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The Parental Origin of X Chromosomes in XX Males Determined Using Restriction Fragment Length Polymorphisms

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SUMMARY

The inheritance of several X-linked restriction fragment length polymorphisms (RFLPs) is examined in seven 46,XX males and their immediate relatives. The XX males are shown to have inherited a paternal and a maternal RFLP allele in each of the five (of seven) families in which these X-linked markers are informative. In the other two families, a maternal X-chromosomal contribution is demonstrated, but a paternal contribution cannot be determined. We conclude that most, if not all, XX males inherit one paternal and one maternal X chromosome.

A segment of single-copy DNA specific to the short arm of the Y chromosome is found to be absent from the genomes of eight XX males. In one of these XX males, an Xp-Yp translocation had previously been inferred from chromosome-banding studies. Our findings argue against mosaicism involving a Y-containing cell line in the XX males examined here, but they do not exclude an X-Y (or Y-autosome) translocation during paternal meiosis. If such a translocation has occurred, the translocation product received by the XX males does not include the Yp-specific sequence tested here.

INTRODUCTION

The unexpected karyotype 46,XX occurs at a frequency of one per 20,000 males in European populations [1]. Numerous hypotheses have been offered to explain

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this appearance of a male phenotype in the absence of any detectable Y chromosome. Here we examine some of the predictions of two of these hypotheses: X-Y interchange during paternal meiosis and mosaicism involving a Y-containing cell line (e.g., 46,XX/47,XXY). Specifically, we test seven XX males for inheritance of the paternal X chromosome, which is required by the X-Y interchange hypothesis. In addition, we examine DNA from eight XX males for the presence of a Y-specific sequence, which is predicted by both hypotheses.

Parental Origin of X Chromosomes

Previous studies relating to the parental origin of the X chromosomes of XX males have been subject to divergent interpretations. In nine of 13 informative families tested, the XX male was found not to express his father's allele for the dominant, X-linked blood-group marker Xg. In the remaining four families, however, the proband did inherit his father's Xg allele [1]. The frequent failure of XX males to express the paternal Xg allele could be due to: (1) both X chromosomes being of maternal origin, or (2) deletion or nonfunction of the *Xg* locus on the paternal X.

Additional insight into the parental origin of the X chromosomes of XX males is derived from population studies of certain X-linked phenotypes among a large number of probands. *Xg* and color blindness map to widely separated positions on the X chromosome, and they are not genetically linked. The absence of color blindness among 58 XX males ([1] and de la Chapelle, unpublished observations, 1983) resembles the female distribution, and this result suggests that two nonidentical alleles function at this locus. In contrast, the proportion of XX males who are Xg(a+) closely resembles that of XY males, and it is significantly different from that of XX females ([1] and R. Sanger and P. A. Tippett, unpublished observations, 1983). Like the family studies of Xg inheritance cited above, these results are consistent with either: (1) the genetic identity of the two X chromosomes or (2) a single functional *Xg* locus.

The X-Y interchange hypothesis [2] was proposed by Ferguson-Smith to explain the failure of some XX males to express the paternal Xg allele. According to this hypothesis, the paternally derived X chromosome of XX males has acquired a male-determining gene from the Y chromosome as a result of an abnormal X-Y translocation during paternal meiosis. In many XX males, this translocation must also have resulted in the loss of the paternal *Xg* locus. This model requires that one of the X chromosomes (an X-Y translocation product) of XX males is paternal in origin.

Here we examine the parental origin of the X chromosomes of seven XX males by means of X-linked RFLPs. Numerous DNA segments cloned from the human X chromosome have been found to detect restriction fragment length polymorphisms [3]. The discovery of these RFLPs greatly increases the number of useful genetic markers on the X chromosome. These X-linked markers also have two striking qualitative advantages: (1) codominant inheritance, allowing simultaneous determination of paternal and maternal contributions in informative families. With Xg, which is a dominant marker, it is very difficult to demonstrate simultaneously a paternal and a maternal X-chromosomal contribution in any given individual;

(2) a "phenotype" that is not dependent upon gene expression. In contrast with Xg, uncertainties with respect to gene expression do not cloud interpretation of these X-linked RFLP data.

