Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3909-3913, June 1986
Genetics

Construction of a map of the short arm of human chromosome 6

(linkage mapping/polymorphic markers/HLA antigen)

ROBIN LEACH*†, ROBERT DEMARS‡, SANDRA HASSTEDT§, AND RAY WHITE*§

*Howard Hughes Medical Institute and the §Department of Human Genetics, University of Utah, Salt Lake City, UT 84132; and ‡Laboratory of Genetics and Department of Human Oncology, University of Wisconsin, Madison, WI 53706

Communicated by L. L. Cavalli-Sforza, January 27, 1986

ABSTRACT Four DNA sequences that reveal restriction fragment length polymorphisms (RFLPs) on the short arm of human chromosome 6 have been identified. Two of these sequences were isolated from recombinant DNA libraries enriched for DNA from human chromosome 6, one was isolated as a subclone from a human DNA segment having homology to an HLA class I sequence, and one was isolated by virtue of its homology to part of the insulin gene. Genetic linkage was determined among these four polymorphic sequences and several genes known to be on chromosome 6: glyoxylase I (*GLO1*), *HLA-DRα*, *HLA-DQα*, and *HLA-B*. Additionally, two of the four RFLPs were regionally localized by using 53 deletion cell lines that had been typed for HLA-A, -B, and -DR, and for glyoxylase I. A genetic map of the short arm of chromosome 6 has been constructed on the basis of linkage studies with the eight markers. The map spans a minimum of 60 recombinational units and will be useful in the study of HLA-associated diseases.

The study of human genetics, and in particular the effort to map the human genome, has long been limited by a lack of sufficiently informative markers. However, recent advances in DNA technology have provided a method for obtaining a large number of genetic markers based on DNA sequence variants frequent in the human population (1). The polymorphisms are revealed as variations in the length of the fragments produced by cleavage with restriction enzymes. They can be detected by the method of Southern (2), using as probes cloned arbitrary single-copy sequences (3) or specific genes [e.g., hemoglobin (4, 5) and insulin (6-8)]. Several hundred evenly spaced genetic markers should be sufficient to map the entire human genome (9, 10). Even though most of them have no known genetic function, restriction fragment length polymorphisms (RFLPs) in arbitrary DNA sequences can provide a large proportion of the necessary markers.

The short arm of human chromosome 6 is of interest to geneticists because the major histocompatibility locus (*HLA*) is located there. Associations, apparently nonrandom, between specific alleles at the *HLA* locus and specific diseases have been observed (11). The biological basis for these associations in many cases is not known; association may result from physical linkage of the disease locus to the *HLA* locus, or it may involve an epistatic interaction. To study the genetics of chromosome 6p as well as HLA-associated disease, we identified DNA-based genetic markers that map to this chromosome and determined their linkage relationships.

In the search for polymorphic DNA markers for chromosome 6, two enriched sources of probes were useful. The first was a recombinant bacteriophage library constructed by flow cytometry of metaphase chromosomes, in which the majority of the human DNA sequences map to human chromosomes 5 and 6 (12). A second recombinant library was constructed by using DNA from a hamster-human cell hybrid that contains human chromosomes 12 and 13 and the short arm of chromosome 6 (13).

Alleles at the *HLA* locus have for some time been defined by their antigenic properties. More recently, by using cloned *HLA* gene sequences as probes, RFLPs have been found that can define variations at the DNA level (14-18). In our study of genetic linkage on 6p, we have used three such RFLPs at the *HLA* locus, as well as the protein marker glyoxylase I.

Five RFLPs, characterized here, were used in the linkage analysis; three of them were arbitrary sequences obtained from recombinant libraries enriched for chromosome 6 DNA, although only two of the three clearly mapped to 6p in the subsequent analyses. The fourth was a sequence with partial homology to the insulin gene (L. Villa-Komaroff, personal communication), which had been regionally localized to chromosome 6 (19). The fifth was isolated from a cloned human segment that has DNA sequence homology to an HLA class I cDNA sequence (20).

We gathered linkage data on the nine polymorphic markers described above, in 25 three-generation Utah kindreds (21). We then used 53 cell lines carrying deletions of chromosome 6p to regionally localize three of the five recently discovered RFLPs.

