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ARTICLE

Reconstructing hominid Y evolution: X-homologous block, created by X–Y transposition, was disrupted by Yp inversion through LINE–LINE recombination

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The human X and Y chromosomes share many blocks of similar DNA sequence. We conducted mapping and nucleotide sequencing studies of extensive, multi-megabase homologies between Yp and Xq21, which do not recombine during male meiosis. We confirmed and built upon previous evidence that a Yp inversion had occurred during evolution: a single contiguous segment of Xq21 is homologous to two non-contiguous segments of Yp. We precisely defined and sequenced the inversion breakpoints, obtaining evidence that the inversion was mediated by recombination between LINE-1 elements in otherwise non-homologous regions. This inversion appears to have followed a single transposition of an ~4 Mb segment from the X to the Y chromosome. These events jointly account for the present arrangement of Yp–Xq21 homologous sequences. Based on Southern blotting studies of primates and of humans drawn from diverse populations, we conclude that both the X–Y transposition and the subsequent, LINE-mediated Yp inversion occurred after the divergence of hominid and chimp lineages but before the radiation of extant human populations. This evolutionary scenario is consistent with our finding of 99.3 ± 0.2% nucleotide identity between the X and Y chromosomes within the transposed region, which suggests that the transposition occurred ~3–4 million years ago, near the time of emergence of *Homo*. Comparative sequencing of the entire human X and Y chromosomes may reveal a succession of transpositions, inversions and other rearrangements underlying the complex pattern of sequence similarities between the present-day sex chromosomes. With the possible exception of cubitus valgus, phenotypic features of Turner syndrome are absent in individuals monosomic for Yp–Xq21 homologous sequences, suggesting that most of the critical 'Turner genes' are found elsewhere on the X and Y chromosomes.

INTRODUCTION

Nucleotide sequence similarities between the human X and Y chromosomes are extensive, totaling at least one quarter of the Y chromosome's euchromatic regions (1,2). Of these 8–10 Mb of X–Y homologous sequences, only 3 Mb comprise the pseudoautosomal regions (pter and qter), where frequent X–Y recombination during male meiosis ensures co-linearity of sequence between the sex chromosomes (3–8). The majority of X–Y homologous sequences are located in the strictly sex-linked regions, from which X–Y recombination is normally excluded. Within these regions, there are at least a dozen blocks of X–Y sequence similarity, which occur in a strikingly different order (and orientation) on the Y as opposed to the X chromosome (9). To account for this complex array of sequence similarities between the strictly sex-linked regions of the X and Y, investigators have postulated the occurrence of numerous inversions, transpositions and other rearrangements during primate evolution (10–15). However, in no case have the postulated rearrangements been reconstructed through nucleotide sequence analysis of breakpoints.

On the human X chromosome, the largest single block of Y-homologous DNA sequence, and the first to have been discovered, is in band q21 (10,15–20). Spanning ~4 Mb, these Xq21 sequences are highly similar to sequences on Yp. At least

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some of these Xq21-homologous sequences are present on Yp by virtue of an X–Y transposition that occurred during human evolution, after divergence of human from chimpanzee and gorilla lineages (10,13,18). However, while the Y-homologous sequences form a single contiguous block in Xq21, the corresponding sequences on Yp are divided into two non-contiguous segments separated by several megabases of Y-specific DNA (2,13,15,19,20). This bipartite arrangement of Xq21-homologous sequences on Yp can be explained most economically by a single transposition followed by a Yp inversion (13,15). We set out to reconstruct conclusively the evolutionary history of these extensive Xq21–Yp homologies by comparative nucleotide sequence analysis of the human sex chromosomes at selected sites within and bordering the homologous regions.