Y-Chromosomal DNA

There is evidence for the presence of a "testis-determining" gene on the short arm of the Y chromosome [4], and it has been hypothesized that XX males carry DNA sequences derived from Yp. The X-Y interchange hypothesis, already described, postulates that one of the X chromosomes of XX males bears a Yp-derived, male-determining gene as the result of an abnormal X-Y translocation during paternal meiosis [2]. Microscopic abnormalities in the distal short arm of one of the X chromosomes of some XX males [5, 6], like the anomalies of Xg inheritance, have been interpreted as evidence of a translocation involving the short arms of the X and Y chromosomes. However, the chromosomes of most XX males appear structurally normal in the light microscope [1, 7]. According to an alternative hypothesis, XX males are undetected mosaics for the presence of a Y chromosome ([8]; reviews in [1] and [9]). The mosaicism hypothesis predicts that DNA from XX males would contain Y-specific sequences, but in a reduced amount.

Human DNA segment *DXYS1* is a site of single-copy sequence homology between the X and Y chromosomes [10, 11], and a probe for *DXYS1* is an ideal tool for examining the genomes of XX males. Not only is *DXYS1* the site of an X-linked RFLP that is of use in determining the parental origin of X chromosomes, but it is also characterized by a Y-specific 15-kilobase (kb)-pair Taq I restriction fragment. This fragment has been mapped to the short arm of the Y chromosome by in situ hybridization ([11] and D. Page and M. Harper, unpublished observations, 1983). In the experiments reported here, a hybridization probe for *DXYS1* is used to examine the genomes of XX males for the presence of this Yp-specific restriction fragment.

MATERIALS AND METHODS

Individuals Studied

Case descriptions and pedigrees have been published. The XX males tested here are identified as follows:

Family	Proband	Reference
1	GM2626	Case 1 in [12]
2	GM2670	Case 2 in [12]
3	LGL105	Case 7 in [13]
4	LGL115	[14]; first XX male reported
5	LGL163	[15]
6	LGL203	Case 6 in [13]
7	LGL208	Case 5 in [13]
	POR101	Case 1 in [6]

GM2626 and GM2670 are second cousins and are remotely related to LGL115. The other cases are sporadic. In the seven cases where parents and siblings were included in this study, paternity was confirmed using a number of autosomal markers [12–15].

Sex-linked Serological Markers

Typing for the blood group Xg and for the antigen 12E7 was performed at the MRC Blood Group Unit, London (courtesy of R. Sanger and P. A. Tippett). The methods have been described [16, 17], and the results reported [12–15].

Preparation of Human DNA

DNA was prepared from the nuclei of peripheral leukocytes or cultured skin fibroblasts by the method of Kunkel et al. [18].

Origin of Hybridization Probes

The DNA hybridization probes used are as follows:

(1) Plasmid pDP34— ([10, 11] and D. Page and M. Harper, unpublished observations, 1983). A 2.2-kb human genomic Eco RI fragment from recombinant phage λ-rHs4813 was subcloned into plasmid vector pDP322, an abbreviated derivative of plasmid pBR322, by methods previously described [10]. This probe hybridizes to a site of single-copy sequence homology on the X and Y chromosomes, specifically to Xq13-q21 and to Yp. This site of X-Y sequence homology has been designated *DXYS1*. When used as a hybridization probe of genomic human DNAs, pDP34 reveals a Taq I RFLP with X-linked alleles of 11 and 12 kb as well as a Y-specific 15-kb fragment.

(2) Phage RC8—[19]. This probe reveals X-linked Taq I RFLP alleles of 3.0, 3.4, and 5.7 kb in addition to an invariant 7-kb fragment. Only two Taq I alleles have been reported previously using this probe (3.2 and 5.3 kb; [3, 19]). We have detected the 3.0-kb allele only in family 7. We suspect that the allelic fragments we measure as 3.4 and 5.7 kb correspond to the previously reported 3.2- and 5.3-kb alleles, respectively, and that it is the 3.0-kb allele that has not been observed before. RC8 has been mapped to Xp21-p223.

(3) Plasmid pD2—[20]. This probe reveals X-linked Pvu II RFLP alleles of 6.0 and 6.6 kb. Its human insert has been mapped to Xp21-pter.

(4) Plasmid L1.28—[21]. This probe reveals X-linked Taq I RFLP alleles of 10 and 13 kb. Its human insert has been mapped to Xp110-p113.

(5) Phage 19-2—[20]. This probe reveals X-linked Msp I alleles of 4.4 and 12 kb, in addition to an invariant 1.2-kb fragment. Its human insert has been mapped to Xq1-q22.

