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Linkage, Physical Mapping, and DNA Sequence Analysis of Pseudoautosomal Loci on the Human X and Y Chromosomes

DAVID C. PAGE,* KRISTINA BIEKER,* LAURA G. BROWN,* SEAN HINTON,* MARK LEPPERT,†
JEAN-MARC LALOUEL,† MARK LATHROP,† MINNA NYSTROM-LAHTI,‡
ALBERT DE LA CHAPELLE,‡ AND RAY WHITE†

*Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142; †Howard Hughes Medical Institute, University of Utah, Salt Lake City, Utah 84132; and ‡Department of Medical Genetics, University of Helsinki, 00290 Helsinki, Finland

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The pseudoautosomal region of the human X and Y chromosomes is subject to frequent X–Y recombination during male meiosis. We report the finding of two pseudoautosomal loci, *DXYS20* and *DXYS28*, characterized by highly informative restriction fragment length polymorphisms (RFLPs). The pseudoautosomal character of *DXYS20* and *DXYS28* was formally demonstrated by comparing their transmission to 45,X and to normal individuals. Studies of the inheritance of these loci reveal that the pseudoautosomal region, though highly recombinogenic, is subject to marked recombinational interference in male meiosis; no double recombinants were observed in 143 triply informative meioses, and the coefficient of coincidence is likely less than 0.45. In female meiosis, linkage of these pseudoautosomal RFLPs to strictly sex-linked RFLPs on the short arm of the X is readily detected; the genetic length of the pseudoautosomal region in female meiosis is at least 4 cM but not more than 18 cM. The genetic map of the human X chromosome is now defined from near the short-arm telomere to band q28 on the long arm. Locus *DXYS20*, which maps near the X and Y short-arm telomeres, is composed of long tandem arrays of 61-bp repeats. Occasional, seemingly random base-pair substitutions within these arrays of 61-bp repeats, in combination with marked variation in the size of the array, generate the high degree of DNA polymorphism at *DXYS20*. © 1987 Academic Press, Inc.

INTRODUCTION

In 1934, Darlington, Haldane, and Koller speculated that recombination might occur between the mammalian X and Y chromosomes, which had been observed to associate in male meiosis. Later studies demonstrated that terminal portions of the short arms of the human X and Y chromosomes pair (Pearson and Bobrow, 1970; Chen and Falek, 1971) and form a synaptonemal complex (Moses *et al.*, 1975) during meiosis in spermatogenesis. Studies using restriction fragment length polymorphisms (RFLPs) have demonstrated that recombination between the distal short arms of the X and Y chromosomes is indeed a frequent event (Cooke *et al.*, 1985; Simmler *et al.*, 1985). Because of this X–Y recombination, these portions of the sex chromosomes do not show strictly sex-linked inheritance. Rather, they exhibit varying degrees of partial sex linkage and in the extreme are inherited as if autosomal, hence the term "pseudoautosomal" (Burgoyne, 1982). Studies of the inheritance of such pseudoautosomal RFLPs have allowed four such loci to be ordered with respect to the centromeres of the sex chromosomes (Rouyer *et al.*, 1986; Goodfellow *et al.*, 1986). Recombination frequencies among these pseudoautosomal loci are strikingly higher in male than in female meiosis (Rouyer *et al.*, 1986).

We now report the identification of two additional highly informative pseudoautosomal RFLPs. The pseudoautosomal character of these two loci is demonstrated in a formal genetic sense by analyzing their transmission to 45,X females. Based on their inheritance in large three-generation human pedigrees, we have ordered these two loci and three previously described loci with respect to one another and with respect to the centromeres of the sex chromosomes. We demonstrate that, in male meiosis, the pseudoautosomal region is subject to marked recombinational interference. In female meiosis, there is no genetic discontinuity between the pseudoautosomal and strictly sex-linked portions of the X chromosome, enabling us to extend the genetic linkage map of the X chromosome to its short-arm telomere. One of the pseudoau-

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TABLE 1

DNA Markers Used in This Study or for Which Previously Published Data (Drayna and White, 1985; Rouyer *et al.*, 1986) Were Included in the Analysis

Locus	Probe	Physical location	Polymorphic enzyme	Reference
DXYS14	29C1	Xp22.3, Yp	Many	(7)
DXYS20	pDP230	Xp22.3, Yp	Many	This paper
DXYS28	pDP411a	Xp22.3, Yp	*Taq*I and others	This paper
DXYS15	113D	Xp22.3, Yp	Many	(40)
DXYS17	601	Xp22.3, Yp	Many	(36)
DXS40	S232	Xp22, Yq	Many (on X)	R. Knowlton, personal communication
DXS143	dic56	Xp22	*Bcl*I	(26)
DXS43	D2	Xp22.1–22.2	*Pvu*II	(1)
DXS9	RC8	Xp22	*Taq*I	(27)
DXS7	L1.28	Xp11.3	*Taq*I	(10)
DXYS1	pDP34	Xq13-q21, Yp	*Taq*I (on X)	(31, 32)
DXS52	St14-1	Xq28	*Taq*I, *Msp*I	(28, 23)

tosomal loci we describe maps very near the short-arm telomere, and it is composed of long tandemly repeated arrays.

MATERIALS AND METHODS

Origin of DNA Hybridization Probes

The DNA probes used are listed in Table 1. Probes pDP230 and pDP411a have not been described previously. Both derive from a library of genomic *Hin*dIII restriction fragments prepared from flow-sorted human Y chromosomes. This library, provided by Marvin Van Dilla, was constructed in λ vector Charon 21A at the Biomedical Sciences Division, Lawrence Livermore National Laboratory (Livermore, CA), under the auspices of the National Laboratory Gene Library Project, sponsored by the U.S. Department of Energy. Plasmid pDP230 (locus *DXYS20*) consists of a 2.2-kb genomic *Hin*dIII fragment cloned into the *Hin*dIII site of pUC13. Plasmid pDP411a (locus *DXYS28*) consists of a 1.9-kb genomic *Eco*RI fragment cloned into the *Eco*RI site of pUC13.

DNA Extraction and Gel-Transfer Hybridization

Human genomic DNAs were prepared from Epstein–Barr virus-transformed lymphoblastoid cell lines (Kunkel *et al.*, 1977), digested with restriction endonucleases, electrophoresed on 0.7% agarose gels, and transferred (Southern, 1975) to nylon membrane. Whole plasmids or purified human inserts were labeled with ^{32}P by nick-translation (Rigby *et al.*, 1977) or random-primer synthesis (Feinberg and Vogelstein, 1984) and hybridized overnight to the human genomic DNA transfers at 42°C in 50% formamide, 5× SSC (1× SSC = 0.15 *M* NaCl, 15 m*M* Na citrate, pH 7.4), 1× Denhardt's (0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 20 m*M* $NaPO_4$, pH 6.6, 50 μg/ml denatured salmon sperm DNA, 1% sodium dodecyl sulfate (SDS), and 10% dextran sulfate. Following hybridization, transfer membranes were washed three times for 15 min each at 60°C in 0.1× SSC, 0.1% SDS and exposed at −80°C for 4 h (pDP230) or 2 days (other probes) with X-ray film backed by an intensifying screen.

Linkage Analysis

Estimation of recombination frequencies and tests of gene order and interference were done with the LINKAGE computer programs (Lathrop and Lalouel, 1984). These programs were modified to allow joint analysis of pseudoautosomal loci, the sex phenotype, and strictly X-linked markers. Thus, if locus 1 is pseudoautosomal, locus 2 is the sex phenotype, and locus 3 is an X-linked marker, the modified program will allow the joint estimation of recombination between loci 1 and 2 in male meioses and of recombination between loci 1 and 3 in female meioses. When several pseudoautosomal markers are considered, sex-specific recombination values can be estimated.