Table 1. Xq21–Yp DNA loci studied

STS	GenBank accession Nos			X–Y nucl	Locus	Plasmid or phage clone				Southern blotting			Reference
name[a]	STS	Y sequence	X sequence	subst./# nucl. compared		Name	insert kb/ restriction enzyme	Vector	Hyb. string.[b]	Restrict. enzyme	Restriction fragment (kb) Y-specific	X-specific	
						λOX18	3.5/*Hin*dIII	λ2010	P	*Taq*I	2.3		26
			AF008133			pDP1015	5/*Eco*RI	pBluescript	P	*Eco*RI	6(4A) & 8(1B)	5	this report
										or *Taq*I	5.2(4A)	13	
			AF008131	4/163		pDP1094	7.3/*Hin*dIII	pBluescript					this report
sXY20	G11978				*DXYS42*	pDP307	0.9/*Hin*dIII	pUC13	H	*Pst*I	3.4	5	1,26
						λBER38	1.5/*Eco*RI	λEMBL3A	H	*Taq*I	5.3	3.2	26
sXY21	G11980	AF005954	AF005953	2/221	*DXYS69*	pDP522b	2.1/*Bam*HI	pUC13	H	TaqI	2.4	3.2	1
sXY22	G31353	AF005956	AF005955	2/231	*DXYS106*								1
sXY23	G11966	AF005957	AF005957	0/99	*DXYS107*								1
					DXYS23	pDP132	3.5/*Hin*dIII	pUC13	H	*Taq*I	4.3 or 7	3.2(intensity)	this report
sXY24	G31354				*DXYS5*	p47a	2.3/EcoRI	pBR327	M	*Taq*I	3.0	8	1,18
					DXYS5	p47z	2.3/*Eco*RI	pBR327	M	*Taq*I	4.3	1.5 and 3.3	16
sXY25	G31355				*DXYS108*								1
sXY26	G31356	AF005958	AF005958	0/170	*DXYS109*								1
sXY27	G31357				*DXYS10*	p41a	0.7/*Eco*RI	pBR322	H	*Msp*I	2.4	2.7	1,6
sXY28	G11967	AF005960	AF005959	1/168	*DXYS110*	λ215	2.1/*Hin*dIII	λCharon 21A	H	*Taq*I	5.5	4.3	1
sXY29	G11968	AF005962	AF005961	2/214	*DXYS111*								1
						λ622	7.3/*Hin*dIII	λCharon 21A	H	*Hin*dIII	7.3	2.8 and 6.3	this report
sXY30	G12026				*DXYS112*								1
sXY31	G12027	AF005963	AF005963	0/165	*DXYS113*								1
sXY32	G31363				*DXYS114*								1
sXY33	G12028	AF005965	AF005964	2/180	*DXYS115*								1
sXY34	G31364				*DYS253*								1
sXY35	G31362	AF005966	AF005966	0/263	*DXYS116*	p17	0.6/*Eco*RI	pBR322	M	*Pst*I	5	6	1,16
sXY36	G12031	AF005967	AF005967	0/138	*DXYS2*	pDP7a	2.1/*Hin*dIII	pUC13	M	*Kpn*I	8	19	1,16
sXY37	G11979	AF005969	AF005968	1/177	*DXYS6*	p16	1.5/*Eco*RI	pBR322	H	*Taq*I	2.1	2.8	1,16
sXY38	G11969	AF005971	AF005970	1/138	*DXYS117*								1
					DXYS7	p13d	1.8/*Eco*RI	pBR322	H	*Taq*I	7	8	16
sXY39	G11970	AF005973	AF005972	4/246	*DXYS118*								1
sXY40	G31358	AF005974	AF005974	0/190	*DXYS8*	pDP61	1.0/*Eco*RI,*Taq*I	pUC8	H	*Taq*I	2.1 or 2.6	2.8	1,16
						pDP1046	3.3/*Hin*dIII	pBluescript	H	*Pvu*II	3.5 and 7.0	2.9 and 6.9	this report
sXY41	G11971	AF005975	AF005975	0/73	*DXYS119*								1
sXY42	G31365				*DXYS120*								1
sXY43	G12011	AF005977	AF005976	2/238	*DYS254*	pDP1045	3.5/*Hin*dIII	pBluescript	H	*Msp*I	4.7	3.1	1
sXY44	G12029	AF005978	AF005978	0/139	*DXYS121*								1
sXY45	G12012	AF005979	AF005979	0/115	*DYS255*	λ103	6.5/*Hin*dIII	λCharon 21A	H	*Msp*I	6	3.7	1
sXY46	G11972	AF005981	AF005980	2/136	*DXYS122*								1
sXY47	G11981	AF005983	AF005982	1/119	*DXYS9*	pDP1057	1.0/*Hin*dIII	pBluescript	H	*Msp*I	3.2	6.5	1,16
sXY48	G31359	AF005985	AF005984	2/282	*DXYS12*	St25–2.7	2.7/*Taq*I,*Eco*RI	pBR322	H	*Taq*I	7.1	2.9	1,17
sXY49	G11973				*DXYS123*								1
sXY50	G11974	AF005987	AF005986	1/129	*DXYS124*								1
sXY51	G12030	AF005988	AF005988	0/229	*DXYS128*	pDP1040	2.9/*Hin*dIII	pBluescript	H	*Taq*I	2.9	3.2	1
						λ238	2.5/*Hin*dIII	λCharon 21A	H	*Taq*I	5.6	7	this report
						pDP1044	4.4/*Hin*dIII	pBluescript	H	*Hin*dIII	4.4	4.6	this report
sXY52	G12024	AF005990	AF005989	2/227	*DXYS4*	p1	1.3/*Eco*RI	pBR322	M	*Taq*I	2.0	6.9 or 10	1,16
						λOX27	6/*Hin*dIII	λ2010	P	*Eco*RI	12		this report
		AF008132		4/262		pDP1034	5.7/*Eco*RI	pBluescript					this report
sXY73	G31360	AF005992	AF005991	1/218	*DXYS1*	pDP34	2.2/*Eco*RI	pDP322	H	*Taq*I	15	11 or 12	1,10

[a]Note: X–Y common STSs (shown here with prefix 'sXY') are numbered according to Vollrath *et al.*, 1992 (ref. 1) (where prefix was 'sY'); e.g. sXY20 is identical to sY20.

[b]Hybridization stringency conditions: H, high; M, medium; P, pre-hybridization of probe followed by high stringency hybridization (see Materials and Methods).

Table 2. X- or Y-specific PCR assays at X–Y homologous loci

STS	X- or Y-specific primer	X–Y common primer	Product size (bp)	Relationship to primary amplification[a]
sXY22-X	GGGAAGAATGAAAGGAAAG	TGGGCATCTTGGAAATCAA	145	nested
sXY22-Y	.......C.........A	....	–	nested
sXY28-X	ACTGTGTGAAACATCCTAC	GATTAAAACAAGGCAATGG	152	nested
sXY28-Y	..................G	....	–	nested
sXY29-X	GTATGTAGTTTTCGGTATG	ATATTTGCTTCCCTTTTCA	190	nested
sXY29-Y	..................A	....	–	nested
sXY37-X	TTTTGTATACCCATAACTTT	TATGTGTTAGCCCTTCTTT	137	none
sXY37-Y	CCAAAATAATCTATTTCTCT	....	120	none
sXY38-X	CATTTTTAATTCTTCTATGTT	ATGCTGCTGTGTTTCTGTGT	141	semi-nested
sXY38-Y	C	....	–	semi-nested
sXY43-X	GGGTTGAAATAAACTGTGC	GAGAAGGAAGAATGTGAAA	162	nested
sXY43-Y	..................T	....	–	nested
sXY47-X	TGGAGGTTGGAGATCTAC	ATCTGTGAAGCACCCACTGT	93	semi-nested
sXY47-Y	..................T	....	–	semi-nested
sXY48-X	GGAGAACAGTATAGAGGT	TTGGACACTTAGATTGCT	185	nested
sXY48-Y	..................A	....	–	nested

X (or Y)-specific primers bear a mismatch at the 3′ end with the homologous sequence on the Y (or X) chromosome.
[a]See Materials and Methods for explanation of nested and semi-nested assays.

RESULTS

Bipartite structure of Xq21-homologous sequences on Yp

We and our colleagues have been constructing and refining DNA-probe-based deletion and physical maps of the human Y chromosome for some years (1,2,19) (unpublished results). Most of the DNA markers employed in constructing these maps have been derived, essentially at random, from recombinant DNA libraries prepared from flow-sorted Y chromosomes. During the course of this work, we identified a large number of X–Y homologous markers, 46 of which we could assign to Yp and Xq21 (Tables 1 and 2). These 46 loci appear to be specific to Yp and Xq21, with no close homologs elsewhere on sex chromosomes or autosomes. On the Y chromosome, we mapped these loci to the short arm by: (i) deletion analysis [usually by Southern blotting; in some cases by PCR-based sequence tagged sites (STS) content mapping] of human XX males, XY females and other individuals carrying partial Y chromosomes (Fig. 1A); and (ii) STS content analysis of a collection of yeast artificial chromosomes (YACs) spanning the euchromatic region of the Y chromosome (2). On the X chromosome, we mapped these loci to band q21 by: (i) deletion analysis (by Southern blotting) of human XY males with choroideremia (an X-linked recessive disorder) due to interstitial deletions of Xq21 (Fig. 1C); and (ii) STS content analysis of X-chromosomal YAC and bacterial artificial chromosome (BAC) clones identified by screening human genomic libraries with previously assigned Yp–Xq21 probes (not shown). Some of the resulting data on Yp–Xq21 loci have been published previously (1,2,19).

