkage analysis.

The two marker loci *RC8* and *L1.28* have previously been reported to flank the *DMD* locus at a distance of 15 centimorgans on either side (5). Our determination of a recombination fraction of 33% between the marker loci *RC8* and *L1.28* provides support for these important linkage relationships.

The observed value of 50% recombination between *Xg* and *RC8* may be a slight overestimation of the distance between these two loci, since it has been shown that a third locus, that for retinoschisis, lies between *Xg* and *RC8*, approximately 25 centimorgans from *Xg* and 15 centimorgans from *RC8* (20).

Taking into account the linkage distances within the two linkage groups plus the intervals demonstrated to be 50 centimorgans or more, the distance from *Xg* (at the distal end of the short arm) to *DX13* (at the distal end of the long arm) is at least 215 recombination units. Neglecting interference, this suggests that the minimal length of the X chromosome is

roughly 260 centimorgans (21). The correlation of these genetic distances with physical distances lends support to the idea of a variable relationship between physical and genetic distance on the X chromosome. For example, the physical distance between *DXYS1* and *S21* is roughly the same as the physical distance between *52A* and *DX13*, but the former two loci are separated by less that half the genetic distance of the latter two. It should further be noted that our data do not support the idea of an obligatory crossover point on the distal portion of Xq.

Although this map of the X chromosome is not yet complete, the majority of loci on the X chromosome must lie within 20 centimorgans of the marker loci mapped in this study. Thus a large fraction of the disease loci on the X chromosome are accessible to linkage studies with these markers. We have already demonstrated their usefulness in this regard by demonstration of the close linkage of marker *52A* with fragile X-linked mental retardation (unpublished data).

1. Kan, Y. W. & Dozy, A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5631–5635.
2. Botstein, D., White, R., Skolnick, M. & Davis, R. (1980) *Am. J. Hum. Genet.* **32**, 314–331.
3. Cavenee, W. D., Dryja, T., Phillips, R., Benedict, W., Godbout, R., Gallie, B., Murphree, A., Strong, L. & White, R. (1983) *Nature (London)* **306**, 770–784.
4. Denaro, M., Blanc, H., Johnson, M., Chen, K., Wilmsen, E., Cavalli-Sforza, L. & Wallace, D. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5768–5772.
5. Davies, K., Pearson, P., Harper, P., O'Brian, T., Safarzi, M. & Williamson, R. (1983) *Nucleic Acids Res.* **11**, 2303–2312.
6. Antonarakis, S., Phillips, J., Mallonee, R., Kazazian, H., Fearon, E., Waher, P., Kronenberg, H., Ullrich, A. & Meyers, D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6615–6619.
7. White, R., Leppert, M., Bishop, D. T., Barker, D., Berkowitz, J., Brown, C., Callahan, P., Holm, T. & Jerominski, L. (1983) in *Proceedings of the International Chromosome Conference*, Lubeck, Germany, ed. Wolf, U. (Allen & Unwin Publ. Ltd., London).

Genetics: Drayna *et al.*

Proc. Natl. Acad. Sci. USA 81 (1984)   2839

8. Davies, K., Young, B., Elles, R., Hill, M. & Williamson, R. (1982) *Nature (London)* **293**, 374–376.
9. Kunkel, L., Tantrahavi, U., Eisenhard, M. & Latt, S. (1982) *Nucleic Acids Res.* **10**, 1556–1578.
10. Southern, E. (1975) *J. Mol. Biol.* **98**, 503–518.
11. Dretzen, G., Bellard, M., Sassone-Corsi, P. & Chambon, P. (1981) *Anal. Biochem.* **112**, 295–299.
12. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
13. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Murray, J., Davies, K., Harper, P., Meredith, L., Mueller, C. & Williamson, R. (1982) *Nature (London)* **300**, 69–71.
15. Page, D., de Martinville, B., Barker, D., Wyman, A., White, R., Francke, U. & Botstein, D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5352–5356.
16. Nussbaum, R., Crowder, W., Nyhan, W. & Caskey, C. T. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4035–4039.
17. Camerino, G. & Mandel, J.-L. (1983) *Nature (London)* **306**, 701–704.
18. Wolski, K., Schmid, F. & Mittel, K. (1975) *Science* **188**, 1020–1022.
19. Chance, P., Dyer, K., Kurachi, K., Yoshitaki, S., Ropers, H.-H., Wieacker, P. & Gartler, S. (1983) *Hum. Genet.* **65**, 207–208.
20. Wieacker, P., Wienker, T., Dallapiccola, B., Bender, K., Davies, K. & Ropers, H. (1983) *Hum. Genet.* **64**, 143–145.
21. Haldane, J. (1919) *J. Genet.* **8**, 299–309.