MATERIALS AND METHODS

Sample Collection and Preparation of DNA. Twenty-five three-generation Utah kindreds were used in the linkage analysis; the pedigrees have been described elsewhere (21). Blood samples were collected from members of these kindreds in acid/citrate/dextrose tubes. A portion of the blood was washed in saline and used for glyoxylase I analysis. High molecular weight DNA was isolated from the blood cell nuclei according to the technique described by Bell *et al.* (8).

Recombinant DNA Cloned Sequences. A recombinant bacteriophage library, constructed in the vector λ-gt*WES*·λ B, was obtained from B. Young (12). The human DNA contained in this library was isolated from the chromosome 6 fraction of a FACS-II cell sorter (Becton Dickinson) by previously described methods (12). A second recombinant bacteriophage library, containing DNA from a human-hamster cell line carrying human chromosomes 12, 13, and 6p, was constructed as described by Cavenee *et al.* (13). A third recombinant bacteriophage library was obtained from T. Maniatis (22).

Two cDNA sequences, homologous respectively to the *HLA* genes *HLA-DRα* (pDRH7) and *HLA-Dqα* (pDCH1), were obtained from C. Auffray (15). A cDNA homologous to the gene *HLA-B8* was obtained from S. Weissman (23). A plasmid (pAGB6) containing a sequence homologous to the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RFLP, restriction fragment length polymorphism; kb, kilobase(s).

†Present address: Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90033.

region of the insulin gene that codes for the prepeptide sequence was obtained from L. Villa-Komaroff.

Cell Lines. Fifty-three cell lines were isolated after γ-irradiation of the human lymphoblastoid line LCL 721, and HLA antigen-loss mutants were selected by using methods previously described (24, 25). These lines were then *HLA* typed at the *A*, *B*, and *DR* loci, and at the *GLO1* (glyoxylase 1) locus. Many of the lines had deletions detected by karyotypic analysis (24, 26) and/or by Southern blotting analysis with probes for *HLA* loci (17, 18, 27–29) and complement loci (30).

To map single-copy sequences to human chromosomes, we used a somatic cell panel containing human–hamster hybrids (13). We obtained two additional hybrid cell lines, CF 34-2-10/5 and CF 34-10-12/3, from T. Mohandas. The major chromosomal complement of hybrid 34-2-10/5 consists of chromosomes 11, 13, and 6p. At the time of preparation, hybrid 34-10-12/3 had a major chromosomal complement of chromosomes 4, 5, 8, 9, 10, 12, 14, 18, and X, as well as a Robertsonian translocation between chromosome 15 and the long arm of chromosome 6 (31).

Genotypic Determinations. Electrophoresis, DNA transfers, hybridization, and autoradiography were performed as described by Maniatis *et al.* (32). Glyoxylase I assays were performed according to a method described by Kompf *et al.* (33).

Statistical Methods. Likelihood analysis (34) was used to estimate the allele frequencies for each marker and the recombination fractions between pairs of markers. Computation of the likelihoods employed the pedigree analysis packages PAP (35) and LINKAGE (36). Maximization was performed with GEMINI (37) as incorporated into both PAP and LINKAGE.

The lod scores were calculated by taking the common logarithm of the ratio of the likelihood at the estimated recombination fraction to the likelihood of a recombination fraction of 0.5. The lod is a standard test statistic for linkage (38). A lod score of 3.0, representing 1000-fold more likely linkage than nonlinkage, is considered sufficient for establishing linkage at a specific recombinational distance. A lod score lower than −2.0 is considered sufficient for excluding linkage at that recombinational distance.

Because the LINKAGE analysis package permits us to examine several loci jointly, it provides the opportunity to further clarify the gene order provided by our linkage data. Specifically, by comparing the relative likelihoods of the maximum likelihood solutions associated with the different gene orders, we can obtain a quantitative estimate of the support provided by linkage data for each possible gene order.