Our mapping studies of Yp–Xq21 homologous loci are completely in accord with those of other investigators (13,15,20–22), yielding three major conclusions, schematically summarized in Figure 1B. (i) In Yp, the Xq21-homologous loci are found in two non-contiguous regions: a more distal segment of 3–4 Mb (deletion intervals 1B–3B; hereafter the ‘1–2–3 block’) and a more proximal segment of several hundred kilobases (the proximal portion of deletion interval 4A), separated by a multi-megabase region of Y-specific sequences (deletion interval 3C through the distal half of interval 4A). Within each of the two regions, X–Y sequence similarity appears to be continuous, or very nearly so. (ii) In Xq21, the Yp-homologous loci appear to be found in a single, essentially uninterrupted region whose size approximates the sum of the two Xq21-homologous regions on Yp. (iii) There is one major difference in the orders of homologous segments in Yp and in Xq21. In Yp, the interval order (distal to proximal) is 1–2–3–4A, while in Xq21 the order of homologous segments (proximal to distal) is 4A-1–2–3. While order appears to be maintained within the 1–2–3 block, the position of interval 4A with respect to that block is inverted on Yp as compared with Xq21.

The arrangement of homologous sequences on Yp and Xq21 is explained most simply by a single X–Y transposition followed by a Yp inversion that separated 4A from the 1–2–3 block (13,15). We focused our efforts on definitively reconstructing this sequence of events (Fig. 2).

Molecular clock analysis consistent with a single X–Y transposition

In the pseudoautosomal regions, meiotic recombination ensures nucleotide sequence identity between the X and Y chromosomes. The Yp–Xq21 homologies fall outside the pseudoautosomal regions, however, and here the sequences of the two chromosomes are similar but not identical (10,13,17,18). Indeed, without occasional X–Y sequence differences, it would have been very