Nucleotide Sequencing

*Hin*dIII–*Xba*I fragments of 0.85 and 1.35 kb were purified from the human insert of plasmid pDP230 and subcloned into bacteriophage M13mp18 and M13mp19 vectors using *Escherichia coli* strain JM109 (Yanisch-Perron *et al.*, 1985). Terminal portions of both *Hin*dIII–*Xba*I fragments were sequenced by dideoxy chain termination (Sanger *et al.*, 1977a) using the universal 17-base primer.

In Situ Hybridization

Metaphase chromosome spreads were prepared from phytohemagglutinin-stimulated cultures of

whole blood from a normal male. Plasmid pDP230 was ^{3}H-labeled by nick-translation to a specific activity of 1.5×10^7 cpm/μg DNA. This probe was hybridized to metaphase chromosomes *in situ* (Harper and Saunders, 1981) in 50% formamide, 2× SSC, 10% dextran sulfate for 14 h at 41°C at probe concentrations of 30 to 50 ng/ml. The slides were coated with photographic emulsion, developed after 11 to 25 days of exposure, and stained with Giemsa. Clusters of grains were scored as single hybridization events.

RESULTS

Two Highly Informative Pseudoautosomal RFLPs

We have been constructing a deletion map of the human Y chromosome using DNA hybridization probes (Vergnaud *et al.*, 1986; Page, 1986). As part of this effort, we have analyzed 100 clones from a library of genomic *Hin*dIII restriction fragments prepared from flow-sorted human Y chromosomes. Fifteen of these clones hybridize to both the X and Y chromosomes. Thirteen of these fifteen X–Y clones detect male-specific restriction fragments on genomic blots, indicating that the homologous loci on the X and Y chromosomes do not recombine with each other (D. C. Page, unpublished results). Most of these sequences belong to the class of X–Y homologous loci typified by *DXYS1*, which are the result of a transposition from the X to the Y during human evolution (Page *et al.*, 1984). In contrast, two of the X–Y clones, plasmids pDP230 and pDP411a, detect RFLPs with no male-specific fragments, suggesting that they might be subject to X–Y recombination.

When hybridized to human genomic DNAs digested with any of a large number of restriction endonucleases, probe pDP230 detects a remarkably polymorphic family of sequences. As shown in Fig. 1, when genomic DNAs were digested with *Taq*I, not only the lengths but also the number of hybridizing restriction fragments varies dramatically from individual to individual. Following *Taq*I digestion, the number of distinct hybridizing fragments varies from 3 in some individuals to 15 or more in others.

In contrast, probe pDP411a detects a more conventional four-allele RFLP when hybridized to human genomic DNAs digested with *Taq*I (Fig. 1). In addition, when hybridized to genomic DNAs digested with, for example *Eco*RI, *Hin*dIII, or *Msp*I, pDP411a detects an insertion/deletion RFLP with at least three alleles (not shown).

Genetic Linkage Analysis

To investigate the inheritance of these RFLPs, probes pDP230 and pDP411a were hybridized to restriction endonuclease-digested DNAs from 44 three-generation families with large sibships (White *et al.*, 1985). The structure of a typical family, Kindred 1418, is shown in Fig. 1 together with autoradiograms obtained after hybridization of probes pDP230 and pDP411a to *Taq*I-digested DNAs.

Despite the complexity of the patterns of restriction fragments detected by pDP230, such family studies reveal that all of this polymorphism derives from a single genetic locus. Though there are on average 8 to 10 third-generation children in these families, we have not observed more than four distinct patterns of hybridization among the children of any family. By comparing those four genotypes with the parental and grandparental genotypes in a family, one can readily recognize two alleles in each parent. In Kindred 1418 (Fig. 1), father 1265's "Y" and "X" alleles (from, respectively, his father 1266 and his mother 1267) are schematically represented to the left, and mother 1264's "P" and "M" alleles (from, respectively, her father 1255 and her mother 1254) are schematically represented to the right of the autoradiogram. Though some alleles consist of only one or two fragments (e.g., alleles Y and M in Fig. 1), most alleles are composed of six or more *Taq*I fragments (e.g., alleles X and P in Fig. 1). These results imply that recombination among the restriction fragments detected by pDP230 occurs rarely, if ever. Those restriction fragments must be in physical proximity, at a single chromosomal locus. This contrasts sharply with the hypervariable restriction patterns detected by some minisatellite sequences (Jeffreys *et al.*, 1985), which reflect crosshybridization to related loci scattered throughout the genome.

As for probe pDP411a, the four-allele *Taq*I RFLP and the insertion/deletion RFLP which it detects are in marked linkage disequilibrium (Table 2). Some of the insertion/deletion alleles differ from each other by less than 100 bp, making it difficult to differentiate among alleles with certainty. In the three-generation families, which were typed for the insertion/deletion RFLP on *Eco*RI digests, we simply scored the alleles as either 1.9 or 2.1 kb. Among either the 1.9- or 2.1-kb alleles, there is undoubtedly some heterogeneity. Nonetheless, linkage disequilibrium between the *Taq*I and *Eco*RI (insertion/deletion) RFLPs is readily apparent. The 1.9- and 2.3-kb *Taq*I alleles virtually always occur together with the 1.9-kb *Eco*RI allele, while the 1.3- and 2.1-kb *Taq*I alleles nearly always occur together with the 2.1-kb *Eco*RI allele. Four of the eight possible haplotypes account for 258 of 259 chromosomes studied (Table 2).

These family studies revealed that the RFLPs detected by pDP230 (locus *DXYS20*) and pDP411a (locus *DXYS28*) are not inherited in a strictly sex-linked manner. In the case of both pDP230 and pDP411a in Kindred 1418 (Fig. 1), father 1265 can pass either of his alleles ("X" or "Y") to his daughters, and the allele ("X") that 1265 passed to both of

