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Genetic linkage map of 46 DNA markers on human chromosome 16

(restriction fragment length polymorphisms/multipoint linkage analysis/autosomal dominant polycystic kidney disease)

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ABSTRACT **We have constructed a genetic linkage map of human chromosome 16 based on 46 DNA markers that detect restriction fragment length polymorphisms. Segregation data were collected on a set of multigenerational families provided by the Centre d'Etude du Polymorphisme Humain, and maps were constructed using recently developed multipoint analysis techniques. The map spans 115 centimorgans (cM) in males and 193 cM in females. Over much of the chromosome there is a significantly higher frequency of recombination in females than males. Near the α-globin locus on the distal part of the short arm, however, there is a significant excess of male recombination. Twenty-seven (59%) of the markers on the map have heterozygosities greater than or equal to 0.50. The largest interval between loci on the sex-average map is 14 cM and the average marker spacing is 3 cM. Using loci on this map, one could detect linkage to a dominant disease on chromosome 16 with as few as 10–15 phase-known meioses.**

In less than a decade from the discovery of the first highly informative human DNA genetic marker (1) and the proposal to construct a human genome map from a set of 150 such markers detecting restriction fragment length polymorphisms (RFLPs) (2), rapid progress has been made in developing high-resolution genetic linkage maps. These emerging genetic linkage maps represent a valuable resource for the study of the organization of the human genome and serve as useful tools for systematic efforts to identify genes responsible for inherited disorders. Following their genetic localization, a number of disease loci have now been cloned and DNA probes that either detect the disease gene directly or detect closely linked polymorphisms are currently used in the diagnosis of a large number of genetic disorders (3, 4).

As an extension of our efforts to refine the localization of autosomal dominant polycystic kidney disease (*PKD1*; ref. 5), which had previously been localized to the short arm of chromosome 16 (6), we have isolated 37 new markers and constructed a genetic linkage map of chromosome 16 consisting of 46 loci. This high-density genetic linkage map of chromosome 16, with an average marker spacing of 3 centimorgans (cM), should facilitate the detection of linkage to other disease genes, serve as a resource for the construction of a physical map of chromosome 16, and contribute to a better understanding of the organization of the human genome.**

MATERIALS AND METHODS

Somatic Cell Hybrids. CH1-16, derived from transformed lymphocytes from a normal individual, is a mouse–human hybrid cell line that contains human chromosome 16 as its only human component. GM6227, subcloned from the mouse–human hybrid cell line 6227-C10, contains a single human chromosome 16 deleted for the region 16p13.11–16pter (7).

Chromosome 16 Markers. Candidate probes were isolated from two human chromosome 16-specific libraries. We constructed a cosmid library in the vector c2RB (8) by using *Mbo* I partial DNA fragments of ≈40 kilobases (kb) from the mouse–human hybrid cell line CH1-16. Cosmids containing human DNA inserts were selected following two rounds of screening with ^{32}P-labeled human genomic DNA.

A phage library (LA16NSO2) was prepared by the Los Alamos National Laboratory, using flow-sorted chromosomal material enriched for chromosome 16 (9). Our characterization of this library revealed that only half of the 109 clones tested contained inserts when fractionated in agarose gels and that the insert size was relatively small (average 2.3 kb). Therefore, to increase the length of DNA screened per clone tested and thereby increase the chance of detecting a polymorphic region, we enriched for phage containing larger inserts by CsCl density fractionation (10). To ensure that phage revealing polymorphisms had inserts derived from human chromosome 16, and to further determine the subchromosomal location of these clones, probes were hybridized in the presence of unlabeled human placental DNA (11) to Southern blots containing total human and mouse DNA as well as DNA from CH1-16 and GM6227.

In addition to probes identified from the chromosome 16-specific libraries, eight other markers were incorporated into the map. These include three probes isolated from a phage genomic library (12), p79-2-23 (*D16S7*) provided by Michael Litt (13), and five probes that detect gene regions on chromosome 16. Probes p3'HVR.64 and p5'HVR.14, which detect variable numbers of tandem repeats near the α-globin locus (*HBA1*) (14, 15), were provided by Andrew Jarman and Douglas Higgs; pHUAP/B2.2, which detects the adenine phosphoribosyltransferase gene (*APRT*) (16), was provided by Peter Stambrook; pBRZ, which detects ζ-globin pseudogene 1 (*HBZP1*) (17), and hp2alpha, which reveals the haptoglobin locus (*HP*) (18), were obtained from the American Type Culture Collection.