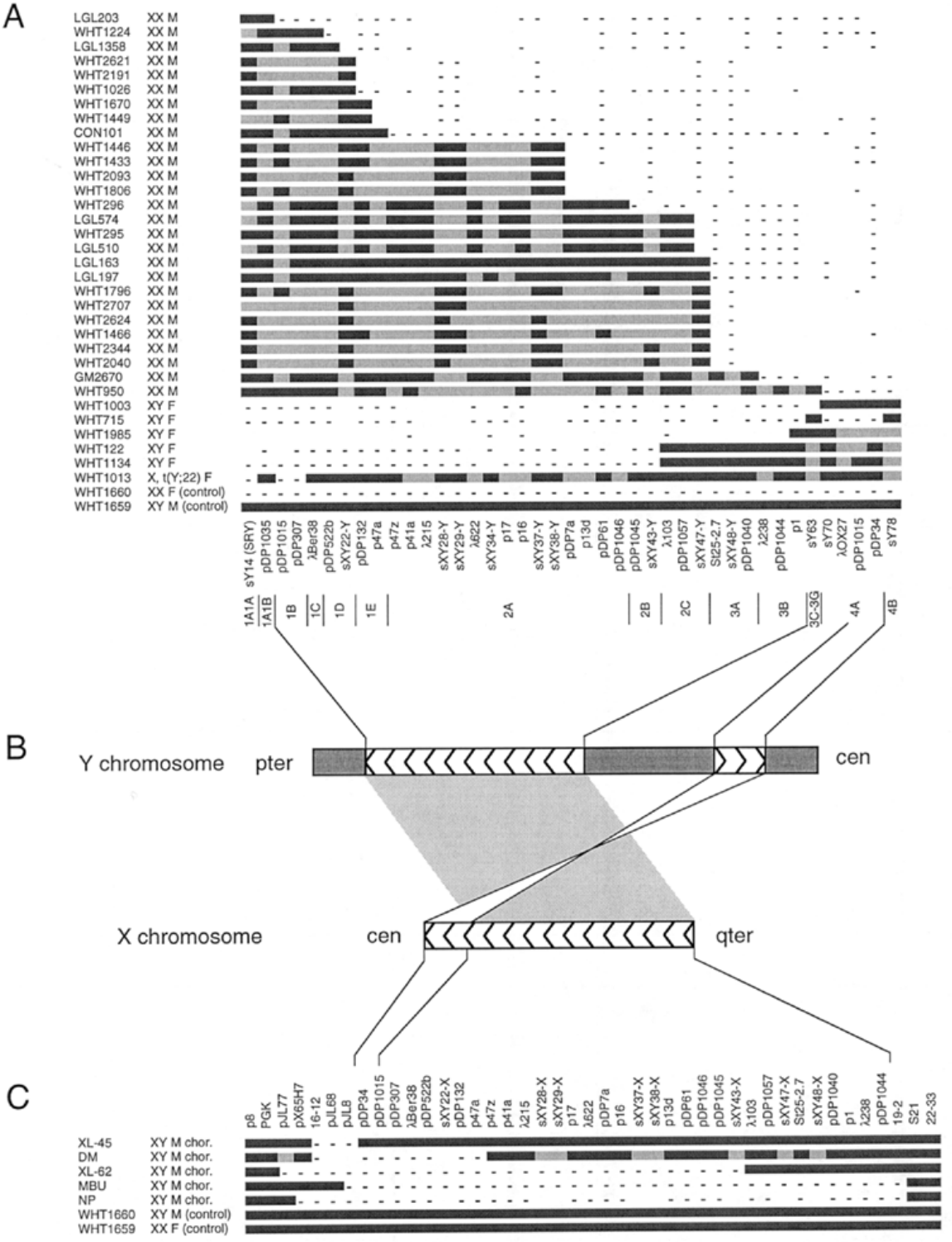

Figure 1. Deletion mapping of homologous sequences on Yp and Xq21. (**A**) Deletion map of Yp (after Figure 2 of Vollrath *et al*., 1992, where some of this data was previously presented). Along the left border are listed 33 individuals carrying part but not all of Yp; most are XX males ('XX M') or XY females ('XY F'). Along the bottom are listed Y-DNA markers whose presence or absence was assessed by PCR (marker names beginning with 'sY' or 'sXY') or Southern blotting. Most DNA markers used are described in Table 1; see reference (1) for details of markers not described in Table 1. Below the markers are listed deletion intervals 1A1A (just proximal to the pter pseudoautosomal region) through 4B (contains centromere). The body of the figure presents experimental data and inferences: experimentally demonstrated presence of a locus in an individual is indicated by a black segment; inferred presence (by interpolation) is indicated by a gray segment; experimentally demonstrated absence is indicated by a minus. Within an interval, the order of loci has not been determined by deletion mapping. (**B**) Cartoon depicting homology between two segments of Yp and a single contiguous segment of Xq21. See also the previous work of Sargent and colleagues (20) and Mumm and colleagues (15). Herringbone shading denotes X–Y homologous regions. Dark shading denotes Y-specific regions. (**C**) Deletion map of Xq21. The individuals with Xq21 deletions are XY males with choroideremia and, in some cases, additional disorders caused by nullisomy for this region of the X chromosome. Most DNA markers used are described in Table 1; see reference (56) for details of markers not described in Table 1. Some data have been published previously; see references (57–61).

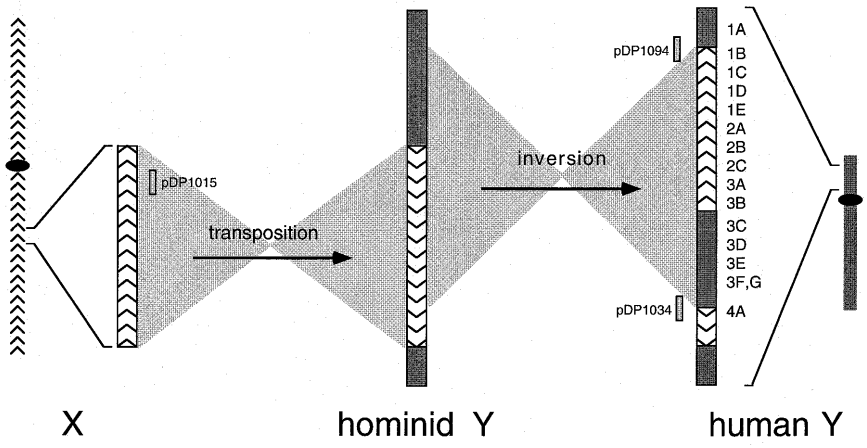

Figure 2. Cartoon of transposition from Xq21 to Yp and subsequent inversion on Yp, both of which are hypothesized to have occurred during hominid evolution. The transposed region and inverted region, which partially overlap, are highlighted. Herringbone shading denotes X-homologous regions, and their polarity. Dark shading denotes Y-specific regions. Deletion intervals on modern human Y are numbered at the right. In interval 4A of modern human Y, the predicted polarity of X-homologous sequences with respect to Ycen and Ypter has been confirmed by radiation hybrid mapping (T. Sawai-Kawaguchi, C. Tilford, H. Skaletsky and D.C.Page, unpublished results). Also indicated are origins of three plasmids for which partial restriction maps and nucleotide sequences are shown in Figure 4. Not drawn to scale.

difficult to carry out deletion mapping studies of the Yp–Xq21 loci using genomic DNAs from individuals with partially deleted X or Y chromosomes. According to the transposition/inversion model, the Yp–Xq21 homologous sequences on the ancestral X and Y chromosomes should have been identical, or nearly so, immediately following the postulated X–Y transposition event, with subsequent fixation of neutral mutations on either the X or the Y accounting for the modest sequence differences observed between the present-day sex chromosomes. If Xq-homologous sequences in both distal and proximal Yp (intervals 1–2–3 and 4A, respectively) were brought to the Y by a single transposition, then one would expect a similar level of X–Y sequence divergence throughout both segments.

To test this 'molecular clock' prediction, we compared the nucleotide sequence of the X and Y chromosomes at 27 STSs (Table 1): 25 drawn from distal Yp (the 1–2–3 block) and two drawn from proximal Yp (interval 4A), all 27 of which have homologs in Xq21. In aggregate, we directly compared nearly 5000 nucleotides from each of the two sex chromosomes. As predicted, we observed a relatively uniform level of X–Y nucleotide divergence (average 0.7 ± 0.2%) throughout the sequences examined (Table 1). In particular, the levels of X–Y divergence were statistically indistinguishable in the 1–2–3 (distal) and 4A (proximal) regions (0.7 ± 0.2% and 1.0 ± 0.5%, respectively).

These direct measures of X–Y divergence are in good agreement with our previous estimates at *DXYS1* (a locus in the proximal region), where comparative restriction mapping of the X and Y chromosomes had yielded an estimate of 0.4 ± 0.4% nucleotide divergence (10). Our findings agree less well with the conclusions of Lambson and colleagues (13), who, on the basis of comparative restriction fragment analysis of seven loci, estimated Xq21–Yp divergence to be 2%. We think it likely that Lambson and colleagues overestimated the degree of X–Y divergence by: (i) underestimating the number of nucleotides screened for X–Y differences (23); and (ii) including in their analysis the restriction enzymes *Taq*I and *Msp*I, whose sites are known to be hypermutable (24). To our knowledge, the ~99.3% X–Y identity observed in the Yp–Xq21 region sets these sequences apart from all other identified, sizeable X–Y homologies, which exhibit either absolute identity (the pseudoautosomal regions) or no more than 98% identity between the sex chromosomes (25). These molecular clock studies are consistent with a single transposition.

Nucleotide definition of the Yp inversion breakpoints

Susbsequent to the transposition from Xq to Yp, an inversion or other rearrangement on Yp must have occurred to account for the bipartite structure of Xq21-homologous sequences there (13,15) (Fig. 2). As we will describe here, studies of the boundaries of the X–Y homologous regions enabled us to identify the Yp inversion breakpoints, thereby securing compelling evidence for the postulated single transposition/inversion sequence.