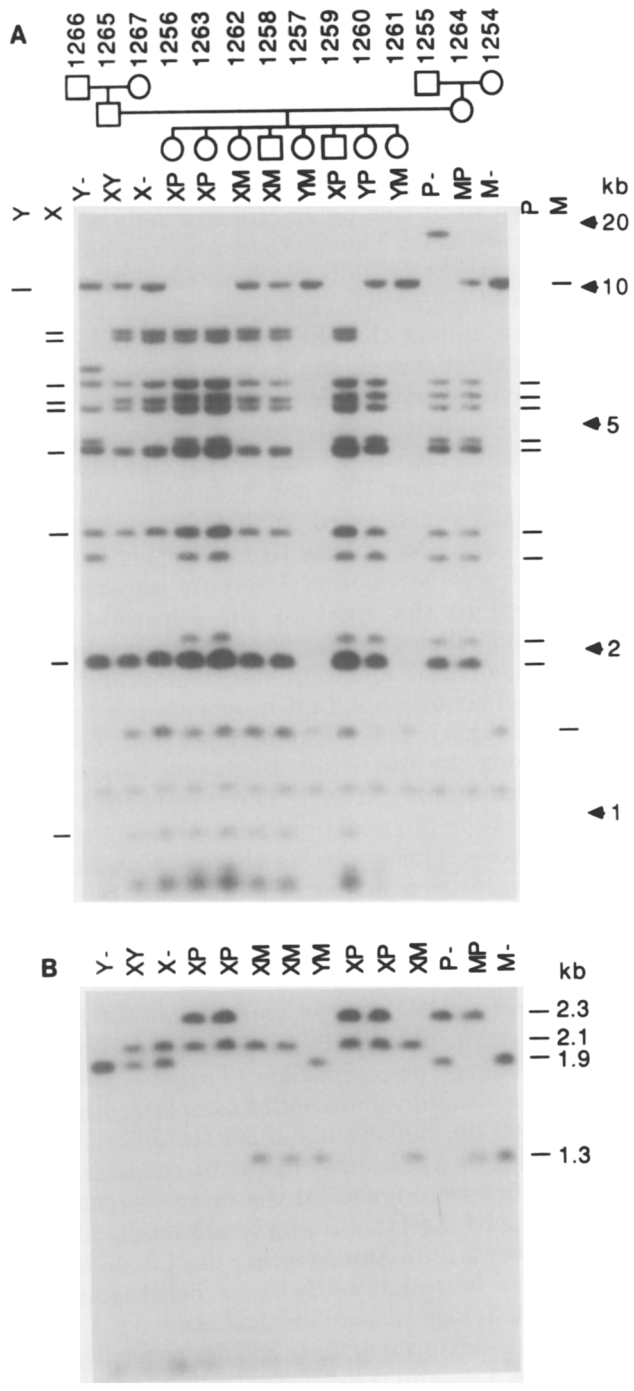

FIG. 1. Pseudoautosomal inheritance in three-generation family K1418 of RFLPs detected by probes (**A**) pDP230 (locus *DXYS20*) and (**B**) pDP411a (locus *DXYS28*). The probes were hybridized to a gel transfer of *Taq*I-digested DNAs. The numbers above the pedigree identify the members of the family. Each lane in the autoradiograms corresponds to the individual above that lane in the pedigree. The letters immediately above the autoradiograms identify the alleles that each individual carries, where "Y" is the allele present on the father's (1265) Y chromosome (inherited from grandfather 1266), "X" is the allele present on the father's X chromosome (inherited from grandmother 1267), "P" is the allele present on the mother's (1264) X chromosome of grandpaternal (1255) origin, and "M" is the allele present on the mother's X chromosome of grandmaternal (1254) origin. To the left and right of autoradiogram A are schematically indicated some of the restriction fragments that comprise the X, Y, P, and M alleles detected by pDP230 in this family.

TABLE 2

***Taq*I/*Eco*RI RFLP Haplotypes at Locus *DXYS28* (Probe pDP411a)**

*Eco*RI fragment	*Taq*I fragment			
	1.9 kb	2.3 kb	1.3 kb	2.1 kb
1.9 kb	156	69	1	0
2.1 kb	0	0	25	8

Note. The numbers of chromosomes found to have various *Taq*I and *Eco*RI fragments are indicated. A total of 259 sex chromosomes were haplotyped.

his sons is that which he received from his mother. As both probes derived from a library made from flow-sorted Y chromosomes, we suspected that the loci detected by pDP230 and pDP411a are "pseudoautosomal." That is, we suspected that those loci are present on both the X and Y chromosomes and are subject to X–Y recombination during normal male meiosis. However, we could not yet exclude the possibility that these DNA sequences are of true autosomal origin.

To distinguish autosomal from pseudoautosomal transmission, we examined the inheritance of the pDP230 RFLP in seven females with a 45,X karyotype (i.e., having a single X chromosome) and their parents. To determine in each case whether the single X chromosome was of maternal or paternal origin, these seven families were also typed for two or more strictly X-linked RFLPs. These strictly X-linked RFLPs revealed the parental origin of the X chromosome in each of the seven 45,X females (Table 3). In families 1, 3, and 5, the X chromosome is of paternal origin. In families 2, 4, 6, and 7, the X chromosome is of maternal origin. (These findings are broadly consistent with estimates, that, in about one-fourth of liveborn individuals with monosomy X, the X chromosome is of paternal origin; Sanger *et al.*, 1977b.)

If the pDP230 RFLP is pseudoautosomal, then a 45,X female should inherit an allele from the parent who contributed the X chromosome, but should not inherit an allele from the other parent, who did not contribute a sex chromosome. If, instead, the pDP230 RFLP is truly autosomal, then a 45,X female should inherit alleles from both parents. In all six 45,X families informative for the pDP230 polymorphism, the results were as predicted for a pseudoautosomal marker (Table 3 and Fig. 2). In each of these six families, the 45,X daughter inherited pDP230-hybridizing fragments from the parent who, as determined using strictly X-linked RFLPs, contributed the X chromo-

TABLE 3

Transmission of *DXYS20* and Strictly X-Linked RFLPs to 45,X Individuals

Family	Person	Relation	DNA probe (locus) pDP230[a] (*DXYS20*)	S232[a] (*DXYS40*)	L1.28[b] (*DXS7*)	pDP34[b] (*DXYS1*)	St14-l[a] (*DXS52*)
1	LGL263	Father			10	11	
	LGL262	Mother			13	11	
	LGL261	45,X female	Paternal	Paternal	10	11	Paternal
2	LGL266	Father			13	12	—
	LGL265	Mother			10, 13	11	—
	LGL264	45,X female	Maternal	Maternal	13	11	—
3	LGL272	Father			13	12	
	LGL270	Mother			13	11, 12	
	LGL271	45,X female	Paternal[c]	Paternal	13	12	Paternal
4	LGL259	Father			—[d]	11	
	LGL258	Mother			—	12	
	LGL257	45,X female	Uninformative	Maternal	—	12	Maternal
5	LGL277	Father			—	11	—
	LGL276	Mother			—	11	—
	LGL275	45,X female	Paternal	Paternal	—	11	—
6	LGL284	Father			—	12	—
	LGL285	Mother			—	11	—
	LGL283	45,X female	Maternal	Maternal	—	11	—
7	LGL252	Father			—	11	—
	LGL251	Mother			—	11	—
	LGL256	45,X fetus	Maternal	Maternal	—	11	—

Note. Each family included an individual with a 45,X karyotype as determined by chromosome banding. All RFLPs were detected on transfers of *Taq*I-digested genomic DNAs.

[a] In the case of probes pDP230, S232, and St14-l, for which most RFLP alleles consist of many restriction fragments, it is indicated only whether the offspring inherited allelic fragments of paternal origin, maternal origin, or both.

[b] In the case of probes L1.28 and pDP34, the lengths in kilobase pairs of the X-linked allelic fragments are given. Probe pDP34 detects a Y-specific 15-kb *Taq*I fragment in addition to the X-linked allelic fragments indicated.

[c] In family 3, the 45,X daughter received an allele at *DXYS20* from her father, but a maternal contribution at the locus cannot be excluded.

[d] Not tested.

some. In none of these families did the 45,X daughter inherit pDP230-hybridizing fragments from the other parent. Such X-linked transmission to 45,X females demonstrates that this polymorphic locus, *DXYS20,* is on the sex chromosomes. Results such as those shown in Fig. 2 suggest that all restriction fragments detected by pDP230 derive from the sex chromosomes. Similarly, the RFLPs detected by pDP411a (*DXYS28*) were transmitted to 45,X females in X-linked fashion (not shown). These 45,X transmission data, together with the fact that transmission to 46,XX and 46,XY individuals is not strictly sex-linked, formally demonstrate that loci *DXYS20* and *DXYS28* are pseudoautosomal.

