

# Multipoint Linkage Map of the Human Pseudoautosomal Region, Based on Single-Sperm Typing: Do Double Crossovers Occur during Male Meiosis?

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## Summary

Sperm typing was used to measure recombination fractions among pseudoautosomal markers and the beginning of the X/Y-specific sequences located at the pseudoautosomal boundary. These experiments included primer-extension preamplification and PCR followed by allele typing using gel electrophoresis. A newly developed data-analysis program allowed the construction of the first multipoint-linkage sperm-typing map, using results obtained on seven loci from three individuals. The large sample size not only confirmed the increased recombination activity of the pseudoautosomal region but allowed an estimate of interference of recombination to be made. The coefficient of coincidence was calculated to be .26 over a physical distance of only ~1,800 kb. The observation of a few sperm presumably resulting from double recombination argues that more than one crossover event can occur in this region during male meiosis.

## Introduction

Several different methods for mapping the human genome are available. Genetic mapping by sperm typing uses PCR to examine the alleles present in single haploid cells (Li et al. 1988; Schmitt and Arnheim, in press). Analyzing several markers and determining potential recombination events among them makes it feasible to investigate even close linkage and to detect rare recombination events, given the potentially unlimited number of sperm available. This im-

portant advantage provided by single-sperm typing has been applied to the study of meiotic recombination frequencies between the human sex chromosomes.

Despite being morphologically dissimilar, the human sex chromosomes pair in male meiosis, and recombination takes place in the pseudoautosomal region (PAR) located on their distal short arms (Chandley et al. 1984; Cooke et al. 1985; Simmler et al. 1985). The PAR is probably important for initiating pairing and the formation of a synaptonemal complex between the sex chromosomes (Hassold et al. 1991; Mohandas et al. 1992). Moreover, a secondary association and recombination at the ends of the long arms of the X and Y chromosomes is also occasionally observed (Speed and Chandley 1990; Freije et al. 1992).

Family studies showed that pseudoautosomal markers can be transferred from one sex chromosome to the other (Cooke et al. 1985; Simmler et al. 1985; Rouyer et al. 1986; Page et al. 1987a). Accordingly, pseudoautosomal loci were ordered relative to the observed gradient of sex linkage; DXYS15 and DXYS17 displayed partial linkage to the sex-specific parts of the chromosomes, with recombination rates <50%, while the telomeric locus DXYS14 showed no linkage. The observation, in family studies, of recombination fractions of 50% for telomeric markers (Rouyer et al. 1986; Page et al. 1987a) lent support to the earlier hypothesis (Burgoyne 1982) of an obligatory crossover between the X and Y chromosome during male meiosis. Rouyer et al. (1986) also suggested that the absence of double-recombination events might signify that only one crossover event could occur in the PAR. Although crossing-over is a regular feature of the sex chromosomes, it is entirely unexpected to encounter such high frequencies of recombination only during male meioses. The X-Y pairing region is ~2,600 kb in length, and recombination occurs at 10-20-fold the expected rate (Brown 1988; Petit et al. 1988). In contrast, during female meiosis, pairing extends over the entire length of the X chromosomes, and recombination in the PAR occurs at the expected genome-average rate (1 cM/Mb) (Renwick 1969; Weissenbach et al. 1987).

Detailed mapping information on markers from the

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PAR is required to further investigate the meiotic behavior and function of this region of the sex chromosomes. This report describes the first multipoint linkage map constructed by single-sperm typing and also provides evidence that, at least occasionally, double crossovers do occur within the PAR during male meiosis.