The most distal boundary of Xq21 homology, in Y deletion interval 1, was localized during the course of a chromosome walk through the sex-determining region of the Y chromosome (see Figure 1 of ref. 26). This chromosome walk, initiated at an X–Y homologous locus defined by probe pDP307 (in deletion interval 1B), extended distally into a 300 kb region (deletion interval 1A) that consists largely of Y-specific sequences, that contains the genes *ZFY, RPS4Y* and *SRY,* and that lies immediately proximal to the pter pseudoautosomal region. About 5 kb distal to the pDP307 locus, we had noted an abrupt transition from X-homologous to Y-specific DNA sequences (see Figure 2 of ref. 26).

To corroborate and characterize this distal boundary of X–Y homology, we compared corresponding X- and Y-derived clones isolated from λ phage libraries of human genomic DNA. [X-derived clones were isolated from a library prepared from a 46,XX female. Y-derived clones were isolated from a library prepared from a 49,XYYYY male (20).] We compared the X- and Y-derived phages by cross-hybridization, by hybridization of subclones and other purified insert fragments to Southern blots of selected human and ape male and female genomic DNAs (e.g. Fig. 3), and by restriction mapping of plasmid subclones (e.g. Fig. 4A). As expected, this analysis revealed that the Y chromosome

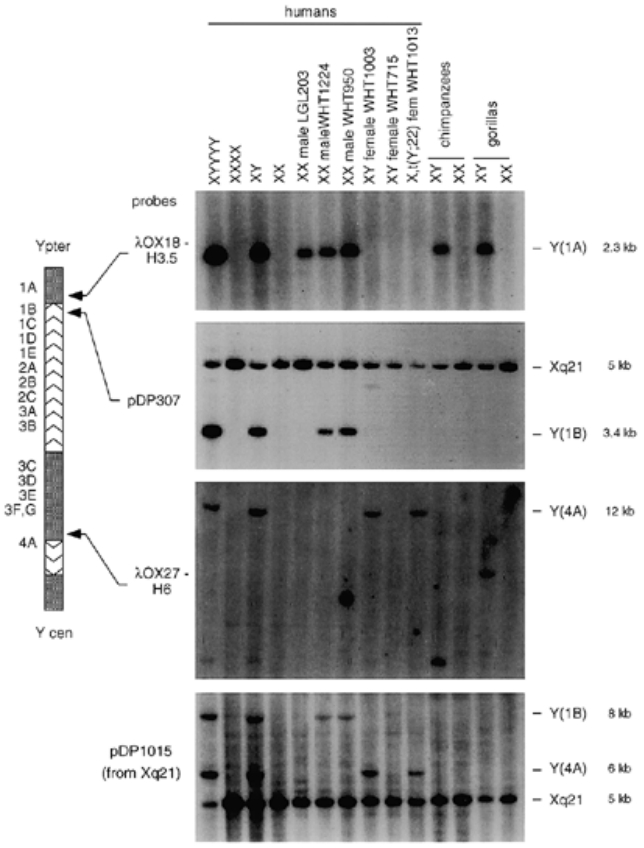

Figure 3. Y- and X-derived DNA probes hybridized to Southern blots of human, chimpanzee and gorilla genomic DNAs. To the left of the autoradiograms are listed four probes, three derived from Yp (cartoon shaded and labeled as in Figures 1 and 2) and one derived from the homologous region of Xq21. To the right of the autoradiograms are listed Y or X origins of hybridizing fragments, the Y deletions intervals to which they map (if applicable) and their sizes. The four autoradiograms were generated using genomic DNAs digested with (from top to bottom) *Taq*I, *Pst*I, *Eco*RI and *Eco*RI. Note that in the case of λOX27-H6, the size of the male-specific restriction fragment detected differs between humans, chimpanzees and gorillas.

DNA sequences are highly and continuously similar to Xq21 until ~5 kb distal to the pDP307 locus. Sequences proximal to this point readily cross-hybridized to their X homologs (e.g. probe pDP307 in Fig. 3). As predicted, more distal sequences on Yp showed no evidence of X homology in humans, chimps or gorillas, but are conserved on the Y chromosome among apes (e.g. probe λOX18-H3.5 in Fig. 3).

By contrast, the entirety of the corresponding X-chromosomal phage contig (45 kb in length, with pDP307 cross-hybridizing near its center) appeared to be homologous to the Y chromosome. However, the X-derived contig was homologous to two widely separated portions of Yp. While the distal half of the X contig cross-hybridized to deletion interval 1, in distal Yp, the proximal half cross-hybridized to deletion interval 4A, in proximal Yp. Probe pDP1015, derived from the center of the X contig, hybridized to both Y intervals 1 and 4A (Fig. 3).

Using DNA fragments derived from the proximal half of the X contig as hybridization probes, we isolated λ phage clones from Y interval 4A. We then compared these Y interval 4A clones with clones from Y interval 1 and from the X chromosome, again employing cross-hybridization, Southern blotting of human genomic DNAs (Fig. 3) and comparative restriction mapping (Fig. 4A). This three-way comparison revealed the following: (i) As on distal Yp (interval 1), X homology is sharply discontinuous in proximal Yp (interval 4A). Beyond the discontinuity lie sequences that are largely Y-specific and that are conserved on the Y chromosome among apes (e.g. probe λOX27-H6 in Fig. 3); and (ii) In Xq21, sequences homologous to distal Yp (interval 1) are immediately adjacent to sequences homologous to proximal Yp (interval 4A). X-derived probe pDP1015, which hybridizes to both intervals 1 and 4A of the Y chromosome (Fig. 3), straddles the junction.

The simplest interpretation of all these findings, together with previous studies from other investigators (13,15,20–22), is as follows (Fig. 2): (i) that the two X-homologous blocks now dispersed to Y intervals 1–2–3 and interval 4A once formed a single, contiguous block on an ancestral Y chromosome (see 'hominid Y' in Fig. 2). Band q21 of the present-day X chromosome retains a close homolog of that single, contiguous block. (ii) That a Yp inversion, occurring at some point during human evolution, broke the single Xq21-homologous block into the present-day interval 1–2–3 and interval 4A blocks. One inversion breakpoint fell a few kilobase pairs from the pDP307 locus, within X-homologous sequences. The other inversion breakpoint fell within a region of Y-specific sequences (represented by λOX18 and λOX27), accounting for the juxtaposition of Y-specific and X-homologous sequences at both the interval 1 and 4A junctions created by the inversion.