RESULTS

Isolation and Identification of Chromosome 6 RFLPs. From the library constructed with DNA from human–hamster cell lines containing chromosomes 12, 13, and 6p, 63 putative single-copy sequences were identified (13). We mapped 2 of these fragments to chromosome 6 by hybridization to a small somatic cell mapping panel. Each of the 2 fragments was subcloned in the plasmid vector pBR322; the subclones were designated p1D12 and p7H4 (D6S7). To identify RFLPs revealed by these two new probes, we hybridized them to Southern transfers of restriction digests of DNAs from nine unrelated individuals. The enzymes used were *Bgl* II, *Bcl* I, *Bam*HI, *Pvu* II, *Msp* I, *Taq* I, and *Eco*RV. No polymorphism was detected by any of the enzymes with the probe p1D12, but *Eco*RV and *Bam*HI each revealed polymorphisms with p7H4. The *Eco*RV polymorphism, with four alleles, was used in our linkage study.

From the recombinant library that had been constructed with DNA from flow-sorted metaphase chromosomes (12), we isolated a total of 26 putative single-copy fragments from a total of 70 recombinant bacteriophage clones. These fragments were radiolabeled and hybridized to Southern transfers containing DNAs digested with the enzymes *Msp* I or *Taq* I. Five polymorphisms were observed. These fragments were subcloned, radiolabeled, and hybridized to DNA from cell lines CF34-2-10/5 and CF34-10-12/3. Two of the fragments, p3C7 (D6S9) and p2C5 (D6S8), showed homology to DNA from the cell line CF 34-2-10/5 and were thus candidates for localization to chromosome 6p. Hybridization of the plasmids to a small somatic cell panel further localized p2C5 to chromosome 6p, and p3C7 to either chromosome 6p or chromosome 11. Both of these markers were used in the linkage analysis. However, subsequent to the Southern blotting analyses it became apparent that the entire short arm of one chromosome 6 had been deleted in cell mutant 721.45.1. The p3C7 sequence was not deleted in this mutant, suggesting that p3C7 may be derived instead from chromosome 11.

In addition to candidate probes from the libraries enriched for sequences from chromosome 6, several other probes proved useful. A third recombinant DNA library (22) was screened with an HLA cDNA sequence from the HLA class I locus *HLA-B8* (23); three putative single-copy sequences were isolated from these bacteriophages. One, designated pCH6 (D6S10), revealed a RFLP useful for the linkage study. We also used an insulin-homologous sequence, pAGB6, which had been isolated elsewhere and regionally localized to chromosome 6 between bands q12 and p24 (19); it reveals a polymorphism with the restriction enzyme *Msp* I. Mendelian inheritance of the five polymorphisms described above, including p3C7, is shown in Fig. 1.

Three cDNAs from *HLA* loci that reveal RFLPs were also used in the linkage analysis. HLA-DQα cDNA (pDCH1) reveals a polymorphism when DNA is digested with the restriction endonuclease *Hin*dIII (15). HLA-DRα cDNA (pDRH7) reveals two polymorphisms, one when hybridized to DNA digested with the restriction enzyme *Eco*RV and the other when hybridized to DNA digested with *Bgl* II (16); haplotypes were determined for this probe. HLA-B cDNA (pDP001) reveals a polymorphism when hybridized to DNA digested with *Eco*RV. This plasmid reveals either the presence or absence of an 8.6-kb band; the presence of the 8.6-kb band is correlated with the HLA-B antigen B8 (14).

Genotypic determinations were made by using nine markers, the eight RFLPs described plus the protein marker glyoxylase I (*GLO1* gene). Fig. 2 presents the maximum likelihood estimates of the percent recombination between pairs of markers, with lod scores. lod scores sufficient for establishing linkage (i.e., greater than 3) were obtained between all pairs of markers except those pairs involving the markers p7H4 and p3C7. Additional lod scores have been calculated at various recombinational distances. These numbers will be reported at the next human gene mapping meeting and are presently available from R.W.

Regional Localizations. Fifty-three lymphoblastoid lines derived by γ-ray irradiation of cell line LCL 721 were used to regionally localize the DNA polymorphisms. These deletion lines were typed for glyoxylase I as well as for HLA-A, -B, and -DR. (The parent line is heterozygous for the RFLPs revealed by the plasmids p3C7, p7H4, and pAGB6). We hybridized these plasmids to Southern transfers of DNA from the deletion lines and the results are presented in Fig. 3. It should be noted that the HLA and glyoxylase I assays measure a loss of phenotypic expression, while the DNA RFLPs used detect an absence of specific DNA sequences.