## Material and Methods

### *Whole-Genome Preamplification (Primer-Extension Preamplification [PEP]) of Single-Sperm DNA, and Analysis of Specific Loci*

Single human sperm cells (donors 2 and 38) that had been sorted by flow cytometry, lysed, and neutralized (Li et al. 1991) were preamplified by PEP (Zhang et al. 1992) modified by using 40  $\mu$ M random primer in a final volume of 50  $\mu$ l. Fifty primer-extension cycles were carried out as described, after an initial denaturation for 4 min at 92°C. The final extension was 7 min at 72°C. Each sample was then divided into aliquots (5  $\mu$ l each) and was analyzed for specific DNA sequences. Except for the MIC2 locus, the PCR amplifications were performed in two stages, using the oligonucleotide primers and PCR conditions listed in table 1. In every case, 2  $\mu$ l (except for steroid sulfatase [STS], for which 5  $\mu$ l were used) of first-round product were taken for the second round of PCR. First- and second-round PCR were repeated using another 5- $\mu$ l aliquot of PEP product when alleles for any of the loci failed to amplify. All PCR amplifications were performed in a 96-well-plate thermal cycler (MJ Research) and were set up using a Biomek 1000 automatic laboratory workstation (Beckman) equipped with an eight-pipette tool. Primers for amplification of DXYS15 and DXYS85 were designed from available sequence data (Vollrath et al. 1992). Primer sequences for DXYS154 were supplied by D. Freije, primers for MIC2 were designed from a human genomic MIC2 sequence (Page et al. 1987b; Schmitt et al. 1993), and primer and sequence information for DXYS17 was supplied by J. Weissenbach (Simmler et al. 1987). Primers for STS were designed from sequence information provided by P. Yen and L. Shapiro, to allow allele-specific amplification of the STS gene (X chromosome) and STS pseudogene (Y chromosome) (Yen et al. 1988).

The allelic differences for DXYS85 and MIC2 are defined by an insertion/deletion polymorphism (Schmitt et al. 1993), while the allelic variation at DXYS17 and DXYS154 results from differences in the number of tandemly repeated sequences. For allele typing at DXYS15, which is based on a single nucleotide substitution (Schmitt et al. 1993), 5  $\mu$ l of the second-round PCR products were digested with 0.3 units of *Fnu*4HI (New England Biolabs) in the buffer supplied. Samples were run in 8% polyacrylamide minigels, stained with ethidium bromide, and photographed under UV illumination. Gel electrophoresis of the DXYS154 products was performed separately, and am-

plification products for DXYS17 were resolved on 2% agarose gels.

### *Single-Sperm-typing Analysis of Recombination between DXYS77 and STS*

The lysed and neutralized sperm from donor X1 were amplified simultaneously for DXYS77 (single nucleotide polymorphism [Schmitt et al. 1993]) and STS, using the conditions described in table 1. In several experiments a second round of amplification was employed, using the same conditions as in the first round except that only 26 cycles at 92°C for 30 s, 60°C for 1 min, and 72°C for 30 s were carried out.

Ten microliters from first- or second-round amplifications were used for dot-blot hybridization with allele-specific probes. Filters were prehybridized individually for 30 min at 52°C (NA28 and NA29), 50°C (O15), and 55°C (NA192) and hybridized at the same temperatures for 1 h, followed by a high-stringency wash for 10 min at 56°C (NA29), 58°C (NA28), 50°C (O15), or 62°C (NA192) (Saiki et al. 1986).

### *Statistical Data Analysis*

For multipoint mapping, data were analyzed by a modification (Lazzeroni et al. 1994) of the MENDEL linkage program (Lange et al. 1988), using standard maximum-likelihood techniques. The sperm-typing version of MENDEL estimates the recombination fraction by considering efficiency, contamination rates, and occurrence of multiple sperm per sample (for details, see Lazzeroni et al. 1994). Interference was analyzed using the THREELOC maximum-likelihood program (Cui et al. 1989; Goradia et al. 1991), which also takes sperm-typing errors into account.