Family Resources. Inheritance of RFLP alleles for the 46 chromosome 16-specific probes was studied in a minimum of 21 three-generation reference pedigrees provided by the Centre d'Etude du Polymorphisme Humain (CEPH) in which

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Abbreviations: RFLP, restriction fragment length polymorphism; PIC, polymorphism information content; lod, logarithm of odds; cM, centimorgan(s); CEPH, Centre d'Etude du Polymorphisme Humain.

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**All CRI probes and linkage data from this study are available to investigators for research purposes.

at least one parent was heterozygous. *HP* and six loci in the region of *PKD1* were mapped on all 40 reference families. Genomic DNA either was provided by the CEPH or was prepared from transformed lymphoblast cell lines (19) obtained from R. White (Howard Hughes Medical Institute and Human Genetics Department, University of Utah Medical Center, Salt Lake City) or purchased from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository. All segregation data were contributed to the CEPH data base, Versions 2 and 3.

Screening for Polymorphisms. Clones were tested for their ability to reveal polymorphism on Southern blots containing DNA from five unrelated individuals digested with nine different restriction enzymes (*Bam*HI, *Bgl* II, *Eco*RI, *Hin*cII, *Hin*dIII, *Msp* I, *Pst* I, *Rsa* I, and *Taq* I). Procedures for restriction enzyme digests, Southern blots, and hybridizations were previously described (12, 20). Both phage and cosmid probes were hybridized in the presence of unlabeled human placental DNA to block the hybridization between repeated sequences in the inserts and genomic DNA on the Southern blots (11). Candidate probes revealing polymorphisms were tested on Southern blots containing DNA from parents of the CEPH families. Based on the parental genotypes, probes that revealed alleles present in reasonable frequencies, generally having polymorphism information content (PIC; ref. 2) values > 0.3, were hybridized to Southern blots of the CEPH families for which at least one parent was heterozygous. Due to our interest in finding additional markers in the region of *PKD1*, in the latter stages of the research we restricted our mapping efforts to probes localized in 16p13 based on results from the hybrid cell lines, unless probes in other regions were highly polymorphic (PIC values > 0.6).

Multipoint Linkage Analysis. Analyses were performed on a MicroVAXII using the computer program CRI-MAP, written in the language C. CRI-MAP (available from P.G.), which uses maximum likelihood multipoint linkage analysis (ref. 21; P.G., unpublished results), has been used to construct maps of many human chromosomes (10, 20, 22). The CHROMPICS option of CRI-MAP, which displays the grandparental origin of alleles for both chromosomes of each CEPH family child and flags those loci involved in apparent double crossover events, was used to check for possible data errors.

The significance of sex differences in recombination between pairs of loci was evaluated by a likelihood ratio test based on likelihoods from two-point linkage analyses. Two-point logarithm-of-odds (lod) tables, computed from the chromosome 16 data with the TWOPOINT option in CRI-MAP, have been submitted to B. J. B. Keats for use by the linkage committee of the Human Gene Mapping Workshop.

RESULTS

Candidate Markers. Of the 424 probes screened on Southern blots, 54% of the cosmid clones containing human inserts and 34% of the phage clones detected polymorphic regions. Based on their hybridization pattern with the somatic cell hybrid lines containing all or a portion of chromosome 16, only half of the phage clones that revealed RFLPs were found to contain inserts that originated from human chromosome 16. The other 17 phage clones were localized to 10 other autosomes by genetic linkage analyses (20), indicating a mixture of chromosomes in the flow-sorted material used in the construction of the phage library. From the probes that revealed polymorphic regions on chromosome 16, a subset of 37 with the highest PIC values was selected to collect genotypic information in the CEPH families.

Table 1 lists the characteristics of the 46 probes that comprise the chromosome 16 map. More than half of the loci reveal polymorphic regions with heterozygosities ≥ 0.50 (seven of these exceed 0.70). In addition to the 37 probes identified from the human chromosome 16-specific libraries, the map includes *D16S7* (13), three loci isolated from a phage genomic library (12) that exhibited two-point lod scores > 6.0 with loci from the chromosome 16-specific libraries, and five probes that detected variable regions in or near four genes physically localized to chromosome 16. These include 3'- and 5'HVR (14, 15), near *HBA1*, *HBZP1* (17), *HP* (18), and *APRT* (16).