(6) Phage S21—[22]. This probe reveals X-linked Taq I RFLP alleles of 2.5 and 2.7 kb, in addition to invariant 1.7- and 4.0-kb fragments. Its human insert has been mapped to Xq213-q22.

(7) Plasmid p22-33—[20]. This probe reveals X-linked Taq I RFLP alleles of 10 and 17 kb. Its human insert has been mapped to Xq24-qter.

(8) Plasmid p43-15—[20]. This probe reveals X-linked Bgl II RFLP alleles of 6 and 9 kb. Its human insert has been mapped to Xq24-qter.

The lengths of restriction fragments given above are our estimates and in some cases differ moderately from published values.

Restriction Digestion, Electrophoresis, Transfer, and Hybridization of DNA

Restriction endonuclease digestions of genomic human DNAs were carried out as directed by the manufacturer (New England BioLabs, Beverly, Mass., or Bethesda Research, Gaithersburg, Md.). The reactions were monitored for completion by transfer of an aliquot containing 0.5 μg of human DNA (immediately after addition of enzyme) into 0.5 μg of phage lambda or plasmid pBR322 DNA for parallel digestion. Complete digestion of the phage or plasmid DNA was taken to indicate that the human DNA was also fully digested. (The patterns of hybridization obtained using gel transfers of these human DNAs were also consistent with complete digestion.) Five μg of restriction-digested human DNA in 5% Ficoll 400, 20 mM EDTA, pH 7.5, 0.1% SDS, and 0.02% bromophenol blue were

separated by electrophoresis on horizontal agarose gels (0.75% agarose in 2.5 mM EDTA, 90 mM Tris-borate, pH 8.3).

The DNA was transferred from the gel to Zetapore (AMF, Cuno Division, Meriden, Conn.) filters according to Southern [23], using 20× SSPE (1× SSPE = 0.18 M NaCl, 1 mM EDTA, 10 mM Na PO_4, pH 7.4). Filters were baked for 2 hrs at 80°C in a vacuum oven and then washed for 2 hrs at 65°C in 0.1× SSC (1× SSC = 0.15 M NaCl, 15 mM Na citrate, pH 7.4), 0.1% SDS. They were prehybridized overnight at 47°C in 50% formamide, 5× SSC, 5× Denhardt's solution (1× Denhardt's = 0.02% Ficoll 400, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin), 50 mM Na PO_4, pH 6.6, and 50 μg/ml denatured salmon sperm DNA.

Filters were then hybridized overnight at 47°C in 50% formamide, 5× SSC, 1× Denhardt's, 20 mM Na PO_4, pH 6.6, 50 μg/ml denatured salmon sperm DNA, and 10% dextran sulfate, typically using 2×10^5 cpm of denatured, ^{32}P-labeled probe DNA per ml. Probe DNA was radiolabeled to a specific activity of approximately 2×10^8 cpm/μg by a two-step nick-translation [24] procedure: 0.25 μg of DNA was incubated for 10 min at 14°C in a final volume of 10 μl containing 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 20 mM beta-mercaptoethanol, and 0.01 ng DNAase I. This nicking reaction was terminated by incubating for 5 min at 65°C. dATP, dGTP, and TTP (final concentration 30 μM each) and [^{32}P]dCTP (3,000 Ci/mmol, final concentration 2 μM; Amersham, Arlington Heights, Ill.) were then added, followed by 8 U of DNA polymerase I (New England BioLabs). This reaction was incubated for 90 min at 14°C. Unincorporated nucleotides were removed by Sephadex G-50 gel filtration.

Following hybridization, filters were washed three times for 15 min each at 55°C in 0.1× SSC, 0.1% SDS. The filters were dried and exposed at −70°C for 1–7 days with Kodak XAR-5 film backed by a Lightning-Plus intensifying screen (DuPont, Wilmington, Del.). Before using filters for subsequent hybridizations, they were stripped of probe DNA by washing for 30 min at room temperature in 0.4 N NaOH. Following quick rinses in water and 20 mM Tris-HCl, pH 7.5, filters were prehybridized as described above and hybridized with another probe.