Fig. 3 also contains data on nine cell lines that have lost markers from more than one chromosome. In these cases, the phenotype and full genotype with all markers are given. Eight

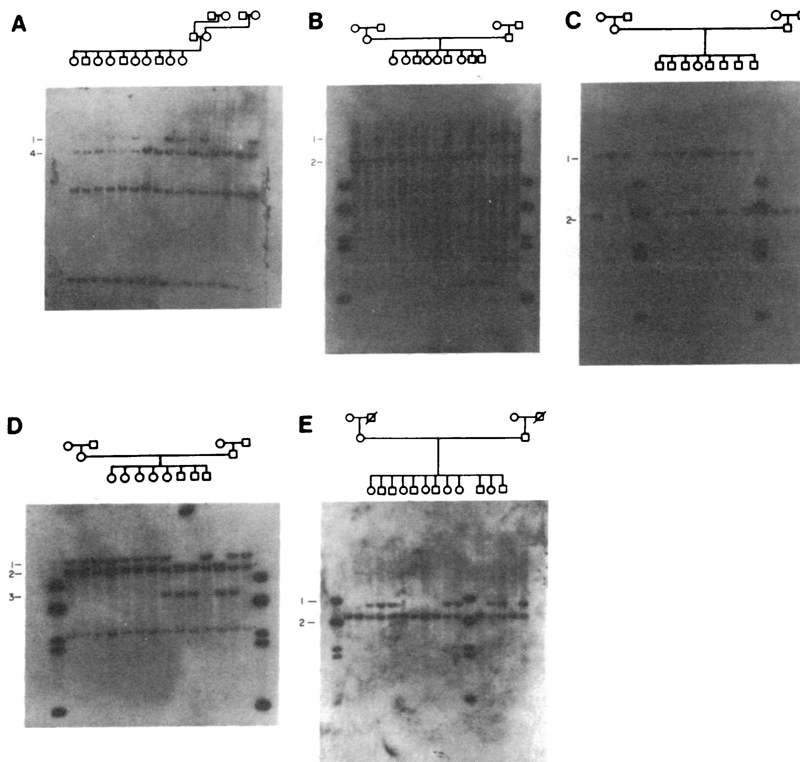

FIG. 1. RFLPs revealed by newly isolated chromosome 6p sequences. Above the Southern blots are the pedigrees (□, man; ○, woman; ⧄, deceased), with the symbol for each person above the lane containing his or her DNA. Some lanes (e.g., left and right in *B*) contain marker fragments. Sequence p7H4* (*A*) revealed a polymorphism when hybridized to DNA digested with the restriction enzyme *Eco*RV. This polymorphism has four alleles in the population [11.0, 10.5, 9.2, and 9.1 kilobases (kb)]; alleles *1* and *4* are present in this family. Sequences p2C5 (*B*), p3C7 (*C*), and pAGB6 (*E*) reveal polymorphisms when hybridized to DNA digested with the restriction enzyme *Msp* I. Each of these three RFLPs has two alleles (7.6 and 5.9 kb; 5.7 and 3.1 kb; 3.3 and 2.8 kb, respectively). Plasmid pCH6 (*D*) reveals a polymorphism when hybridized to DNA digested with the restriction enzyme *Taq* I; this RFLP has three alleles, at 5.7, 5.0, and 3.7 kb.
*Marker designations by the Eighth International Workshop on Human Gene Mapping (Helsinki, 1985; ref. 39): p7H4 = *D6S7*; p2C5 = *D6S8*; p3C7 = *D6S9*; and pCH6 = *D6S10*.

of these lines [721.180.2 (ref. 41), 721.144.5 (ref. 18), 721.58.5, 721.146, 721.147, 721.148, 721.149, and 721.132] were derived by a second γ-ray irradiation of deletion line 721.45.1 and were isolated on the basis of their loss of additional HLA antigens. Line 721.120 appears to have two deletions, one of which includes all *HLA* loci and *GLO1* on one homologue; the second deletion includes the region of DNA homologous to p7H4 on the other homologue. This was the only line observed that contained deletions on both homologues after a single γ-ray exposure.