Multipoint Linkage Map. The computer program CRI-MAP (10, 20) was used to generate the multipoint linkage maps.

Table 1. Loci on chromosome 16

Probe*	Locus	Enzyme(s)†	Het (PIC)‡	Meioses§
CRI-O2	*D16S38*	B	0.21 (0.19)	73
CRI-O3	*D16S39*	P	0.52 (0.38)	173
CRI-O15	*D16S40*	T	0.62 (0.45)	172
CRI-O43	*D16S41*	T	0.29 (0.29)	85
CRI-O66	*D16S42*	P	0.31 (0.29)	103
CRI-O84	*D16S43*	H, P	0.83 (0.79)	278
CRI-O89	*D16S44*	Bg	0.62 (0.45)	206
CRI-O90	*D16S45*	D, E	0.74 (0.52)	350¶
CRI-O91	*D16S46*	M, T	0.64 (0.60)	215
CRI-O95	*D16S47*	E	0.50 (0.43)	159
CRI-O101	*D16S48*	H	0.45 (0.33)	144
CRI-O114	*D16S49*	E	0.43 (0.40)	169¶
CRI-O119	*D16S50*	T	0.55 (0.36)	162
CRI-O120	*D16S51*	B	0.64 (0.55)	220
CRI-O123	*D16S52*	H	0.33 (0.26)	92
CRI-O125	*D16S53*	P	0.55 (0.36)	175
CRI-O126	*D16S54*	E, Hc	0.57 (0.48)	189
CRI-O128	*D16S55*	Hc	0.24 (0.21)	72
CRI-O129	*D16S56*	Bg, E	0.38 (0.36)	264¶
CRI-O131	*D16S57*	B	0.29 (0.29)	80
CRI-O133	*D16S58*	H	0.43 (0.33)	248¶
CRI-O134	*D16S59*	M	0.26 (0.21)	122¶
CRI-O136	*D16S60*	Hc	0.83 (0.81)	444¶
CRI-O144	*D16S61*	E	0.48 (0.40)	198¶
CRI-O149	*D16S62*	T	0.31 (0.31)	106
CRI-O327	*D16S63*	H	0.38 (0.33)	276¶
CRI-O373	*D16S64*	H	0.64 (0.55)	197
CRI-O377	*D16S65*	Bg, Hc, T	0.76 (0.76)	224
CRI-O383	*D16S66*	P	0.57 (0.36)	189
CRI-O391	*D16S67*	Hc, T	0.67 (0.60)	221
CRI-O393	*D16S68*	Bg, P	0.64 (0.52)	217
CRI-P84	*D16S69*	B	0.38 (0.29)	132
CRI-P85	*D16S74*	Bg	0.50 (0.38)	196
CRI-P130	*D16S70*	M	0.62 (0.45)	195
CRI-P400	*D16S71*	B	0.43 (0.36)	139
CRI-P403	*D16S72*	M	0.57 (0.38)	178
CRI-P477	*D16S73*	T	0.40 (0.38)	128
CRI-L223	*D16S76*	T	0.48 (0.40)	160
CRI-L922	*D16S77*	H, Hc	0.50 (0.43)	163
CRI-R99	*D16S75*	H	0.64 (0.62)	227
3'HVR	*D16S85*	M	0.93 (0.93)	573¶
5'HVR		R	0.79 (0.79)	257
p79-2-23	*D16S7*	T	0.64 (0.62)	201
pHUAP	*APRT*	Bg, T	0.29 (0.29)	92
pBRZ	*HBZP1*	Bg, Hc	0.79 (0.76)	257
hp2alpha	*HP*	M	0.55 (0.43)	339¶

*CRI probes designated with the letters O and P were from chromosome-specific libraries prepared in cosmid and phage vectors, respectively. Probes with the letters R and L were isolated (12) from a Charon 4A library, with the L probes isolated by screening for single-copy sequences.

†Restriction enzyme abbreviations: B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; M, *Msp* I; P, *Pst* I; R, *Rsa* I; T, *Taq* I; D, *Dra* I.