RESULTS

Parental Origin of X Chromosomes

To gain information as to the parental origin of the X chromosomes of the XX males, seven probands and their immediate relatives were typed for as many as eight X-linked RFLPs. The results are shown in table 1. Figure 1 shows two autoradiograms representative of those from which the data in table 1 were derived. All of the probes except RC8 detect polymorphisms with just two alleles. With respect to parental origin of the X chromosomes, a two-allele RFLP is fully informative only if the mother is homozygous for one allele and the father is hemizygous for the second allele. Such is the case, for example, in family 7 when examined with probe pDP34 (fig. 1*A*); the mother, LGL207, is homozygous for the 11-kb allele, while the father, LGL206, is hemizygous for the 12-kb allele. The proband's 11-kb allele is thus of maternal origin and his 12-kb allele of paternal origin. In this manner, a paternal and a maternal X-chromosomal contribution is demonstrated in five of the seven XX male families tested (by probe pDP34 in families 1, 2, and 3; by pDP34 and RC8 in family 7; and by p22-33 in family 6). Probes S21 and p43-15 demonstrate a maternal contribution in family 4. The question of a paternal contribution in family 4 is treated below (see DISCUSSION). In family 5, probes pD2 and L1.28 demonstrate a maternal X-

TABLE 1

SEGREGATION OF RFLPs AND SEROLOGICAL MARKERS

FAMILY	PERSON	RELATION	DNA PROBE (RESTRICTION ENZYME) pDP34 (Taq I)	RC8 (Taq I)	pD2 (Pvu II)	L1.28 (Taq I)	19-2 (Msp I)	S21 (Taq I)	p22-33 (Taq I)	p43-15 (Bgl II)	Xg(a)	12E7
1	GM2672	Father	11,15	...	...	...	...	...	...	...	+	...
	GM2671	Mother ...	12	...	...	...	...	...	...	...	+	...
	GM2626	XX male ..	11, 12	...	...	...	...	...	10, 17	...	+	...
2	GM2624	Father	11, 15	...	...	...	...	...	...	...	+	...
	GM2625	Mother ...	12	...	...	...	...	...	...	...	+	...
	GM2670	XX male ..	11, 12	...	...	...	...	...	10, 17	...	+	...
3	LGL103	Father	11, 15	...	...	...	...	...	10	...	+	+
	LGL104	Mother ...	12	...	...	...	...	...	10	...	+	+
	LGL105	XX male ..	11, 12	...	...	...	...	...	10	...	+	+
4	LGL118	Father	11, 15	3.4	6	13	4.4	2.7	10	6	+	+
	LGL117	Mother	11, 12	3.4	6, 6.6	13	4.4	2.5, 2.7	10	6, 9	−	−
	LGL115	XX male ..	11	3.4	6	13	4.4	2.5, 2.7	10	6, 9	−	−
	LGL116	Brother ...	12, 15	3.4	6	13	4.4	2.7	10	9	−	−
	LGL119	Sister	11, 12	3.4	6	13	4.4	2.7	10	6	+	+
5	LGL162	Father	11, 15	3.4	6.6	13	4.4	2.7	10	6	+	+
	LGL161	Mother ...	11, 12	3.4	6, 6.6	10, 13	4.4	2.5, 2.7	10	6	+	+
	LGL163	XX male ..	11	3.4	6, 6.6	10, 13	4.4	2.7	10	6	−	+
	LGL164	Brother ...	12, 15	3.4	6	10	4.4	2.7, 2.5	...	6	−	+
6	LGL201	Father	12, 15	...	...	...	...	...	17	...	+	+
	LGL202	Mother ...	11, 12	...	...	...	...	...	10	...	+	+
	LGL203	XX male ..	12	...	...	...	...	...	10, 17	...	+	+
	LGL204	Brother	11, 15	...	...	...	...	...	10	...	−	+
	LGL205	Sister	12	...	...	...	...	...	10, 17	...	+	+
7	LGL206	Father	12, 15	3.0	...	13	...	2.7	10	...	+	...
	LGL207	Mother ...	11	3.4, 5.7	...	10, 13	...	2.7	10	...	+	...
	LGL208	XX male ..	11, 12	3.0, 3.4	...	13	...	2.7	10	...	+	...
	LGL209	Brother ...	11, 15	5.7	...	10	...	2.7	10	...	...	...
	POR101	XX male ..	11	...	...	...	...	...	10	...	+	...

NOTE: Only the allelic restriction fragments detected are given. For example, probe RC8 detects an invariant 7-kb Taq I fragment in all individuals tested in addition to the allelic fragments indicated. A "+" under 12E7 indicates high-level expression of 12E7, while a "−" indicates low-level expression. All blank entries were not tested.