The genomic DNA homologous to plasmid p3C7 was present in two copies in all 53 cell lines analyzed. This result was to be expected in view of the recent evidence from mutant 721.45.1, mentioned above, that the p3C7 sequence is not present on chromosome 6p.

Establishing the Map Order. The physical locations of the *GLO1* locus and the *HLA* loci on chromosome 6p have been established (42), giving the map order centromere–*GLO1*–*HLA(D, B, C, A)*. Because linkage of p7H4 to the other loci characterized in this study was not detected by pedigree analysis, we depend completely on the deletion mapping data presented above to place it distal to the *HLA* cluster, giving us centromere–*GLO1*–*HLA*-p7H4. The clone pCH6 has been shown by restriction mapping (H. Orr, personal communi-

	pAGB6	GLO	pDCH1 (DQα)	pDRH7 (DRα)	pDP001 (B8)	pCH6	p2C5	p7H4	p3C7
pAGB6	–	3.9	5.3	14.7	3.1	5.8	3.2	0.1	0.1
GLO	8.0 ±5.6	–	24.8	17.44	5.5	11.4	12.0	0	0.1
pDCHI (DQα)	10.6 ±5.2	6.9 ±2.1	–	52.81	14.8	33.8	23.0	0.4	0.01
pDRH7 (DRα)	3.9 ±2.3	5.2 ±2.1	0	–	13.3	28.9	21.0	0.2	0.4
pDP001 (B8)	5.6 ±5.6	6.3 ±4.5	0	0	–	9.6	7.1	0.1	0.2
pCH6	13.5 ±4.8	6.3 ±2.8	0.7 ±0.7	0	0	–	11.7	0.4	0.1
p2C5	16.6 ±6.9	9.7 ±3.2	2.9 ±1.7	30 ±1.7	5.2 ±3.8	6.1 ±3.0	–	0.4	0.2
p7H4	42.3 ±9.9	50 ±6.8	41.6 ±6.1	40.2 ±8.3	45.2 ±9.0	40.6 ±8.1	61.8 ±8.2	–	0.2
p3C7	44.8 ±9.3	43.9 ±8.7	51.7 ±8.1	39.7 ±6.2	37.4 ±9.9	44.5 ±7.3	41.7 ±7.0	59.2 ±7.8	–

FIG. 2. Maximum likelihood estimates of the recombination percentages with lod scores for nine polymorphic markers, based on two-factor crosses. Maximum likelihood estimates of the percent recombination are given ± standard error below the diagonal. The lod scores appear above the diagonal. Marker designations are given in the legend for Fig. 1 and the text.

cation) to be located within only a few kilobases of the *HLA-B* locus; for the calculations below, it has been included within the *HLA* haplotype. The solution of the map order, therefore, reduces to orienting the loci defined by the clones pAGB6 and p2C5 with respect to *GLO1* and *HLA*.

The support for each of the 12 possible orders of these four loci has been calculated by using the program ILINK from the package LINKAGE (36). The two most favored orders are pAGB6-*GLO1*-*HLA*-p2C5 (order 1) and *GLO1*-*HLA*-p2C5-pAGB6 (order 2). Each of the other 10 possible orders is excluded by negative odds of more than 100:1 when sex differences are taken into account.

The two favored orders, however, cannot be distinguished by the linkage analysis, which provides only slight support (2:1 odds) favoring order 2 over order 1 when possible sex differences are ignored, and slight support for order 1 over order 2 (1.4:1 odds) when sex differences are permitted. Fortunately, the deletion analysis eliminates the order *GLO1*-*HLA*-p2C5-pAGB6, as this order would place pAGB6 distal to *GLO1* and *HLA*. We are, therefore, left with the order pAGB6-*GLO1*-*HLA*-p2C5-p7H4 as the order most strongly supported by both linkage and deletion analysis. Fig. 4*A* indicates the maximum likelihood recombination values for this gene order, considering all five loci jointly but not taking into account possible sex differences in recombination rates.