‡Heterozygosity (Het) and PIC estimates are based on parents in the CEPH families.

§Number of informative meioses in the CEPH families.

¶Those loci for which >21 CEPH families were studied.

Throughout the analyses, sex-average recombination fractions were assumed for each interval. Final sex-specific maps were also generated. During map construction, probes were sorted by their informativeness and a pair of highly informative linked loci (CRI-O136 and a haplotyped locus consisting of 3'HVR, 5'HVR, and *HBZP1*) was chosen as the nucleus of the map. Subsequent loci were selected in order of their informativeness and placed in each possible position. Maximum likelihood estimates of recombination fractions were calculated, and a locus was added to the map if one placement was at least 10,000 times more likely than alternative placements. Once a map of all loci that were uniquely placed at 10,000:1 odds was constructed, the locus with the fewest number of permissible positions was added, and the entire process was repeated with succeeding loci tested in each position with respect to each possible order. To facilitate the initial analyses, "phase known" data, consisting of data from families in which the parents were not identical heterozygotes and the grandparental origin of each child's alleles could be determined, were used to generate an initial map. The complete genotypic data set was analyzed subsequently. For those loci not uniquely placed at a criterion of 10,000:1 odds, the program computed the likelihoods for all possible positions with respect to the uniquely placed loci and a map was generated for the most likely placement of all loci. Triplets of adjacent loci were then permuted within this map and log-likelihoods were computed to determine a final map with the highest likelihood. Maximum likelihood estimates of recombination fractions were converted to map distances by using the Kosambi mapping function (23).

The sex-specific and sex-average multipoint maps are shown in Fig. 1 along with an ideogram of chromosome 16 indicating the subchromosomal localization of *HBA1* to 16p13.3 (24), *HP* to 16q22.1 (25), and both *APRT* and *D16S7* to 16q24 (26, 13). The subchromosomal location of 16 other loci on the map was determined by hybridization to the hybrid cell lines CH1-16 and GM6227.

Twenty-one loci (one locus consisting of the three haplotyped probes in the α-globin gene cluster that served as one of the pair of loci used as the nucleus of the map) can be placed in a unique order with odds > 10,000:1. The majority of the remaining 23 markers were well positioned on the map; 17 could not be ordered with respect to one other uniquely placed locus on the map with odds of 10,000:1 (9 of these showing no recombinants with the uniquely placed locus). Six of the less informative probes could only be regionally localized at 10,000:1 odds. When the criterion for order during map construction was reduced to odds of 1000:1, only one additional locus (CRI-R99) was uniquely placed.

The sex-average map in Fig. 1 shows the most likely positions, and ranges when appropriate, from the multipoint analysis at odds of 10,000:1. All loci included in the map were connected by a chain of lod 6 linkages. The current map is different in several respects from a previously published map of chromosome 16 (20) using 41 of the markers presented here. First, spurious crossovers between CRI-O90 and CRI-O327 were revealed in CEPH family 1334 following additional testing based on an inconsistency in their order between the CEPH and 19 *PKD1* families (5). Second, the protein polymorphism data for *HP* submitted to the CEPH (27) and included in the previous map were replaced with RFLP data since the CHROMPICS option of CRI-MAP revealed multiple crossovers between *HP* and several other loci. The DNA results changed the position and order of several probes on the long arm of the chromosome. Third, we added three additional markers (*D16S7, HBZP1*, and *APRT*). Lastly, segregation data from additional CEPH families for several probes, particularly in the region of the α-globin locus, have increased the resolution of the map.

The length of the sex-average map is 149 cM. There are, however, statistically significant differences in recombination rates between the sexes. Based on a likelihood ratio test from two-point linkage data between the 21 uniquely ordered loci, females show significantly higher recombination frequencies than males in three intervals on the long arm of the chromosome. These include O391–O101 and O377–O91 ($P < 0.01$) and O3–O377 ($P < 0.001$). Despite the overall longer length of the female map, which exceeds that of the male by 78 cM, there is a significant excess of male recombination in the distal region of the short arm near the α-globin locus. For example, based on two-point linkage analyses, the maximum likelihood estimates for the recombination fraction in females and males between 3'HVR and CRI-O327 are 0.01 and 0.14, respectively ($P < 0.01$).