Genetic Linkage in Male Meiosis

We then analyzed the inheritance of the pDP230 (*DXYS20*) and pDP411a (*DXYS28*) RFLPs in the large three-generation families in a search for genetic linkages. We first examined their inheritance in male meiosis, where an X and a Y are paired. In pseudoautosomal linkage studies, the sex phenotype—male or female—serves as a centromere-linked marker that is always informative in male meiosis. In two-point analysis of male meioses, we observed about 10% recombination between *DXYS20* and *DXYS28* (14 recombinants in 143 phase-known meioses) and about 38% recombination between *DXYS28* and the sex phenotype (68 recombinants in 179 phase-known meioses). However, no linkage was detectable between *DXYS20* and the sex phenotype (146 recombinants in 300 phase-known meioses). Three-point data strongly suggested the order *DXYS20–DXYS28*–sex, in which case no double recombinants were observed; the order *DXYS28–DXYS20*–sex would require 14 double recombinants, while the order *DXYS20*–sex–*DXYS28* would require 52 double recombinants (first line in Table 4). Examining the eight phase-known, triply informative male meioses in Kindred 1418 (Fig. 1), for example, one observes two recombinants between *DXYS20* and *DXYS28* (1260 and 1261), three

TABLE 4

Three Point Crosses among Genetic Markers on the X and Y Chromosomes in Male Meiosis

Locus A	Locus B	Locus C	Number of double recombinants assuming order		
			A–B–C	A–C–B	B–A–C
DXYS20	*DXYS28*	Sex	0	52	14
(*DXYS14–DXYS20*)	*DXYS28*	*DXYS15*	0	0	2
(*DXYS14–DXYS20*)	*DXYS15*	*DXYS17*	0	2	1
(*DXYS14–DXYS20*)	*DXYS15*	Sex	0	9	5
(*DXYS14–DXYS20*)	*DXYS17*	Sex	0	3	9
DXYS28	*DXYS15*	Sex	0	3	0
DXYS15	*DXYS17*	Sex	0	3	2

recombinants between *DXYS28* and sex phenotype (1258, 1257, and 1259), three nonrecombinants (1256, 1263, and 1262), and no double recombinants.

The finding, among 143 triply informative male meioses, of 66 single but no double recombinants suggested that recombination among these markers might be subject to marked interference. (If there were no interference, one would have expected about 6 or 7 double recombinants among these 143 meioses.) We tested for interference, assuming the order *DXYS20–DXYS28*–sex, and found the evidence for it statistically significant (χ^2 of 16.9 with 1 degree of freedom). One can describe the degree of interference in terms of a coefficient of coincidence, defined as the ratio of observed to expected double recombinants; a coincidence of 0 indicates complete interference, and a coincidence of 1 indicates the absence of interference. In the case at hand, as no double recombinants were observed, the best estimate of coincidence is 0. The upper bound to this estimate of coincidence, defined as the coincidence for which the likelihood of the resulting map is reduced by 1 lod unit, is 0.45. Assuming complete interference and the order *DXYS20–DXYS28*–sex, we estimate 10.2% recombination between *DXYS20* and *DXYS28*, 35.7% recombination between *DXYS28* and sex, and, therefore, 45.9% recombination between *DXYS20* and sex.

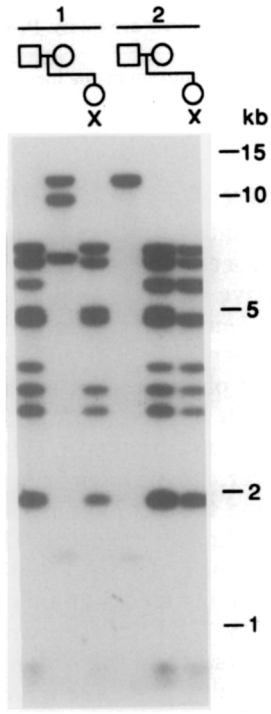

FIG. 2. X-linked transmission of *DXYS20* RFLP to 45,X females. Probe pDP230 was hybridized to a gel transfer of *Taq*I-digested DNAs from families 1 and 2, each of which includes a daughter with a 45,X karyotype.

Five of the three-generation families we studied (K1332, K1346, K1413, K1418, K1421) had previously been typed for RFLPs at the pseudoautosomal loci *DXYS14*, *DXYS15*, and *DXYS17* (Rouyer *et al.*, 1986). Comparing these published results with our *DXYS20* and *DXYS28* data, we set out to construct a map of five pseudoautosomal loci (and sex phenotype) by means of multipoint linkage analysis. The most likely order among these loci is shown at the right in Fig. 3. This order accounts for each meiotic product on the basis of no more than one pseudoautosomal crossover; inclusion of the data for *DXYS14*, *DXYS15*, and *DXYS17* does not reveal any double recombinants. (The results of all informative three-point crosses are shown in Table 4.) Cooke *et al.* (1985) have demonstrated that locus *DXYS14* is within 20 kb of the short-arm telomeres of the X and Y chromosomes. It is of note, then, that we did not observe any recombination between *DXYS20* and *DXYS14* in 32 informative male meioses. This close genetic linkage suggests that *DXYS20* is also near the short-arm telomeres. The sex phenotype serves as a centromere-linked marker in male meiosis, defining the proximal boundary of the pseudoautosomal region. The map of the X and Y in male meiosis shown at the right in Fig. 3 was derived using the LINKAGE computer programs (Lathrop *et al.*, 1984), where the

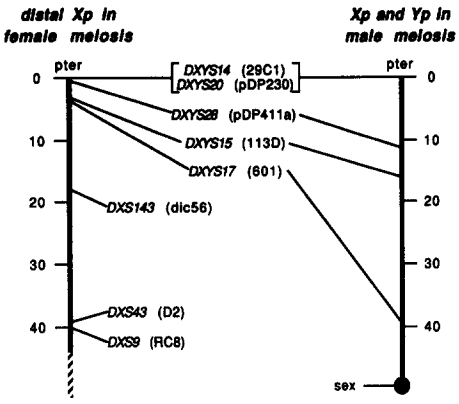

FIG. 3. Comparative genetic linkage maps of (**left**) the distal short arm of the X chromosome in female meiosis and (**right**) the distal short arms of the X and Y chromosomes in male meiosis. Genetic distances are given in centimorgans. The names of the polymorphic DNA loci are italicized, followed in parentheses by the names of the corresponding probes. The positions of five pseudoautosomal loci (*DXYS14, DXYS20, DXYS28, DXYS15, DXYS17*) are shown on both maps. The positions of three strictly X-linked loci (*DXS143, DXS43, DXS9*) are shown on the map of distal Xp in female meiosis. The sex phenotype (male or female) serves as a centromere-linked marker on the map of Xp and Yp in male meiosis.

estimates have been obtained from joint analysis of all loci assuming complete interference. Taking the "telomeric" complex locus (*DXYS14,DXYS20*) as the point of reference, then, moving toward the centromere, *DXYS28* is at 11.4 cM, *DXYS15* is at 16.0 cM, *DXYS17* is at 39.5 cM, and the sex phenotype is at 49.4 cM. If interference is not complete, then the distances in centimorgans are actually somewhat greater.

Genetic Linkage in Female Meiosis

We next analyzed the inheritance of *DXYS20* and *DXYS28* in female meiosis, again in the large three-generation pedigrees. Strikingly, no recombination was detected between these pseudoautosomal loci in 130 informative, phase-known female meioses. In Kindred 1418, for example, mother 1264's alleles ("P" and "M") at the *DXYS20* and *DXYS28* RFLPs cosegregate in all eight phase-known meioses (Fig. 1). These findings differ markedly from the 11% recombination observed between these markers in male meiosis. These results corroborate the finding of Rouyer *et al.* (1986) that recombination among pseudoautosomal markers is much greater in male than in female meiosis.