Sex Differences. Differences in recombination rates between male and female meioses have been reported (43), leading to the supposition that there may be a generally higher rate of recombination in females. More recently, however, examination of sex-specific differences in recombination on the short arm of human chromosome 11 has indicated that the situation is somewhat more complex: significant differences were found between male and female recombination rates in some regions of 11p, but with an excess in male recombination (44). Recent work with chromosome 13 indicates an excess of female recombination (unpublished data). Since these results suggest a need for two linkage maps to describe the frequencies of recombination in male and female meioses, we used the LINKAGE routines to estimate recombination rates, jointly for the four linked loci, separately in males and females (Fig. 4*B*). Because independent iteration of each interval failed to give a significant improvement in the likelihood, the values reported in Fig. 4*B* are those found by imposition of a constant ratio of recombination between males and females. The maximum likelihood estimate of the ratio of female recombination frequency to male recombination frequency was 1.23 (standard error ±0.12). Sex differences in recombination frequency were not significant by this method of calculation.

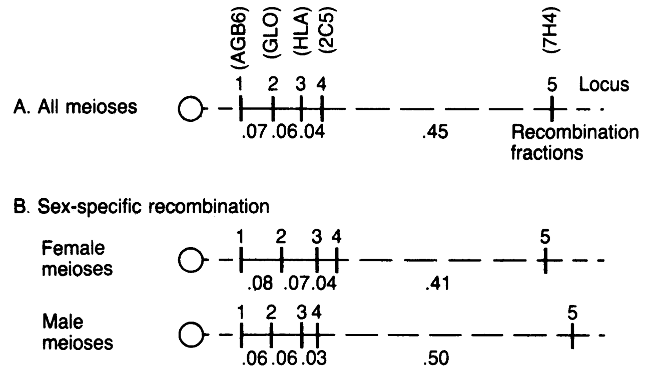

FIG. 4. Combined and sex-specific genetic maps of the short arm of chromosome 6, derived from four-factor analysis of the closely linked markers and two-factor analysis for the interval 2C5-7H4, with constant ratios maintained between intervals. Map distances are in recombination fractions. The distances between the *HLA* loci and other markers were calculated by using the *HLA* loci as a single haplotype. The Eighth International Workshop on Human Gene Mapping (Helsinki, 1985) has designated markers 2C5 and 7H4 as *D6S8* and *D6S7*, respectively.

Polymorphic markers	Cell lines with deletions in one homologue										Cell lines with deletions in both homologues		
	721.64 .76 .80 .108	721.74 .87 .109	721.50 .145	721.2 .17 .21 .24 .26 .29	721.3 .13 .19 .31 .34 .40 .116 .117 .118.1	721.22 .77.1 .78 .123 .125 .126	721.111 .121	721.115 .124	721.112	721.1 .38 .45.1 .92 .110 .113 .114 .127 .131	721.120	721.58.5 .132 .144.5 .146 .147 .148 .149	721.180.2 *
pAGB6	+	+	+	+	+	+	+	+	−	−	− +	+ −	+ −
Glyoxylase I	+	+	+	+	+	+	−	−	−	−	− +	+ −	+ −
HLA-DR	+	+	+	+	−	−	−	−	−	−	− +	+ −	− −
HLA-B	+	+	−	−	−	−	−	−	−	−	− +	+ −	− −
HLA-A	−	−	−	−	−	−	−	−	−	−	− +	− −	− −
p7H4	+	−	+	−	+	−	+	−	+	−	+ −	+ −	+ −
p3C7	+	+	+	+	+	+	+	+	+	+	+ +	+ +	+ +

FIG. 3. Regional localization of RFLPs by deletion mapping. The data were gathered on 53 deletion lines, all derived from line LCL 721; the cell lines are arranged in order of increasing number of missing markers. Lines that were genotypically identical as judged by the group of seven markers are listed together. The polymorphic markers (see marker designations in Fig. 1 legend) are arranged to fit a pattern of a single deletion event; where the deletion was detected in one homologue the information is presented for only that homologue. A + represents the detection of the appropriate allele for that homologue; a − is the absence of the appropriate allele. The glyoxylase I and HLA typing were done by using classical electrophoretic and serological techniques; therefore, the information given represents the phenotype of the mutant cells for those alleles. With the DNA markers, the genotype is given.