To confirm the occurrence and reconstruct the details of this inversion, we then sequenced both the interval 1 junction (contained in plasmid pDP1094) and the 4A junction (plasmid pDP1034) as well as the single, homologous segment of the X chromosome (plasmid pDP1015). Comparative restriction mapping of the three plasmids suggested that the Y inversion breakpoint fell within sequences homologous to a 0.6 kb *Spe*I–*Xmn*I fragment on the X chromosome (Fig. 4A). Sequencing of this segment of the X chromosome, and of the partially homologous segments of Y intervals 1 and 4A, confirmed this inference. The three sequences were aligned readily about a 9 bp segment (CATTATTCT) that they all shared (Fig. 4B). To the 5′ side of the CATTATTCT, the X chromosome and Y interval 1 sequences were nearly identical, with no insertions or deletions and only occasional nucleotide substitutions. Conversely, 3′ of the CATTATTCT, the X chromosome and Y interval 4A sequences were nearly identical, again with no insertions or deletions and only a few nucleotide substitutions. These nucleotide sequence findings are completely consistent with a Yp inversion during human evolution, and they suggest that the inversion was mediated by aberrant but homologous recombination between similar or identical 9 bp sequences on distal and proximal Yp. We surmise that the Xq21 sequence we determined closely resembles one of the Yp sequences that participated in this homologous recombination event, and that the other 'parental' sequence can be reconstructed from the Y-specific (non-shaded) portions of the Y interval 4A and interval 1 sequences shown in Figure 4B. Curiously, though the two parental sequences show little similarity apart from the CATTATTCT 9mer that they share, both parental sequences correspond to segments of LINE-1 repeats. It appears that the Yp inversion was mediated by

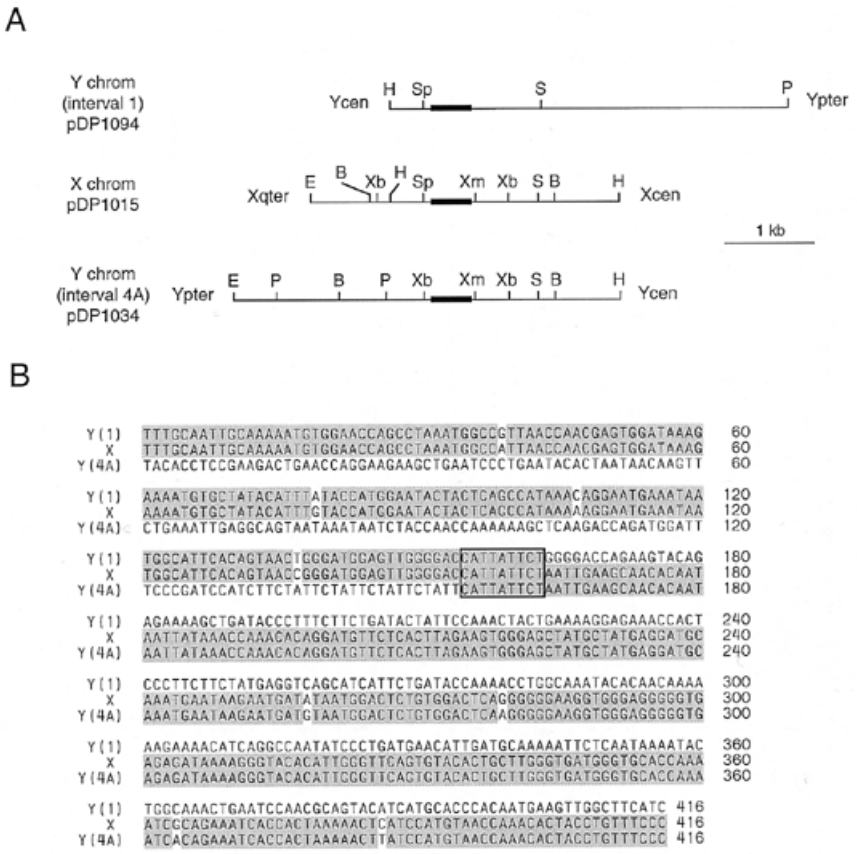

Figure 4. Defining Yp inversion breakpoints by (A) restriction mapping and (B) nucleotide sequence analysis of partially homologous portions of Y interval 1 (plasmid pDP1094), the X chromosome (plasmid pDP1015) and Y interval 4A (plasmid pDP1034). (**A**) Both Yp inversion breakpoints fall within sequences homologous to an *Spe*I–*Xmn*I fragment on the X chromosome: the *Spe*I site is shared with Y interval 1, and the *Xmn*I site is shared with Y interval 4A. Restriction site abbreviations: B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sac*I; Sp, *Spe*I; Xb, *Xba*I; Xm, *Xmn*I. Only portions of inserts of pDP1094, pDP015 and pDP1034 are shown; thick lines indicate 416 bp regions whose sequences are shown in (B). (**B**) Nucleotide sequences. Regions of pairwise nucleotide sequence identity are shaded; the 9 bp region of identity among all three sequences is boxed. GenBank accession numbers: pDP1094, AF008131; pDP1015, AF008133; pDP1034, AF008132.

recombination between two LINE-1 elements that were aligned out of register (Fig. 5).

Transposition and inversion during hominid evolution

When during mammalian evolution did the X–Y transposition and the subsequent Yp inversion occur? By quantitative Southern blotting studies of human and ape genomic DNAs, we previously had demonstrated that the *DXYS1* locus is present on human Yp (in interval 4A) by virtue of an X–Y transposition that occurred after the divergence of the human and chimpanzee lineages (10). If the entirety of the Xq21–Yp homology resulted from a single X–Y transposition, then one would expect parallel findings when hybridizing other Xq21–Yp probes to Southern blots of human and ape genomic DNAs. Indeed, our laboratory and others have obtained similar results for other human Xq21–Yp loci, including several in the distal Yp (interval 1–2–3) block (13) (e.g. pDP307 and pDP1015 in Fig. 3). These results confirm that the X–Y transposition responsible for this extensive X–Y homology occurred after the divergence of the human line from that of our closest extant relatives, chimpanzees—a split that is thought to have occurred between 4 and 8 million years (Myr) ago (27,28).