DISCUSSION

Our genetic linkage map of 46 markers on chromosome 16 spans 193 cM in females and 115 cM in males [7 cM longer than a previous estimate based on chiasma counts in males (28)]. While the most distal markers may still lie some distance from the telomeres, they appear to extend over much of the chromosome based on their location with respect to *HBA1* and *APRT*, which have been localized to 16p13.3 and 16q24, respectively. The average spacing between loci is 3 cM on the male map and 4 cM on the female map, with the largest interval between markers only 14 cM on the sex-average map.

A primary value of genetic linkage maps is that fewer meioses are required to detect new linkages, since adjacent loci increase their mutual informativeness and facilitate linkage analyses (29). This is especially important in the localization of disease genes where the number of meioses in families harboring the disorder may be limited. Based on the heterozygosity values of loci on multipoint maps, the expected number of meioses necessary to detect linkage between a marker on the map and a hypothetical dominant disease on the chromosome can be determined (10). Depending on the position of the disease locus on chromosome 16, between 10 and 15 phase-known meioses will be required to detect linkage with a high degree of certainty.

We have used the genetic linkage map of chromosome 16 to refine the position of the locus for autosomal dominant polycystic kidney disease (*PKD1*), which initially had been localized to the short arm of chromosome 16 based on its linkage to 3'HVR (6). Based on segregation data from nine markers within 25 cM of 3'HVR (sex-average map) in 19 *PKD1* families, the most likely position of *PKD1* was found to lie between 3'HVR and CRI-O327 (odds of 170:1; ref. 5). Markers flanking *PKD1* should facilitate efforts to identify the gene as well as to provide an improved method for presymptomatic diagnosis of the disorder.

In addition to medical applications, genetic linkage maps are providing interesting biological insights into genome organization. For example, there appears to be a nonrandom distribution of polymorphic regions along chromosomes with the most variable being preferentially localized to distal regions. In linkage analyses of 403 loci on 23 human chromosomes (20), 11 of 28 probes (39%) with PIC values > 0.70 were located within the terminal 5% of the genetic linkage maps. Many of these probes may reveal polymorphic regions composed of variable numbers of tandem repeats (VNTRs), which have been observed to lie preferentially, though not exclusively, in telomeric regions (30–32). We observe the same phenomenon on chromosome 16, where several hypervariable loci detecting VNTRs are found within the α-globin gene cluster (14, 15, 33, 34) in the distal region of the short arm.

Genetic linkage maps are also revealing interesting facts about genetic recombination. Sex differences in the fre-