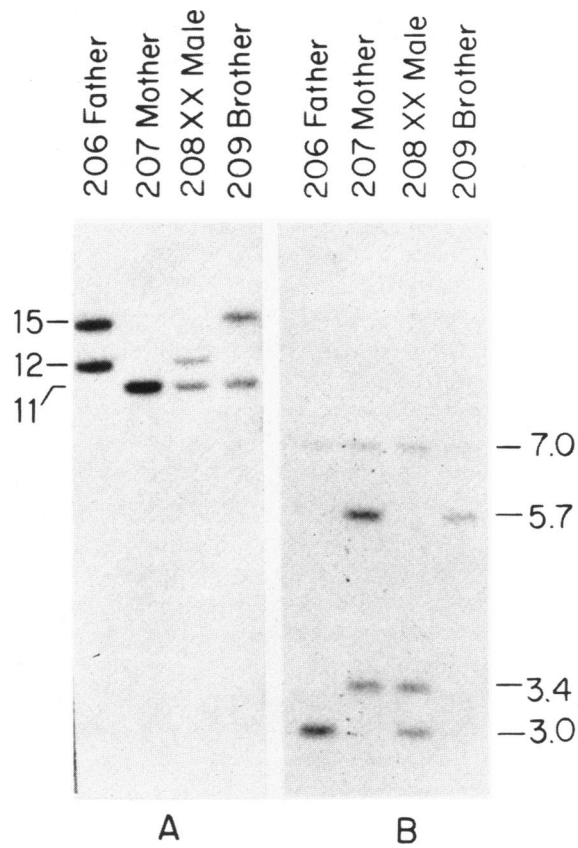

FIG. 1.—Hybridization of Taq I-digested DNAs from family 7 with pDP34 (*A*) and with a partially purified Taq I fragment from RC8 (*B*). Probe pDP34 (*A*) detects a Y-specific 15-kb band and X-linked alleles of 11 and 12 kb. The RC8 probe detects X-linked alleles of 3.0, 3.4, and 5.7 kb in addition to the weaker, nonpolymorphic band at 7.0 kb.

chromosomal contribution, but, by chance, none of the X-linked RFLPs provides definitive information with respect to a paternal contribution.

Y-Chromosomal DNA

In addition to its X-linked Taq I RFLP alleles, probe pDP34 detects a Yp-specific 15-kb Taq I fragment ([10, 11] and D. Page and M. Harper, unpublished observations, 1983). This Yp-specific fragment was not detected, even in reduced amount, in the genomes of the eight XX males tested. (See, for example, LGL208 in fig. 1*A*.) In the case of XX male POR101, chromosome-banding studies have been reported as evidence that one of the X chromosomes is an Xp-Yp translocation product [7]. Using probe pDP34, we have readily detected 10% mosaicism for a 46,XY cell line in an individual previously thought to be exclusively 45,X (D. Page and A. de la Chapelle, unpublished observations, 1983). The eight XX males examined here show no evidence of mosaicism involving a Y-containing cell line.

Serological Markers

Table 1 also shows the results of Xg and 12E7 typing of many of these individuals. Of note: XX males LGL115 and LGL163 are Xg(a−) while their fathers are Xg(a+). We have argued that the segregation of Xg and 12E7 provides evidence of an X-Y interchange in paternal meiosis in family 5 [15].

DISCUSSION

Parental Origin of X Chromosomes

In the XX male families included in this study, X-linked RFLP analysis documents a maternal and a paternal X-chromosomal contribution in five of the seven probands (table 1). In family 5, one of the two remaining families, a maternal contribution is demonstrated, but a paternal contribution cannot be determined. At first glance, the situation in family 4 appears similar: a maternal contribution is revealed, but the markers appear uninformative with respect to a paternal contribution. However, the situation is actually more complex. The X loci defined by probes pDP34 and S21 have been shown to be linked; among 32 informative meioses, only four recombinations were observed [22]. In family 4, the mother (LGL117) is heterozygous at both the pDP34 and S21 loci, while the XX male son (LGL115) is heterozygous at S21 but not at pDP34. Assuming tight linkage of the pDP34 and S21 loci, the mother's coupling phase can be established by examining the two other children, LGL116 and LGL119; in the mother, 12 kb (at pDP34) is evidently in coupling with 2.7 kb (at S21). It is possible that this XX male inherited one nonrecombinant (11/2.5) and one recombinant (11/2.7) haplotype, both from his mother. However, it is more likely that he inherited an 11/2.5 (pDP34/S21) haplotype from his mother and an 11/2.7 haplotype from his father. This XX male, LGL115, does not express his father's allele for Xg.