We then looked for evidence of genetic linkage, in female meiosis, between pseudoautosomal loci *DXYS20* and *DXYS28* and strictly X-linked RFLPs on Xp. Several of the same three-generation families had previously been typed for numerous strictly X-linked RFLPs, from which a genetic linkage map of the X chromosome was constructed by multipoint analysis (Drayna and White, 1985). Among the X-linked RFLPs previously typed were *DXS143* (probe dic56), *DXS43* (probe D2), and *DXS9* (probe RC8), all of which map to Xp22, the most distal band on the X short arm. Multipoint analysis of *DXYS20, DXYS28, DXS143, DXS43,* and *DXS9* yielded the female meiotic linkage map shown at the left in Fig. 3. Notably, linkage was readily detected between the pseudoautosomal markers *DXYS20* and *DXYS28* and the strictly X-linked Xp22 RFLPs. Among those strictly X-linked Xp22 RFLPs, we confirmed the order and approximate genetic distances among *DXS143, DXS43,* and *DXS9* previously reported by Drayna and White (1985). Multipoint analysis suggests that, relative to *DXYS20* and *DXYS28* (which showed no recombinants) and moving toward the centromere, *DXS143* is at 18 cM, *DXS43* is at 39 cM, and *DXS9* is at 40 cM (Fig. 3). The female meiotic map shown at the left in Fig. 3. also includes three previously reported pseudoautosomal loci, *DXYS14, DXYS15,* and *DXYS17.* The approximate positions of these loci on the female map are based on the findings of Rouyer *et al.* (1986); we did not reanalyze their linkage data.

This genetic linkage analysis unambiguously maps the pseudoautosomal sequences distal to the strictly sex-linked portion of the short arm of the X chromosome. These studies extend the known linkage map of the X chromosome (Drayna and White, 1985) to its short-arm telomere.

We observed approximately 17% recombination between *DXYS20* and *DXYS28,* on the one hand, and *DXS143,* a strictly X-linked locus, on the other. About 15% recombination has also been observed between *DXYS20* and a strictly X-linked RFLP at *DXYS40,* which itself is tightly linked to *DXS143* (R. Knowlton, personal communication). Given that *DXYS20* maps very near the short-arm telomeres of the sex chromosomes, these findings set an upper limit to the genetic length of the pseudoautosomal region in female meiosis. The finding of about 4% recombination between *DXYS14* and *DXYS17* in female meiosis (Rouyer *et al.,* 1986) sets an approximate lower limit to this estimate. Therefore, the pseudoautosomal region is at least 4 cM but no more than 18 cM in length in female meiosis. Moreover, these studies demonstrate that there is far less recombination across the entirety of the pseudoautosomal domain in female meiosis than in male meiosis. Rouyer *et al.* (1986) demonstrated that recombination among certain pseudoautosomal loci was markedly reduced in female compared with male meiosis. Those studies, however, could not exclude the possibility that the pseudoautosomal region as a whole is equally recombinogenic in male and female meiosis, with recombination in male meiosis tending to occur more

distally in the pseudoautosomal region, and recombination in female meiosis tending to occur more proximally. There may be considerable nonlinearity of female and male linkage maps for other human chromosomes (see discussion in Leppert *et al.*, 1986). The analysis reported here suggests that in only a minority of female meioses do the X chromosomes recombine within their pseudoautosomal regions.

Physical Mapping of DXYS20 (pDP230) and DXYS28 (pDP411a)

As described above, genetic linkage analysis of RFLPs at *DXYS20* and *DXYS28* demonstrated that (1) both loci map to the X and Y chromosomes, (2) on the X chromosome, both loci map distal to strictly X-linked RFLPs previously assigned to Xp22, and (3) both loci are closely linked to pseudoautosomal loci previously assigned to Xp22 and Yp by physical methods (Cooke *et al.*, 1985; Simmler *et al.*, 1985). These conclusions, based on linkage mapping, were confirmed and refined by physical mapping studies.

First, probes pDP230 and pDP411a were hybridized to Southern transfers of DNAs from human–rodent somatic cell hybrids retaining X or Y chromosomes. At moderate stringencies, both probes hybridized little, if at all, to rodent DNAs. When hybridized to *Taq*I-digested DNAs prepared from hybrids 3E7 and 853, in which the Y is the only cytogenetically detectable human chromosome (Marcus *et al.*, 1976; P. Goodfellow, personal communication), both probes detect fragments of the same length as those seen in normal human males or females. Similar results were obtained using *Taq*I-digested DNAs prepared from hybrids AHA-11aB1 and t60-12, in which the X is the only human chromosome detectable cytogenetically (Dorman *et al.*, 1978; H. Willard, personal communication). These results confirm the localization of *DXYS20* and *DXYS28* to the X and Y chromosomes.

Probes pDP230 and pDP411a were then tested on hybrids carrying deleted human sex chromosomes. The results map both probes to the most distal portions of Xp and Yp. Neither probe produced a hybridization signal with either hybrid 85-13/8, which contains a terminally deleted X, Xqter–Xp22.3 (Curry *et al.*, 1984), or hybrid 97-1/5, which contains an X–Y translocation product, Xqter–Xp22.3::Yp11–Yqter (Bernstein *et al.*, 1978; Bernstein, 1985; Geller *et al.*, 1986). Similarly, no hybridization was observed with either probe with hybrid Y.iso1T14, which contains an isochromosome Y, Yqter–Yp11::Yp11–Yqter (M. Rocchi, personal communication). Both of the last two hybrids, 97-1/5 and Y.iso1T14, contain all Y-specific DNA sequences for which we have tested by hybridization (unpublished results).

Finally, by *in situ* hybridization to metaphase chromosomes of a normal male, we mapped locus *DXYS20* (pDP230) to Xp22.3 and Yp11.3, the most distal bands on the short arms of the X and Y (Fig. 4). Of 71 male metaphase spreads analyzed, 36 showed labeling of band Yp11.3, and 20 showed labeling of band Xp22.3. Of a total of 478 autoradiographic grains on all chromosomes examined, 36 grains were found at band Yp11.3, and 22 grains were found at band Xp22.3. There was no significant labeling of any other site on the sex chromosomes or of any autosome. Band Yp11.3 was more heavily labeled than was band Xp22.3. This may be because this normal male has different *DXYS20* alleles on his X and Y chromosomes, such that there are more copies of the pDP230 repeat on his Y than on his X chromosome.

Nature of DNA Polymorphism at DXYS20 (pDP230)

Probe pDP230 detects unusually polymorphic and complex patterns of restriction fragments at *DXYS20*. Accordingly, we set out to explore the basis of the variability at this locus. Several observations suggested that the human insert of plasmid pDP230 derives from a region of tandemly repeated DNA sequences. First, while the insert of pDP230 is only 2.2 kb in length, it detects in genomic DNA a multitude of restriction fragments, many of comparable or greater length (Figs. 1 and 2). Since these hybridizing fragments are transmitted as a single genetic locus, they must be tightly clustered at one chromosomal location. Second, the highly complex pattern of hybridization to human genomic DNA is unaffected by substantial increases or decreases in the stringency of hybridization and washing (not shown). This suggested that the repetitive sequences detected by pDP230 are all very highly related to one another, but are not closely related to any other sequences in the genome. Third, when the human insert of pDP230 was divided into 0.85- and 1.35-kb portions by digestion with *Xba*I (Fig. 5A) and the resulting fragments were subcloned and hybridized to human genomic DNA, the same highly complex pattern was observed (not shown). This demonstrated that the human insert of pDP230 is itself internally redundant. For these reasons, we determined the nucleotide sequence of much of the human insert of plasmid pDP230.