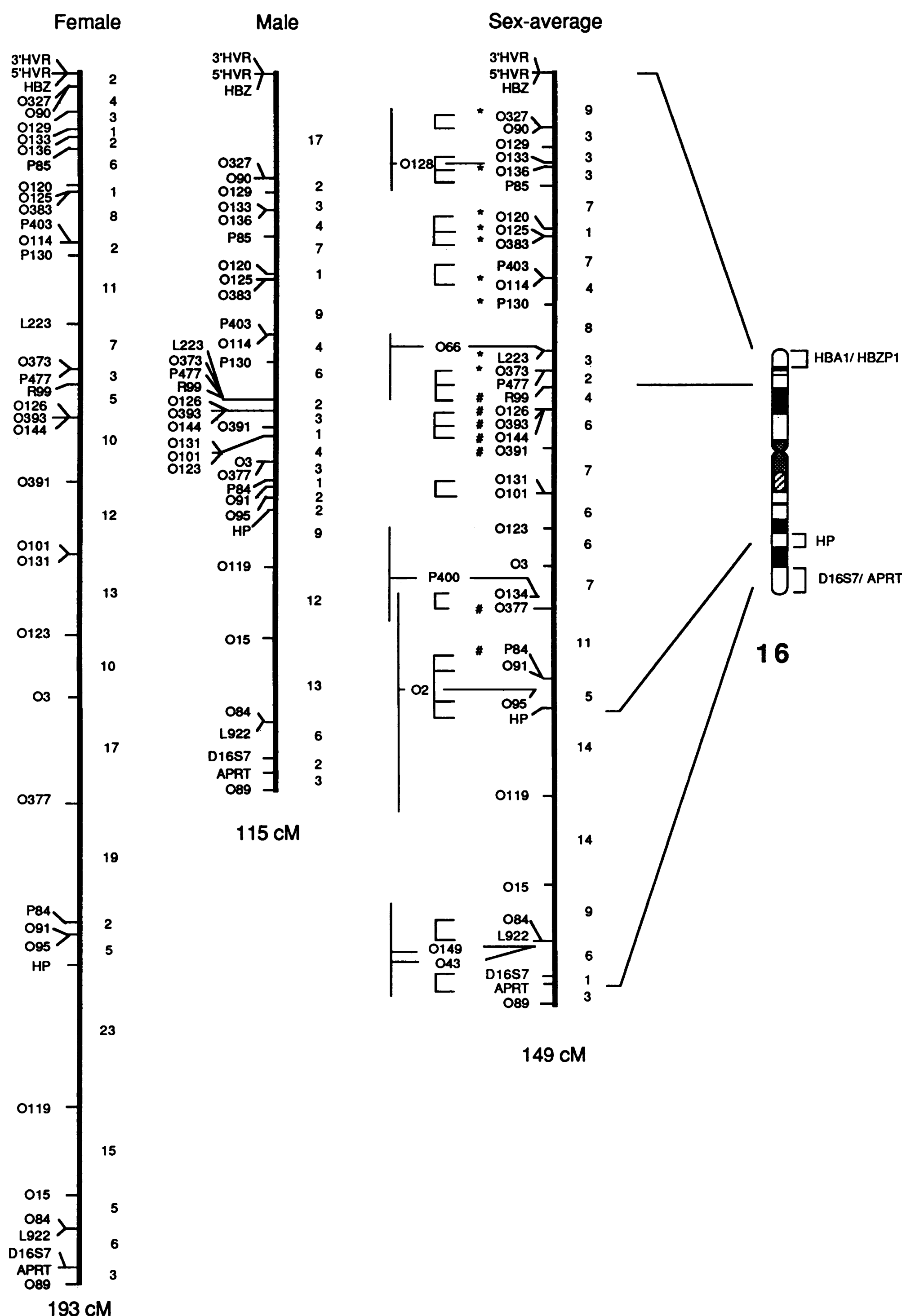

FIG. 1. Genetic linkage map of chromosome 16. Loci are arranged in their most likely order with map distances indicated in centimorgans. Horizontal hash marks connect loci to the vertical line where the most likely position is favored over alternative locations by at least 100:1. All possible positions of loci not uniquely placed at odds of 10,000:1 are indicated on the sex-average map in the following manner: brackets enclose a set of loci whose mutual order cannot be determined if odds of 10,000:1 are required; a caret connects two probes with no observed recombinants between them; if the genetic location of a locus is poorly resolved relative to a neighboring region, a line to the left of the chromosome indicates the range of possible positions at a criterion of 10,000:1. *, Localized to 16p13.1–16pter; #, localized to 16p13.1–16qter.

quency of recombination have been observed in a number of animal species. In general, and in agreement with Haldane's Rule (35), the heterogametic sex has a lower rate of recombination. The best documented cases of sex differences in the frequency of recombination in mammals (other than humans) are the horse and *Mus musculus*, where a significant excess of female recombination was observed in both species for many chromosomal regions (36, 37). In *M. musculus*, however, despite the overall increased rate of recombination in female meioses, there were specific regions that exhibited an excess of male recombination (37, 38).

Throughout the human genome a higher rate of recombination in female meioses now seems to be a fairly general observation, with the genetic maps of female autosomes

≈90% longer than those of males (20). However, as observed in *M. musculus*, there are certain chromosomal regions within which males exhibit higher recombination rates. In general, these appear to be preferentially located in distal chromosomal regions, with an excess of male recombination observed for the distal regions of chromosomes 10 (22), 11 (20), 12 (39), 15 (40), 17 (41), 19 (42), and 20 (43). Our data from chromosome 16 further support an overall higher rate of recombination in female meioses, with a significant excess of male recombination limited to the distal region of the short arm near the α-globin gene cluster. As high-density genetic maps are used as signposts in the development of physical maps, it should be particularly interesting to compare genetic and physical estimates of distance in those regions where differences in male and female recombination rates are observed.

Note Added in Proof. Since submission of this manuscript, another genetic linkage map of human chromosome 16 has been published (44).

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