Can we estimate more precisely when the transposition occurred? Li and colleagues have studied the relative rates of accumulation of presumably neutral nucleotide substitutions on the primate X and Y chromosomes (29). A simple analysis of the data of Li and colleagues suggests that, among primates, the rate of X–Y divergence (at selectively neutral sites) is ~0.2% per Myr. Based on our measurement of 0.7 ± 0.2% nucleotide sequence divergence between the homologs on human Yp and Xq21, we propose that the X–Y transposition occurred ~3–4 Myr ago, probably a few million years after the hominid–chimpanzee split, perhaps near the time of the emergence of our genus, *Homo*, but well before the emergence of our species, *H.sapiens*. This estimate is at odds with the conclusions of Lambson and colleagues, who suggested that an Xq–Yp transposition had occurred ~8 Myr ago. As discussed earlier, Lambson and colleagues appear to have overestimated X–Y sequence divergence, and this in turn caused them to overestimate the age of the transposition.

If the X–Y transposition occurred ~3–4 Myr ago, how much more recently did the Yp inversion occur? Did the inversion occur before or after the primary radiation of human racial groups, estimated to have occurred perhaps 100 000–200 000 years ago (30–33)? To address this question, we tested genomic DNAs from males of diverse racial ancestry (African, Asian and Caucasian) by Southern blotting for the presence of one or more

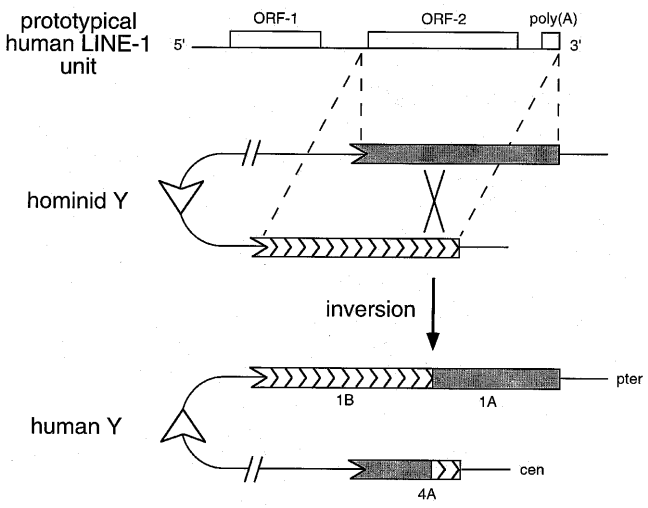

Figure 5. Recombination between LINE-1 elements generated Yp inversion. At the top is schematically diagrammed a full-length, prototypical human LINE-1 element, showing the location of two long open reading frames (ORFs) and the polyadenylated tail. Below is shown a model of inversion, involving recombination between two LINE-1 repeats aligned out of register, as indicated. Note that only a few hundred base pairs surrounding the recombination breakpoints have been sequenced (Figure 4B), and thus the LINE-1 elements involved were not necessarily full length. Deletion intervals on modern human Y chromosome are indicated.

restriction fragments specific to the distal inversion junction, in interval 1. All human males examined were found to contain these fragments (not shown). We conclude that the Yp inversion occurred before the radiation of human racial groups.

DISCUSSION

In summary, it appears that a massive X–Y transposition occurred ~3–4 Myr ago, subsequent to the human–chimpanzee split but prior to the emergence of *H.sapiens*, creating the single largest block of sequence similarity between the hominid X and Y chromosomes. Subsequently, but prior to the radiation of human racial groups, a Yp inversion, mediated by homologous recombination between misaligned LINE-1 elements, disrupted that block of X homology, generating the bipartite structure found on the present-day human Y chromosome.

While this Yp inversion is, to our knowledge, the largest rearrangement in a primate genome to be defined at the nucleotide level, many other comparable or even larger rearrangements have been recognized through comparative chromosome banding studies. In the great apes, for example, known differences among species include almost two dozen pericentric inversions as well as a host of transpositions, paracentric inversions and telomeric fusions (34,35). Perhaps many of these evolutionary rearrangements involved homologous recombination between interspersed repetitive elements—the mechanism responsible for the Yp inversion studied here and also responsible for numerous mutations and chromosome abnormalities arising in human populations (36–38).

The Y chromosome may be uniquely tolerant of inversions and other rearrangements during evolution. For other chromosomes, all of which normally undergo recombination throughout their lengths, large inversions may impair meiosis, cause repeated abortions or result in recombinational aneuploidy among offspring (39,40). By contrast, the bulk of the Y chromosome shows strictly sex-linked (as opposed to pseudoautosomal) inheritance, and here the absence of recombination during meiosis means that inversions arising in one generation would be unlikely to disrupt meiosis or create gene imbalances in subsequent generations. Moreover, there appears to be a low density of single-copy genes in the strictly sex-linked region of the human Y chromosome. Recent findings suggest that the majority of transcription units in the strictly sex-linked region are members of Y-specific gene families (41–44). Thus, assuming that breakpoints are randomly distributed and that positions effects are modest, Y inversions should rarely disrupt non-redundant functions. Indeed, Y inversion polymorphisms, associated with no phenotypic abnormalities, are frequently observed in human populations (45).

Investigators have postulated numerous inversions or other rearrangements on the Y chromosome during human evolution to explain: (i) the complex pattern of sequence homologies to the human X chromosome; and (ii) occasional discrepancies in maps of the Y chromosome based on naturally occurring deletions (e.g. refs. 10–14,46). Apart from the Yp inversion described in this report, these postulated events have yet to be reconstructed at the nucleotide level. The insights into X–Y transposition and Yp inversion reported here were gained from focused comparisons of X and Y nucleotide sequence, on a very modest scale. During the next several years, large-scale sequencing of the human sex chromosomes should make possible a comprehensive nucleotide comparison of the X and Y chromosomes. We anticipate that comprehensive X–Y sequence analysis will provide definitive evidence of multiple inversions and other Y rearrangements that occurred during human evolution.

Implications for Turner syndrome

Because the Xq21–Yp homologies are massive and characterized by such high X–Y sequence similarity, they merit close scrutiny for genes that play critical roles in Turner syndrome. Turner syndrome, a complex phenotype classically associated with monosomy X, probably results from haploinsufficiency of certain genes common to the X and Y chromosomes (47). Embryos with a 45,X karyotype develop as phenotypic females with poor viability *in utero*. Those that survive to term usually develop ovarian failure, short stature and specific anatomical abnormalities that include webbing of the neck (48). Different ‘Turner genes’ may be responsible for different aspects of the phenotype (49).