Four portions of pDP230 totaling 1138 nucleotides were sequenced (Figs. 5A and 5B). All four stretches were found to consist entirely of tandem repeats of essentially the same 61-base unit. As shown in Fig. 5B, the individual 61-base monomers do differ from one another, primarily by base substitutions. However, a consensus sequence is readily apparent, with only about 5% of the nucleotides diverging, apparently at random, from that consensus. The consensus sequence has a GC content of 49%. Given that we sequenced only a single strand of portions of the human insert of pDP230, detailed statistical analysis of the base-pair

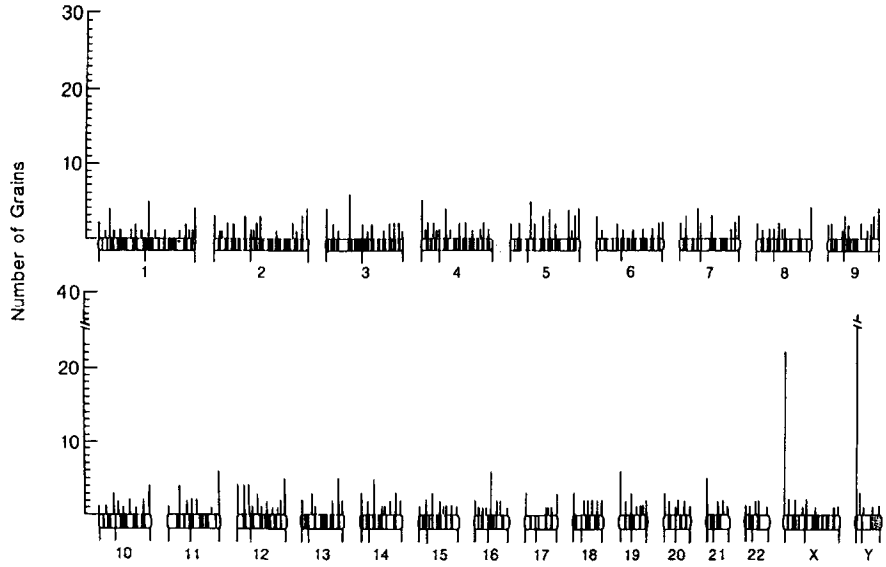

FIG. 4. *In situ* hybridization of probe pDP230 to metaphase chromosomes of a 46,XY male. This histogram summarizes the localization of grains on the chromosomes.

substitutions by which the actual sequence of pDP230 differs from the consensus seems premature. We do note that, of the six possible types of substitutions, three (the two transitions, A:T to G:C and G:C to A:T, and the transversion C:G to G:C) account for 44 of the observed 61 base-pair deviations from the consensus. The consensus sequence does not closely resemble any other satellite sequence of which we are aware.

When gel transfers of human genomic DNAs digested with a variety of restriction endonucleases are probed with pDP230, the patterns of hybridization fall into two classes (Fig. 6). On this basis, restriction enzymes can be divided into what we will call, for purposes of discussion, Classes A and B. Many restriction enzymes, including *Bam*HI, *Eco*RI, *Pvu*II, *Sac*I, and *Bcl*I, yield one to three hybridizing bands, 10 kb or more in length, in most individuals. In many individuals (e.g., 1265 and GM1416), these "Class A" enzymes yield one or more bands at least 20 to 30 kb in length (field-inversion gel electrophoresis autoradiograms not shown).

In contrast, other restriction enzymes, including *Alu*I, *Taq*I, *Hin*dIII, *Xba*I, and *Bgl*II, produce five or more hybridizing bands, generally 0.5 to 10 kb in length, in most individuals (Fig. 6). In many individuals (e.g., 1265), these "Class B" enzymes yield a dozen or more bands, most less than 10 kb.

Comparison of the recognition sequences of the various restriction endonucleases tested with the 61-bp consensus sequence offers a possible explanation for this dichotomy. In all, 19 restriction enzymes were examined (listed in Fig. 5C). Of these 19 enzymes, only *Hae*III cuts the consensus sequence. *Hae*III is also the only enzyme that produces no signal when conventional gel transfers of digested human genomic DNAs are hybridized with pDP230 (not shown). The pDP230-hybridizing sequences are cut by *Hae*III into such small fragments that they would run off the end of gels such as those shown in Fig. 6.

Of the remaining 18 enzymes, none cuts within the 61-bp consensus repeat. However, 10 of these enzymes have what we refer to as "potential sites" within the consensus. That is, the recognition sequence for each of these 10 enzymes differs from the consensus by a single base-pair substitution. These "potential sites" and the base-pair substitutions required to actualize them are shown in Fig. 5C. While only 2 of 9 Class A enzymes (listed above the consensus in Fig. 5C) have potential sites, 8 of 9 Class B enzymes (listed below *Hae*III in Fig. 5C) have such potential sites within the consensus.

These results suggest the following model: the 61-bp repeats occur in largely uninterrupted, tandem arrays, tens of kilobases in length. That is, the entirety of the human insert of pDP230—and a substantial block of adjacent sequence in the genome—is composed of tandem arrays of these 61-bp repeats. The number of copies of the repeat (and hence the length of the array) is very polymorphic in the population. Class A enzymes cut outside but rarely cut within these arrays; they detect what appear to be "insertion–deletion" polymorphisms (Wyman and White, 1980) in large restriction fragments. Class B enzymes yield a greater number of smaller pDP230-hybridizing fragments because scattered base-pair substitutions turn potential sites within the array into