## Results

### *Multipoint Linkage Map of the PAR, Using Sperm Typing*

Recombination among the sex-chromosome-specific-marker STS/STS pseudogene; the short-arm pseudoautosomal markers DXYS15, DXYS85, DXYS17, MIC2, and DXYS77; and the long-arm pseudoautosomal marker DXYS154 were analyzed. The STS and STS pseudogene are representative of the strictly sex-linked portions of the X and Y chromosomes and serve as a genetic marker for the pseudoautosomal boundary.

The polymorphic markers DXYS15, DXYS85, and STS were all typed on a total of 903 sperm from donors 2 and 38 (348 and 555 samples, respectively). A subset of the 903 sperm were also typed for DXYS17, DXYS154, and MIC2. Since the recombination fractions were similar for both individuals (data not shown), the data were pooled for mapping analysis.

The typing of 903 sperm from donors 2 and 38 was performed by taking a separate aliquot for each marker, from

**Table 1****Oligonucleotide Primers and PCR Conditions**

Locus	Name	Sequence (5' to 3')	Primer Concentration		Other Conditions <sup>a</sup>
STS	NA26	GAGTGAACTCACTCAGCAC	.2	2.0	1-3, 5-7
STS	NA27	CCTTAGGAACCAGGAGATAC	.2		
STS	NA168	ACCGTACTTGCATGAGAAGCTGTCCCAAAGGA		.5	
STS	NA169	TGGGAGACTGTCCCAGAGGT		2.0	
MIC2	NA269	CAAATGCAGCTGATAAAA	.1		1-3
MIC2	NA271	AGAGCTTCCTGTTTCTCC	.1		
DXYS154	O104	GGCTGAATTCATTATTATTCTAATAG	.2	.9	1, 2, 4-6
DXYS154	O106	GAACAGGCAAAGATGCCCACTCTC	.2		
DXYS154	O105	ATGAAATTATCTGTTTGCTGATGAC		.9	
DXYS85	O72	ACCACAGGCCTATCGTG	.2		1-3, 5, 6
DXYS85	O73	TTTGAGGGCCTATCGTG	.2	.1	
DXYS85	O83	TAGGTCCTCTAGGTGCAGGA		.1	
DXYS17	O132	AGAGTACGAGAGGGAGGAGT	.2		1, 2, 4-6
DXYS17	O129	AAAGAAATTCTGGTGATATC	.2	.5	
DXYS17	O128	GCACATGCTGTGCTCGGTAG		.5	
DXYS77	NA172	GGAGTAAGACGCCATCTCAA	.2		7
DXYS77	NA173	GATGTCGGCAAAGTGAACC	.2		
DXYS15	O76	TATTTATGGAAATTGCCCCC	.2		1-3, 5, 6
DXYS15	O77	TAATACAAGCCAGACGAGCC	.2	.1	
DXYS15	O88	CACACATCACTGGAAATAGACTG		.1	