These results suggest that most, if not all, XX males inherit one X chromosome from their mothers and one from their fathers. This conclusion is drawn from sporadic cases (LGL105, LGL203, and LGL208) as well as from familial cases (GM2626 and GM2670). This result is consistent with the absence of color blindness among 58 XX males. It is also logically satisfying in that the alternative—inheritance of two X chromosomes from one parent and none from the other—requires the coincidence of two meiotic nondisjunctions (one in each parent). The failure of many XX males (e.g., LGL115) to express their fathers' Xg alleles must reflect either its deletion or nonfunction on the paternal X chromosome of those XX males.

X-Y Interchange Hypothesis

Our finding that XX males have one paternal and one maternal X chromosome is compatible with the X-Y interchange model but does not provide a specific test of it. Among the specific predictions of the X-Y interchange model are that: (1) XX males should be hemizygous for DNA sequences normally found on the distal portion of Xp, and (2) the distal short arm of one of the two X chromosomes of XX males should bear DNA sequences derived from Yp. With the increasing use of DNA hybridization probes of known subchromosomal origin, these predictions are becoming testable.

Our present study demonstrates that the Yp-specific restriction fragment at locus *DXYS1* is absent from the genomes of eight XX males. In one of these XX males, an Xp-Yp translocation had previously been inferred from chromosome-banding studies [6]. However, our results do not rule out the presence of other Yp-derived sequences in the genomes of XX males. Rather, if any of the XX males examined here has inherited an X-Y translocation product, our results simply imply that the translocation product must not carry a Yp-derived *DXYS1* locus. Thus, the X-Y interchange hypothesis is neither confirmed nor contradicted.

In this context it is interesting to note that the segregation of Xg and 12E7 in family 5 provides evidence of an X-Y interchange in that family [15]. Table 1 shows the results of Xg and 12E7 typing. The proband, XX male LGL163, expresses high levels of 12E7 but is Xg(a−). This male was the first Xg(a−) individual with no Y chromosome for whom high 12E7 expression was reported. Since a Y-linked gene ("*Yg*") controls the expression of 12E7 in Xg(a−) individuals [25], the high expression of 12E7 in the proband must be due to the presence in his genome of material from the Y chromosome. This material could not come from his mother, so an interchange at paternal meiosis is the most likely explanation. According to this interpretation, the proband's paternal X chromosome has lost its *Xg* gene but carries the "*Yg*" gene (and perhaps a testis-determining gene) that is normally on Yp [15].

Mosaicism

Undetected mosaicism involving a Y-containing cell line has been suggested as an explanation of maleness in the apparent absence of a Y chromosome ([8]; reviews in [1] and [9]). While cytogenetic studies can never absolutely exclude mosaicism, the study of many mitoses from many tissues can render mosaicism quite unlikely. Such studies have consistently failed to demonstrate mosaicism in most XX males. Studies of quinacrine-stained interphase nuclei of testicular origin, originally interpreted as providing evidence of Y-chromosomal material [8], are inconclusive [9]. The eight XX males examined here show no trace of the Y-specific 15-kb Taq I fragment detected by probe pDP34. Using hybridization probe pDP34, we have detected 10% mosaicism for a 46,XY cell line in a female previously thought to be simply 45,X (our unpublished observations, 1983). Our results therefore argue against any of the eight XX males in this study being mosaics for the presence of a Y chromosome.

In conclusion, the experiments described here demonstrate that most, if not all, XX males inherit one X chromosome from their fathers as well as one from their mothers, resolving what had been a matter of ambiguity. Also reported here is the absence of a Yp-specific DNA sequence from the genomes of all eight XX males examined. Definitive testing of the X-Y interchange hypothesis awaits further studies using other probes of X and Y chromosomal DNA.

NOTE ADDED IN PROOF: The presence of certain Y-specific DNA sequences in the genomes of three XX males has been reported recently (GUELLAEN G, CASANOVA M, BISHOP C, ET AL.: Human XX males with Y single-copy DNA fragments. *Nature* 307:172–173, 1984). A previously unreported 2.9-kb Taq I allele at the locus defined by probe RC8 has been observed in a British family

(PEMBREY ME, DAVIES KE, WINTER RM, ET AL.: Clinical use of DNA markers linked to the gene for Duchenne muscular dystrophy. *Arch Dis Child.* In press, 1984). This 2.9-kb allele may be identical to the 3.0-kb allele that we observed in family 7.

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