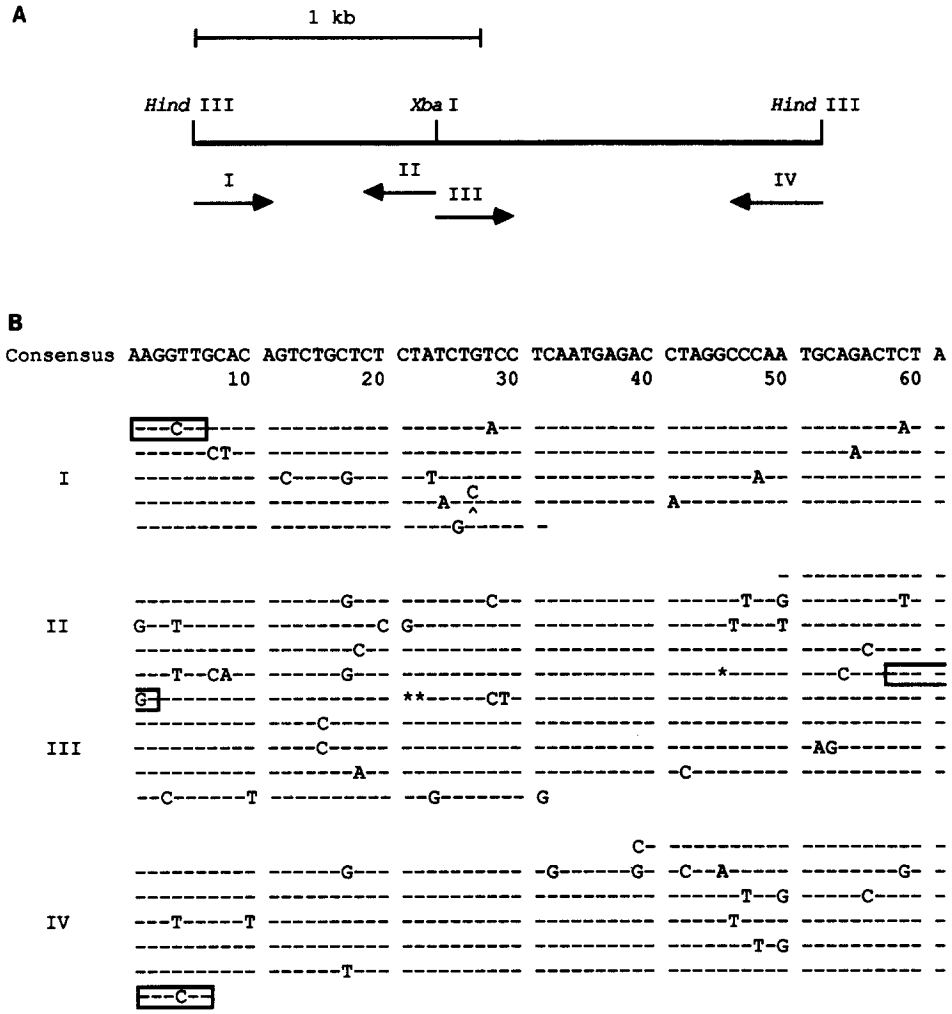

FIG. 5. Tandem arrays of 61-bp repeats at locus *DXYS20*. (**A**) Restriction endonuclease map and sequencing strategy for the human insert of plasmid pDP230. (**B**) Nucleotide sequence of portions of the human insert of pDP230. The nucleotide sequence of each of the regions examined (I, II, III, and IV) consists of tandemly arranged, imperfect 61-bp repeats. The sequences of I, II, III, and IV are shown below and related to the consensus of these 61-bp repeats. Identity with the consensus is indicated by a dash. Bases that differ from the consensus are given. Deletions with respect to the consensus are indicated by asterisks. There is one insertion with respect to the consensus—a cytosine inserted between positions 26 and 27 in the fourth repeat of I. The two *Hin*dIII sites and the single *Xba*I site are enclosed in boxes. (**C**) "Potential" restriction sites within the 61-bp consensus repeat at *DXYS20*. *Hae*III is the only enzyme among these 19 whose recognition sequence occurs within the consensus repeat unit. "Potential sites" (see text) for other restriction enzymes are indicated by dashes; base-pair substitutions required to realize those potential sites are indicated. The potential *Hin*dIII and *Xba*I sites were realized in the portion of *DXYS20* represented by pDP230 (B).

actual sites. As predicted by this model, the actual *Hin*dIII and *Xba*I sites within pDP230 occur at potential sites (Fig. 5).

Comparison of hybridizations obtained with the Class B enzymes *Alu*I and *Hin*dIII suggests that not all potential sites are realized at equal frequencies. In the consensus repeat, there are four potential *Alu*I sites, one of which coincides with the single potential *Hin*dIII site (Fig. 5C). In each of the three unrelated individuals examined, the hybridization patterns are strikingly similar with *Alu*I and *Hin*dIII (Fig. 6). In particular, there is a ladder of fragments (with an approximately 120-bp spacing) between 1 and 2 kb observed in either *Alu*I or *Hin*dIII digests of individuals 1265 and GM1416. These findings suggest that, of the four potential *Alu*I sites, the one most frequently realized is that which coincides with the potential *Hin*dIII site.

DISCUSSION

The meiotic behavior of the pseudoautosomal regions of the human X and Y chromosomes is most unusual. It is estimated that, in humans, a genetic distance of one centimorgan typically corresponds to a physical distance of one megabase. The short arm of the Y chromosome is, judging by its cytogenetic appearance, perhaps 10 to 15 megabases in length. Since

C

```
Bam HI   GGATCC
Eco RI   GAATTC
Pvu II   CAGCTG
Sac I    GAGCTC
Bcl I    TGATCA
Apa I    GGGCCC                                          G-----
Eco RV   GATATC
Kpn I    GGTACC
Pst I    CTGCAG                                                  C-----

Consensus        AAGGTTGCACAGTCTGCTCTCTATCTGTCCTCAATGAGACCTAGGCCCAATGCAGACTCTA

Hae III  GGCC                                  -G--       -G--  ----

Alu I    AGCT    --C-          A---    -G--              -G--
Taq I    TCGA                       --G-       --G-                          --G-
Hind III AAGCTT  ---C--
Xba I    TCTAGA  G-                                                          ----
Bgl II   AGATCT                                        ---T--
Dra I    TTTAAA  --                                                          -T--
Mbo I    GATC                  -A--  G---              --T-
Msp I    CCGG
Rsa I    GTAC          -T--
```

FIG. 5—*Continued.*

the vast majority of DNA sequences derived from Yp are strictly Y-linked, not pseudoautosomal (Simmler *et al.*, 1985; D. C. Page, unpublished results), the pseudoautosomal region is likely no more than several megabases in length. Given these considerations, the roughly 50% rate of recombination across the region in male meiosis (Rouyer *et al.*, 1986; this paper) is remarkably high. Moreover, in male meiosis, the human pseudoautosomal region is subject to a degree of meiotic interference (coefficient of coincidence 0, with confidence limit of 0.45) that is unparalleled, to our knowledge, in mammalian genetics. In this regard, the behavior of the human pseudoautosomal region contrasts sharply with the behavior of the mouse pseudoautosomal region, which does not appear to be subject to marked interference in male meiosis (Soriano *et al.*, 1987). Finally, the rate of recombination across the entirety of the human pseudoautosomal region is at least 3-fold and perhaps 10-fold greater between an X and a Y in male meiosis than between two X chromosomes in female meiosis (Rouyer *et al.*, 1986; this paper). In mouse, too, pseudoautosomal recombination is much more frequent in male than in female meiosis (Soriano *et al.*, 1987).

It has been argued that the pseudoautosomal region of the mammalian sex chromosomes undergoes, in male meiosis, a "single, obligatory" X–Y recombination (Burgoyne, 1982; Rouyer *et al.*, 1986). According to this model, in every normal male meiosis (at least in those giving rise to viable gametes) there is one crossover between the X and Y chromosomes. The model predicts (1) 50% recombination in male meiosis between genetic markers at either end of the pseudoautosomal region and (2) no double (or triple) recombinants in the pseudoautosomal region. However, nothing short of meiotic tetrad analysis would provide a full test of the model. Our findings as to the inheritance of pseudoautosomal RFLPs in male meiosis are consistent with this hypothesis. It should be emphasized, however, that neither our data nor previously published data (Rouyer *et al.*, 1986) constitute proof of such a single, obligatory crossover. The pseudoautosomal region has a genetic length of approximately 50 cM in male meiosis. This implies that most male meioses do involve one X–Y recombination event, but it is entirely consistent with some male meioses having zero or two X–Y recombination events. As predicted by the single, obligatory crossover model, there is marked recombinational interference in the pseudoautosomal region in male meiosis. However, this does not exclude the possibility that, in some normal human male meioses, the X and Y chromosomes undergo two recombination events. One should note that, in the pseudoautosomal region of the mouse sex chromosomes, double recombination events do occur during male meiosis (Soriano *et al.*, 1987).