<sup>a</sup> 1 = PCR buffer as described by Li et al. (1991). 2 = First round—100  $\mu$ M dNTP (200  $\mu$ M for STS and DXYS77). 3 = First round—for DXYS15, DXYS85, MIC2, and STS—cycling conditions were 92°C for 2 min, followed by 10 cycles of 92°C for 30 s and 60°C (55°C for MIC2) for 4 min. This was followed by 30 cycles using the same conditions as above, except that the annealing/extension step was reduced to 3 min. 4 = First round for DXYS154 and DXYS17—cycling conditions were 94°C for 3 min, followed by 10 cycles of 92°C for 45 s and 60°C (55°C for DXYS17) for 4 min. This was followed by 12 cycles using the same conditions, except that the annealing/extension step was reduced to 3 min. A final extension step was at 72°C for 5 min. Hotstart PCR for the DXYS17 locus required an extra step of 75°C for 3 min before the start of the cycling conditions, during which a primer/water mixture was added to the samples. 5 = Second round—100  $\mu$ M dNTP (20  $\mu$ M for STS). 6 = Second round—cycling parameters for the second round of PCR (after an initial denaturation of 92°C for 3 min) were 23 cycles of 92°C for 45 s, 55°C for 1 min, and 72°C for 2 min and a final extension at 72°C for 5 min, for DXYS17; 25 cycles of 92°C for 30 s, 60°C (65°C for STS) for 1 min, and 72°C for 30 s, for DXYS15, DXYS85, and STS; and 23 cycles of 92°C for 45 s and 60°C for 1 min and a final extension at 72°C for 5 min, for DXYS154. 7 = Special conditions for subset of experiments involving dot-blot analysis of PCR products (multiplex PCR with primers NA26, NA27, NA172, and NA173)—potassium-free PCR buffer (Li et al. 1991), 200  $\mu$ M dNTP, 0.1  $\mu$ M of primers NA26 and NA27. Cycling conditions consisted of 11 cycles at 92°C for 30 s and 60°C for 4 min and 31 cycles at 92°C for 30 s and 60°C for 3 min. Allele-specific oligonucleotide probes were NA192, 5'-ATCTGCCCATGGTGTGTT-3'; O15, 5'-GTCGTTATCGTTGTTTTGA-3'; NA28, 5'-ATCTGCATCGCAGGGGCTG-3'; and NA29, 5'-CAGCCCCTGCAATGCAGTT-3'. Probes NA28 and NA29 were used for STS, and O15 and NA192 were used for DXYS77.

single sperm that had been preamplified by PEP. This procedure can provide multiple copies of the DNA sequences present in individual sperm, by repeated rounds of primer extension with random oligodeoxyribonucleotides consisting of a collection of all possible 15-mers. PCR was repeated with additional aliquots, to type specific loci for those samples in which specific alleles failed to amplify. Direct experiments were designed to estimate contamination rates by adding all the PCR reagents to a well that contained water instead of a sperm. No PCR product was obtained for any of the alleles from such wells, which constituted  $\sim$ 10% of every experiment and were not included in the statistical analysis; this result is consistent with the absence of contamination.

Linkage analysis (MENDEL) of the data from donors 2 and 38 considered only orders consistent with the fact that the STS/STS pseudogene and DXYS154 are not located in the PAR of the sex-chromosome short arms. The most

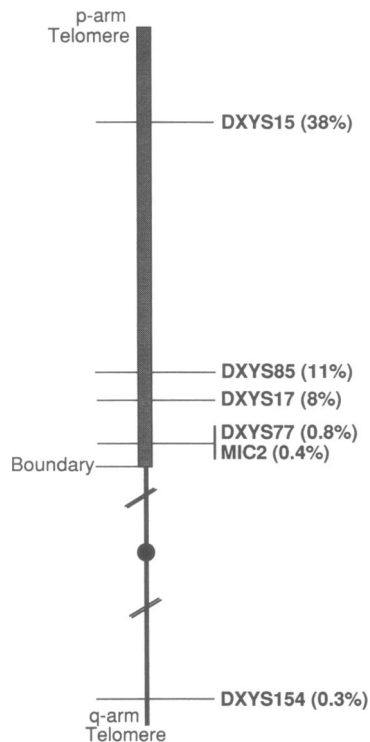
likely order was DXYS15–DXYS85–DXYS17–MIC2–STS–DXYS154 (for details on linkage analysis, see fig. 1). The recombination fractions and their standard errors for each interval are shown in table 2. A second possible order is identical with the first, but with the tightly linked markers DXYS85 and DXYS17 exchanged. The difference in log-likelihood between these two orders is 4.3, which provides odds of  $\exp(4.3) = 74:1$ —reasonably strong in favor of the former order.

In a separate experiment that typed single sperm directly without PEP, the segregation pattern of the sex-chromosome-specific-marker STS/STS pseudogene and the pseudoautosomal polymorphism DXYS77 was analyzed by typing 2,005 sperm from donor X1. The estimate for the recombination fraction between DXYS77 and STS was 0.767% (95% confidence interval 0.083%–1.451%). The large sample size allowed recombination between the markers to be measured at this very high resolution. The

pseudoautosomal locus DXYS77, which has been physically located very close to the pseudoautosomal boundary, was added to the linkage map (fig. 1).