*Recent analysis of mutant 721.180.2 shows that the HLA-A and -B (40), as well as the complement genes (30), are present on one homologue. The deficient expression of the HLA-A and -B antigens results from a post-transcriptional defect (40). The deficiency in HLA-DR expression results from homozygous deletion of a DNA segment between the complement gene cluster (between *HLA-B* and *HLA-DR*) and the *HLA-DPα2* locus (between *GLO1* and *HLA*) (unpublished data). The apparent discrepancy between HLA-A and -B phenotypes and genotypes does not affect the derived linkage map.

DISCUSSION

Four RFLPs have been identified that map to the short arm of chromosome 6; a fifth was characterized but did not map to 6p when cell deletion analysis was combined with the linkage data. The major histocompatibility locus in man is also located on chromosome 6p and has been used as a genetic marker to study numerous human diseases. Linkage disequilibrium, a nonrandom association among alleles at different loci, has been observed between many HLA alleles and specific diseases (11). The biological basis of these associations in most cases is not known; they may be the result of physical linkage of the disease locus to HLA or they may involve an epistatic interaction. The RFLPs described here will be useful in studying diseases associated with HLA.

Linkage disequilibrium has also been demonstrated among the different *HLA* loci, by analysis of the frequencies of *HLA* haplotypes (determined by classical serological typing methods) in many populations (39). Until recently, analysis of the *HLA* region at the DNA level was not possible. In this study, however, we have used RFLPs for determination of inheritance at the *HLA* locus and have observed linkage disequilibrium between the *HLA* RFLPs (20), confirming the disequilibrium phenomenon at the DNA level.

Eight markers were finally used in the construction of our genetic map of the short arm of chromosome 6. These markers could be localized with respect to one another with good confidence through a combination of deletion mapping and linkage analysis; four of them are included in the *HLA* haplotype. The combined and sex-specific maps have good precision and will provide reference points for mapping additional loci in this region.

We thank B. Young for the use of the chromosome 6 recombinant DNA library and C. Auffray, L. Villa-Komaroff, and S. Weissman for providing DNA clones. We also thank Candace Brown for excellent technical assistance in determining the glyoxylase I phenotypes. The work was supported by the Howard Hughes Medical Institute and by National Institutes of Health Training Grant 5-T32-GM0746407 (R.L.) and National Institutes of Health Grant A1-15486 (R.D.). R.W. is an Investigator of the Howard Hughes Medical Institute.