The coexistence of highly informative pseudoautosomal RFLPs, marked pseudoautosomal interference in male meiosis, and limited pseudoautosomal recombination in female meiosis is of practical importance in human gene mapping. Many traits appear to be inherited in autosomal fashion but have not been assigned to an autosomal linkage group (McKusick, 1986). It should be borne in mind that a few such traits may actually be pseudoautosomal. Our findings suggest that linkage analysis using a very small num-

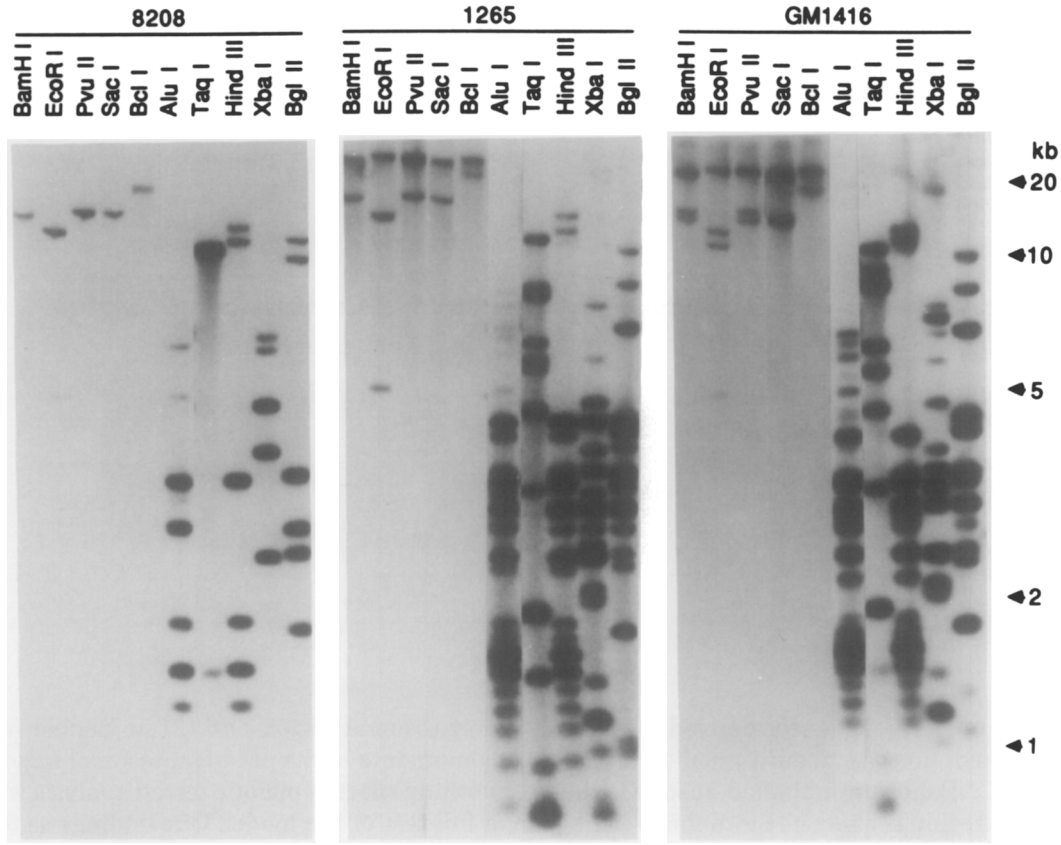

FIG. 6. Hybridization of pDP230 to genomic DNAs of three individuals cut with various restriction endonucleases. 8208 is a 46,XX female, 1265 is a 46,XY male, and GM1416 is a 48,XXXX female. 8208, 1265, and GM1416 were run on separate gels; accordingly, the sizes in kb are approximate.

ber of pseudoautosomal and X-linked RFLPs should establish whether a given trait is pseudoautosomal or truly autosomal. About 50% of male gametes are nonrecombinant for the pseudoautosomal region; the entire region is inherited as a block. In these nonrecombinant male gametes, the inheritance of the entire pseudoautosomal domain can be followed with two genetic markers, one at the centromere-proximal end and one near the telomere. The sex phenotype provides a universally informative centromere-linked marker, and *DXYS14* and *DXYS20* provide highly informative RFLPs near the telomere. To obtain information from the roughly 50% of male gametes which are single recombinants requires the addition of a third marker between the centromere- and telomere-linked markers. In female meiosis, given the limited recombination distance across the pseudoautosomal region, one can use highly informative pseudoautosomal RFLPs (with or without strictly X-linked Xp22 RFLPs) to monitor the inheritance of the entire region with reasonable accuracy. Indeed, in the case of epidermolysis bullosa simplex, in which partially sex-linked inheritance had been suspected, a pseudoautosomal location was excluded by the inheritance of the *DXYS20* RFLP and the sex phenotype in one large pedigree (D. C. Page and T. Gedde-Dahl, unpublished results).

Pseudoautosomal RFLPs will also be useful in linkage studies of strictly X-linked traits, especially those mapping to band Xp22. Even the most distal pseudoautosomal loci, *DXYS14* and *DXYS20,* both highly polymorphic, are no more than 18 cM and perhaps as little as 4 cM from strictly X-linked loci. Pseudoautosomal RFLPs should figure prominently in any effort to refine the genetic linkage map of distal Xp.

Pseudoautosomal locus *DXYS20* is composed of tandem arrays of a 61-bp repeat unit that appear to extend for tens of kilobases. Scattered base-pair substitutions result in the actual sequence of these repeats diverging from the consensus at, on average, 5% of nucleotides. As we have illustrated, this divergence can be examined directly, by nucleotide sequence analysis, and indirectly, by hybridization ("potential restriction site") analysis. *DXYS20* may serve as a good model system for examining the spectrum of human mutation at the nucleotide level. For example,

the low but significant nucleotide divergence from the consensus at *DXYS20* may provide opportunity for quantitative estimates of the relative frequencies of various classes of base-pair substitutions.

Two other pseudoautosomal loci, *DXYS15* and *DXYS17*, are composed of tandem arrays of, respectively, 21- to 29- and 28- to 33-bp repeat units (Simmler *et al.*, 1987). The repeats at *DXYS15* and *DXYS17* are not similar to each other, nor is either similar to the repeat reported here at *DXYS20*. Perhaps the pseudoautosomal region is largely made up of a series of distinct tandem arrays, no two alike, with an array of one repeat unit followed by an array of a quite different repeat unit. Such a series of satellite-DNA loci, each with its own distinctive repeat unit, might be responsible for the peculiar properties of the pseudoautosomal region in male meiosis. This series of satellite loci may play a role in aligning or pairing the pseudoautosomal portions of the X and Y chromosomes in male meiosis. Subsequently, one or more of these satellite-DNA loci may provide a particularly favorable substrate for recombination in male meiosis.

The number of kilobases of DNA hybridizing to pDP230, i.e., the size of the *DXYS20* locus, varies dramatically from sex chromosome to sex chromosome. Preliminary experiments with Southern blots made after field-inversion electrophoresis (Carle *et al.*, 1986) suggest that the size of the locus varies from about 10 to 50 kb. Perhaps other pseudoautosomal satellite-DNA loci are similarly variable in size. If so, the cumulative effect of size variation at several satellite-DNA loci may be significant variation in the size of the pseudoautosomal region among sex chromosomes. Incongruity between the sizes of the pseudoautosomal regions of paired X and Y chromosomes in male meiosis may predispose to aberrant X–Y recombination events, such as give rise to XX males (Petit *et al.*, 1987; Page *et al.*, 1987).

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