### Interference of Recombination in the PAR

Analysis of the data by MENDEL cannot take interference into account. Therefore the large sperm-typing data set from donor 38 was reanalyzed for three loci by using the sperm-typing computer program THREELOC (Goradia et al. 1991). For DXYS15, DXYS85, and STS the analysis of 555 sperm from donor 38 gave overall efficiencies (probabilities of detecting an allele if it is present) of ~85% (range 78%–92%); the data are also consistent with little contamination, since the average contamination parameter is 0.8%. The majority (94%) of the samples received a single sperm, and only 1.8% of the samples were estimated to have two sperm. Similar to the estimates obtained by MENDEL, the order of the three markers when THREELOC is used is DXYS15–DXYS85–STS, with this



**Figure 1** Multipoint genetic map of the human PAR, established by sperm typing. The short-arm PAR is a blackened rectangle. DXYS154 falls within the long-arm PAR, whose exact boundary is not known. The values in parentheses next to the pseudoautosomal markers are the recombination fractions with respect to the STS/STS pseudogene, which serves as a genetic marker for the strictly sex-linked portions of the X and Y chromosomes. Knowledge of the physical location of several of the markers was used in constructing the linkage map; all the markers except DXYS154 and the STS/STS pseudogene had been localized to the PAR of the X/Y chromosome short arms. DXYS77 is very close to MIC2, but their relative order cannot be determined on the basis of our results.

**Table 2**

**Simultaneous Multilocus Estimates, Derived from MENDEL, of Recombination Fractions and Their 95% Confidence Intervals, for Pooled Data from Donors 2 and 38**

Interval	Recombination Fraction (95% conf. interval <sup>a</sup> ) (%)
DXYS15–DXYS85 .....	26.92 (23.28–30.56)
DXYS85–DXYS17 .....	2.61 (.00–5.33)
DXYS17–MIC .....	7.18 (4.16–10.20)
MIC–STS .....	.41 (.00–1.51)
STS–DXYS154 .....	.30 (.00–1.20)

NOTE.—Donor 2 was typed for DXYS15, DXYS85, MIC2, and STS; donor 38 was typed for the same loci and for DXYS17 and DXYS154. Sperm typing efficiencies estimated by MENDEL for all alleles were 76%–93%. These are per-sample efficiency rates calculated by disregarding contamination, since the estimated levels of contamination were negligible (0.5%); the actual per trial efficiency rates are lower. The efficiency of single-sperm sorting was very high, with ~94% of the wells containing one sperm, 3%–5% of the wells containing no sperm, and up to ~2.8% of the wells containing two sperm.

<sup>a</sup> The standard errors are not only a reflection of the sample size but also take PCR errors into consideration.

order being >10 orders of magnitude more likely than the next most likely order. The recombination fraction in the first interval is ~28%, and that in the second interval (between DXYS85 and the pseudoautosomal boundary) is ~10%. In the absence of interference, the expected number of double recombinants is 15 for donor 38 (sample size 555); yet only three sperm samples appear to fall into the double-crossover category. Such a discrepancy between the expected and the much smaller number of apparent double crossing-over events suggests a high degree of interference. Because of the role that sperm-typing errors can play in generating observed genotypes, the THREELOC program, which takes these errors into account, was used to provide a maximum-likelihood estimate of interference. The degree of interference is described 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. Analysis of the data from donor 38 estimates the coincidence parameter to be .26 with the 95% confidence interval just overlapping 0 and ranging up to .60. Maximum-likelihood values can also be computed for these data for each of the three alternative hypotheses: an unspecified degree of interference, no interference, and complete interference. If  $L_1$  is the maximum likelihood under the null hypothesis and  $L_2$  is the maximum likelihood under the general model, then the likelihood-ratio statistic  $2\ln(L_2)/L_1$  follows an approximate  $\chi^2$  distribution with 1 df. In these data, this statistic for the hypothesis of no interference is 9.27, with an associated  $P$  value <.005. Unfortunately, standard large-sam-