Although the Xq21–Yp sequences constitute about a third of all X–Y homology, theoretical considerations and karyotype–phenotype correlations lead us to predict that no more than a few critical Turner genes will be found there. While Turner genes are expected to escape X inactivation (47,49), we suspect that most or all genes in the Yp-homologous region of Xq21 would undergo X inactivation. Homologous genes on the hominid X chromosome probably would have undergone X inactivation prior to the Yp transposition—a condition unlikely to have been altered by the appearance of a homolog on the Y chromosome.

These theoretical considerations are bolstered by karyotype–phenotype observations. Among the individuals whose genomic DNA we studied is a 46,XY male with an interstitial Xq21 deletion spanning the entirety of the Yp-homologous region (patient NP in Fig. 1). In this case, the interstitially deleted X chromosome was inherited from the mother, who was obviously

fertile, and it was also present in a phenotypically unremarkable sister (50). This 46,XY male, his mother and his sister appear to be monosomic for all Xq21–Yp homologous sequences, just as a 45,X Turner female would be. Both the boy and his mother exhibited cubitus valgus, a frequent finding in Turner syndrome, suggesting that a gene for this particular Turner trait might be located among the Xq21–Yp sequences. However, the mother and sister were reported to exhibit no other Turner features (50), suggesting that monosomy for Xq21–Yp homologous sequences is not responsible for other components of the Turner phenotype. With the possible exception of a gene for cubitus valgus, the search for Turner determinants should be directed primarily to other regions of the sex chromosomes containing X–Y common sequences.

MATERIALS AND METHODS

DNA markers

All DNA markers employed are listed in Table 1, Table 2 or Figure 1. Most of these probes derived from a library of genomic *Hin*dIII restriction fragments prepared from flow-sorted human Y chromosomes. This library, kindly provided by Marvin Van Dilla, was constructed in λ phage 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 US Department of Energy. Several of the remaining probes were generous gifts of Jean Weissenbach and derived from a cosmid library prepared from a human–rodent hybrid cell line retaining the human Y (17,51,52). Four Yp–Xq21 hybridization probes used here (pDP7a, pDP61, pDP1057 and St25–2.7) are plasmid subclones of previously reported clones (p7b, p115, p8j and St25/2, respectively) (17,18). Additional probes were derived from chromosome walking in λ phage libraries of human genomic DNAs (26).

Southern blot analysis

Human or ape genomic DNAs were prepared from blood or cultured cell lines, digested with restriction endonucleases, electrophoresed on 0.75% agarose gels and transferred to nylon membranes (26). Human inserts purified from recombinant plasmids or λ phage clones were labeled with ^{32}P by random-primer synthesis (53). The resulting probes were hybridized overnight to Southern blots at concentrations of 5×10^5–3×10^6 c.p.m./ml at 42°C ('moderate' stringency) or 47°C ('high' stringency) in 50% formamide, 5× SSC (1× SSC = 0.15 M NaCl, 15 mM Na citrate pH 7.4), 1× Denhardt's (0.02% Ficoll 400, 0.02% polyvinyl pyrrolidone, 0.02% bovine serum albumin), 1% SDS, 50 mM $NaPO_4$ pH 6.6 and 0.01% yeast tRNA. Prior to hybridization, some probes were pre-hybridized with an excess of sonicated human genomic DNA (54). Following hybridization, blots were washed three times for 20 min each at 65°C in 0.1× SSC, 0.1% SDS.

Comparative nucleotide sequencing of X and Y chromosomes

At many Yp–Xq21 STSs (Table 1), we separately determined the nucleotide sequences of the X and Y chromosomes by dideoxy chain termination (55). Y chromosomal sequencing templates were derived by PCR amplification from appropriate Y chromosomal YACs (2). X chromosomal sequencing templates were derived by PCR amplification from human female genomic DNA. In all cases, both strands of the PCR products were sequenced, and only regions sequenced to high confidence were used in X–Y comparisons. Portions of the human genomic inserts of X-derived plasmid pDP1015 and Y-derived plasmids pDP1094 and pDP1034 were also sequenced (Fig. 4).

X- and Y-specific PCR assays at X–Y homologous loci

For most Yp–Xq21 loci, the previously reported PCR assays (1) do not discriminate between the X and Y chromosomes. We took advantage of occasional X–Y nucleotide substitutions to devise X- or Y-specific PCR assays at eight loci (Table 2), in most cases employing a nested (four-primer) or semi-nested (three-primer) strategy. For all nested and semi-nested assays, PCR was first carried out using human genomic DNAs, STS primers and conditions as previously reported (1). These primary amplification products were then diluted 1:10, and 1 µl was transferred to a second PCR reaction; in this case, the primers were those listed in Table 2. Each primary amplification involved two X–Y common primers, and each secondary amplification involved one X–Y common and one X- or Y-specific primer. In the case of semi-nested (three-primer) assays, the X–Y common primer used in the secondary amplification is identical to one of the primers used in the primary amplification.

To achieve X or Y specificity, secondary amplifications were optimized as follows. All reactions were performed in 20 µl of 5 mM NH_4Cl, 10 mM Tris (pH 8.2 at 25°C), 1.5 mM $MgCl_2$, 50 mM KCl, 0.5 mM dNTPs and 1–1.2 U of *Taq* DNA polymerase. Each primer was at a concentration of 0.35 µM (assays sXY29-X, -Y), 0.5 µM (sXY47-X, -Y), 1 µM (sXY22-X, -Y; sXY28-X, -Y; sXY43-X, -Y; sXY48-X, -Y) or 1.95 µM (sXY38-X, -Y). The cycling protocol included an initial 5 min denaturation (94°C); 18 cycles of 1 min denaturation (94°C), 1.5 min annealing, 1 min extension (72°C); and finally 10 min at 72°C. Annealing temperatures were 53°C (assays sXY38-X, -Y); 55°C (sXY47-X, -Y); 57°C (sXY28-X, -Y; sXY48-X, -Y); 58°C (sXY29-X, -Y); or 59°C (sXY22-X, -Y; sXY43-X, -Y). All reactions were performed using an MJ Research thermal cycler. PCR products were analyzed by gel electrophoresis in 2% agarose.

In the case of STS sXY37, X-specific and Y-specific primers were chosen within and flanking a 17 bp insertion on the X chromosome, and no primary amplification was required. PCR conditions were as above except that 100 ng of human genomic DNA and 1.5 U of Taq DNA polymerase were used, primer concentrations were 1 µM, annealing was at 42°C and 35 cycles were performed.

In the case of Y-specific loci whose presence or absence was scored by PCR, primers and cycling conditions were as previously described (1).

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