1. Botstein, D., White, R., Skolnick, M. & Davis, R. (1980) *Am. J. Hum. Genet.* **32**, 314–331.
2. Southern, E. (1975) *J. Mol. Biol.* **98**, 503–517.
3. Wyman, A. & White, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6754–6758.
4. Kan, Y. & Dozy, A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5631–5635.
5. Jeffreys, A. (1979) *Cell* **18**, 1–10.
6. Ullrich, A., Dull, T., Gray, A., Brosius, J. & Sures, I. (1980) *Science* **209**, 612–614.
7. Bell, G., Pictet, R. & Rutter, W. (1980) *Nucleic Acids Res.* **8**, 4091–4109.
8. Bell, G., Karam, J. & Rutter, W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5759–5763.
9. Lange, K. & Boehnke, M. (1982) *Am. J. Hum. Genet.* **34**, 842–845.
10. Bishop, D. & Skolnick, M. (1980) in *Banbury Report 4: Cancer Incidence in Defined Populations*, eds. Cairns, J., Lyon, J. & Skolnick, M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 421–433.
11. Ryder, L., Svejgaard, A. & Dausset, J. (1981) *Annu. Rev. Genet.* **15**, 169–187.
12. Boncinelli, E., Goyns, M., Scotto, L., Simeone, A., Harris, P., Kellow, J. & Young, B. (1984) *Mol. Biol. Med.* **2**, 1–14.
13. Cavenee, W., Leach, R., Mohandas, T., Pearson, P. & White, R. (1984) *Am. J. Hum. Genet.* **36**, 10–24.
14. Cann, H. M., Ascanio, L., Paul, P., Marcadet, A., Dausset, J. & Cohen, D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1667–1668.
15. Auffray, C., Ben-Nun, A., Rous-Dosseto, M., Germain, R., Seidman, J. & Strominger, J. (1983) *EMBO J.* **2**, 121–124.
16. Ehrlich, H., Stetler, D., Saiki, R., Goldstone, P. & Pious, D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2300–2304.
17. Orr, H., Bach, F., Ploegh, H., Strominger, J., Kavathas, P. & DeMars, R. (1982) *Nature (London)* **296**, 454–456.
18. Orr, H. & DeMars, R. (1983) *Immunogenetics (N. Y.)* **18**, 489–502.
19. Shows, T., Eddy, R., Grezschik, K., Gonzalez, A. & Villa-Komaroff, L. (1984) *Cytogenet. Cell Genet.* **37**, 582 (abstr.).
20. Leach, R. (1984) Dissertation (Univ. of Utah, Salt Lake City, UT).
21. White, R., Leppert, M., Bishop, D., Barker, D., Berkowitz, J., Brown, C., Callahan, P., Holm, T. & Jerominski, L. (1984) *Chromosomes Today* **8**, 23–32.
22. Maniatis, T., Hardison, R., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. & Efstratiadis, A. (1978) *Cell* **15**, 687–701.
23. Sood, A., Pereira, D. & Weissman, S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 616–620.
24. Kavathas, P., Bach, F. & DeMars, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4251–4255.
25. DeMars, R., Chang, C. & Rudersdorf, R. (1983) *Hum. Immunol.* **8**, 123–139.
26. Kavathas, P., DeMars, R. & Bach, F. (1980) *Hum. Immunol.* **4**, 317–324.
27. Orr, H. & DeMars, R. (1983) *Nature (London)* **302**, 534–536.
28. Auffray, C., Kuo, J., DeMars, R. & Strominger, J. (1983) *Nature (London)* **304**, 174–177.
29. Roux-Dosseto, M., Auffray, C., Lillie, J., Boss, J., Cohen, D., DeMars, R., Mawas, C., Seidman, J. & Strominger, J. (1984) *Proc. Natl. Acad. Sci. USA* **80**, 6036–6040.
30. Whitehead, S., Colfen, H., Chang, C. & DeMars, R. (1985) *J. Immunol.* **134**, 641–643.
31. Mohandas, T., Sparkes, R., Shulkin, J., Sparkes, M. & Moldjono, S. (1980) *Cytogenet. Cell Genet.* **28**, 116–120.
32. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
33. Kompf, J., Bissbort, S., Gussman, S. & Ritter, H. (1975) *Humangenetik* **27**, 141–145.
34. Edwards, A. M. F. (1972) *Likelihood: An Account of the Statistical Concept of Likelihood and its Application to Scientific Inference* (Cambridge Univ. Press, New York).
35. Hasstedt, S. & Cartwright, P. (1981) *PAP: Pedigree Analysis Package, Revision 2*. Technical Report Number 13, Department of Medical Biophysics and Computing, Univ. of Utah, Salt Lake City.
36. Lathrop, M. & Lalouel, J.-M. (1984) *Am. J. Hum. Genet.* **36**, 460–465.
37. Lalouel, J.-M. (1979) *GEMINI: A Computer Program for Optimization of General Nonlinear Functions*. Technical Report Number 14, Department of Medical Biophysics and Computing, Univ. of Utah, Salt Lake City.
38. Morton, N. E. (1962) in *Methodology in Human Genetics*, ed. Burdette, W. J. (Holden-Day, San Francisco), pp. 17–52.
39. Mi, M. & Morton, N. (1966) *Vox Sang.* **11**, 434–449.
40. DeMars, R., Rudersdorf, R., Chang, C., Petersen, J., Strandtmann, J., Korn, N., Sidwell, B. & Orr, H. T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8183–8187.
41. DeMars, R., Chang, C., Shaw, S., Reitnauer, P. & Sondel, P. (1984) *Hum. Immunol.* **11**, 77–97.
42. Bakker, E., Pearson, P., Meera Kahn, P., Schrender, G. & Madam, K. (1979) *Clin. Genet.* **15**, 198–202.
43. Renwick, J. (1968) *Eur. Soc. Hum. Genet.* **2**, 7–8.
44. White, R., Leppert, M., Bishop, D., Barker, D., Berkowitz, J., Brown, C., Callahan, P., Holm, T. & Jerominski, L. (1985) *Nature (London)* **313**, 101–105.