**Table 3**

**Sperm-typing Results for the Additional Markers DXYS17, MIC2, and DXYS154 for Three Presumed Double-Recombinant Sperm from Donor 38 (parental haplotypes: ABCDEF and abcdef)**

	DXYS15	DXYS85	DXYS17	MIC2	STS	DXYS154
1 .....	a	B	c	d	e	...
2 .....	A	b	...	D	E	F
3 .....	A	b	...	D	E	F

NOTE.—An ellipsis (. . .) indicates that no PCR product was detected.

ple statistical theory does not apply to the hypothesis of complete interference, because in this case the coincidence coefficient occurs on its lower boundary of 0. Thus a formal test cannot be performed, and no valid *P* value can be cited. However, the likelihood-ratio statistic of 5.43 for a comparison of complete interference with incomplete interference is strongly suggestive of incomplete interference. Thus it appears that in the human PAR double crossovers can occasionally take place during male meioses.

In addition to these statistical tests, we examined each of the three apparent double-crossover sperm in more detail. Because each sperm was preamplified by PEP, the presumed double-recombinant samples could be studied for additional loci in order to confirm the typing results. For each of the apparent double-recombinant sperm, three additional markers—DXYS17, MIC2, and DXYS154—were analyzed using a separate aliquot from the PEP reaction. The typing results are shown in table 3. The data for DXYS154 and MIC2 support the notion of double recombination taking place and confirm the typing results for STS; additionally, both crossing-over events must have fallen within the PAR of the short arm, since no exchange is detected in the MIC2-STS interval or the STS-DXYS154 interval. Amplification of a large DNA fragment from the VNTR polymorphism at DXYS17 failed for two of the double-recombinant sperm; however, DXYS17 typing results for the third sperm indicate that one of the recombination events occurred in the interval DXYS85–DXYS17. In conclusion, these results also support the idea that double crossovers can occur within the PAR during male meiosis.

## Discussion

### Genetic Map of the Human PAR

The results of the experiments reported here show the usefulness of PEP for multilocus mapping, including the fact that typing results can be confirmed and PCR analysis can be repeated in cases where one or several alleles are not amplified from any particular aliquot of PEP product. The mapping data obtained were analyzed with a new

sperm-typing version of the MENDEL linkage analysis program, which estimates the recombination fractions and finds the best order of the markers along the chromosome. Unlike the previous sperm-typing programs, MENDEL allows the pooling of data from different donors where sperm typing has been carried out on only a subset of markers in each individual. The MENDEL program output also reports estimates for the efficiencies of sperm typing, as well as contamination rates, and includes standard errors for these estimates. However, the program cannot take interference into account. Apparently, this is not critical for estimating recombination fractions of the markers that we studied in the PAR, since the output from the MENDEL analysis for the six loci entered in a combined analysis do not differ much from several three-locus combination runs with THREELOC, which does take interference into account (data not shown). However, it is to be expected that incorporating interference into the model would improve the ability to order multiple loci and to estimate interval widths.

Physical maps of the PAR that are based on pulsed-field gel electrophoresis (PFGE) and YACs have been established and include the loci DXYS15, DXYS17, DXYS85, and MIC2 (Brown 1988; Petit et al. 1988; Foote et al. 1992; Wapenaar et al. 1992; Slim et al. 1993). These maps correspond very closely to the genetic linkage map derived by sperm typing. Three of our markers (DXYS15, DXYS17, and MIC2) had been typed in earlier family studies of the PAR, and the recombination fractions are similar to our estimates (Rouyer et al. 1986; Page et al. 1987a; Henke et al. 1993). Moreover, the recombination fractions reported for the pseudoautosomal markers in this study provide a high-resolution documentation of the suggestion (Rouyer et al. 1986; Page et al. 1987a; Henke et al. 1993) that recombination in this region is significantly higher than the average genome rate during male meioses (table 4).

The pseudoautosomal boundary is considered a junction between the pseudoautosomal and sex-specific part of the chromosome. The data from this study suggest that even DNA segments very close to the boundary may have the same unaltered high recombination rates characteristic of the human PAR during male meiosis. The analysis of 2,005 sperm gave a recombination fraction of 0.77% over the 25-kb segment estimated to lie between DXYS77 and the boundary. This is 31 times higher (table 4) than the expected frequency for a segment of this size, based on estimates of the recombination fraction derived for the whole genome (Renwick 1969). With DXYS77 located so close to the boundary, it seems that there is no special structural feature of the boundary that alters the high pseudoautosomal recombination rates for very nearby distal markers. Recombination immediately proximal to the boundary must be sufficiently low to account for the significant sequence differences between the X and Y chro-

**Table 4****Correspondence between Physical and Genetic Distances Estimated by Sperm Typing**

Interval	Distance (A) (kb)	Recombination Fraction (B) (%)	A/B	Increase
STS-DXYS77 .....	25	.77	32 <sup>a</sup>	31-fold <sup>a</sup>
STS-DXYS85 .....	800	11	73	14-fold
STS-DXYS15 .....	1,900	27	70	14-fold
Whole genome .....	3 × 10 <sup>6</sup>	~3,000	1,000	...

<sup>a</sup> Estimate depends on the assumption that no recombination took place outside the PAR; estimates for the other intervals would not change even if the recombination fraction for the STS-DXYS77 interval were entirely due to events outside the PAR.

mosomes in this region (Ellis et al. 1989). The sex-chromosome-specific loci (STS and STS pseudogene) used to mark the pseudoautosomal boundary were not physically located close to the respective boundaries themselves (Yen et al. 1988). It might therefore be possible that some of the recombination events between the boundary and DXYS77 actually occurred between the boundary and the STS markers. Information about the frequency of XX males and the location of the recombination events that lead to such a condition suggests that the recombination rate between the boundary on the short arm and STS is <0.01% (de la Chapelle 1981). However, since it is possible that other classes of X-Y exchanges may not be recoverable as viable fetuses, we cannot completely exclude the possibility that recombination between DXYS77 and the boundary is less than we observed. It is interesting to note that studies using whole-genome amplification and additional markers in the X- and Y-specific regions could be designed to identify sperm that resulted from exchange events outside the PAR and to locate their position and estimate their frequency.

Two CA-repeat markers (including DXYS154 [Arveiler et al. 1989; Freije et al. 1992]) from the newly identified second PAR on the distal long arm of the human sex chromosomes have recently been shown to recombine with one another at a male-specific frequency of ~2% (four recombination events in 195 informative meioses; 95% confidence interval 0.03%–4.08%) in CEPH reference pedigrees (Freije et al. 1992). Typing of 505 sperm samples identified several sperm recombinant between the STS/STS pseudogene and DXYS154. The recombination fraction estimated by sperm typing is 0.3% (95% confidence interval 0%–1.2%) and is slightly lower than that reported elsewhere, although the confidence intervals of both estimates overlap. This lower frequency could also be due to the possibility that DXYS154 is relatively close to the proximal boundary of this second PAR. If so, recombination between DXYS154 and the STS/STS pseudogene could in fact be lower than that observed between DXYS154 and the other marker (Freije et al. 1992).

**Interference Analysis in the Human PAR**

The analysis of large sample sizes made possible by sperm typing permitted the detection of double crossing-over in the human PAR. Traditional pedigree analysis, albeit on a smaller sample, failed to detect any such events (Rouyer et al. 1986; Page et al. 1987a). Using PEP made confirmation of these events possible by looking for exchanges of additional flanking markers in the same samples.

Typing errors cannot be absolutely ruled out as an explanation for sperm in the double-crossover category. When three adjacent loci are considered, a typing error involving the middle locus would yield an apparent double-crossover. However, in sperm samples typed for DXYS85, the STS/STS pseudogene, and DXYS154, no apparent double recombinants were observed, suggesting that such typing errors are not frequent. In addition, the fact that each of the two reciprocal classes of double recombinants was observed seems to exclude a simple repetitive error that would have contributed to our findings. Finally, another possibility is that the exchange of the central marker is caused by only a single genetic recombination event. This refers to the formation of a single Holliday junction within which the polymorphic nucleotide at DXYS85 exists as a base-pair mismatch. If the Holliday junction is resolved so that flanking markers are not exchanged (patch formation) and the mismatch is corrected to the allele found on the other homologue, then an apparent "gene-conversion-like" event mimicking a double crossover will be observed. The frequency of such events in the human genome remains unknown.

Analysis of the apparent double-crossover sperm by typing the three additional markers (DXYS17, MIC2, and DXYS154) that were available placed both recombination events within the PAR. Confirmation of the typing result for the middle marker DXYS85 by a proximal marker DXYS17 separated from it by 3% recombination showed inconclusive results; for two of the three apparent double-recombinant sperm from donor 38, DNA amplification for DXYS17 failed, presumably because of the large size of the

expected PCR product. The outcome for the third sperm indicates that one of the two crossovers must have fallen within the interval separating DXYS17 and DXYS85—an event expected to occur in  $\sim 1/4$  of the recombinants in the 11% interval that separates DXYS85 from the pseudoautosomal boundary. Additional markers tightly linked to DXYS85 need to be identified in order to localize the recombination events more precisely.

Maximum-likelihood linkage analysis using the computer program THREELOC for the sperm-typing data from donor 38 (3 apparent double-recombinant sperm among a sample size of 555) estimated a value of .26 for the coefficient of coincidence. Statistical evidence for the occurrence of double crossing-over in donor 38 comes from maximum-likelihood analysis of the sperm-typing data under the hypothesis of the coefficient of coincidence constrained to the value 0 (total interference; no double crossing-over), versus allowing interference to vary between 0 and 1. Our estimate of the coefficient of coincidence (.26) is consistent with the earlier maximal estimate of .45, which was based on a much smaller number of meioses (Page et al. 1987a) and is similar to the value of .35 estimated for non-sex chromosomes in humans (data from chromosomes 1 and 10 [Pascoe and Morton 1987; Morton and Collins 1990; Shields et al. 1991]).

How the numbers and locations of recombination events are regulated along chromosomes is a long-standing unanswered question. Studies in yeast have demonstrated higher rates (centimorgans per kilobase pair) of meiotic recombination for smaller chromosomes than for larger chromosomes (Kaback et al. 1992; Riles et al. 1993). At least a single crossover would appear essential for regular segregation of sex chromosomes in humans, since nondisjunction of the X and Y chromosomes seems to be associated with reduced recombination (Hassold et al. 1991). However, on the basis of the physical distance (1,800 kb) between DXYS15 and the PAR boundary, we would have expected that recombination would occur at a frequency of .018, instead of the observed frequency of  $\sim .4$ , and that interference would be complete. In a report on mice, double recombination in the PAR was observed without any significant interference (Soriano et al. 1987), which could be related to any difference in size between the two pairing regions. The existence of a mechanism for controlling recombination that responds directly to chromosome size seems plausible. But, whereas most models for interference assume that the interference depends inversely on the physical distances (Fox 1973; King and Mortimer 1990), data from the PAR suggest that interference depends primarily on overall genetic distance, an inference in agreement with recent analysis of data from *Drosophila* and *Neurospora* (Foss et al. 1993).

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