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Steroid Sulfatase Gene in XX Males

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Summary

The human X and Y chromosomes pair and recombine at their distal short arms during male meiosis. Recent studies indicate that the majority of XX males arise as a result of an aberrant exchange between X and Y chromosomes such that the testis-determining factor gene (*TDF*) is transferred from a Y chromatid to an X chromatid. It has been shown that X-specific loci such as that coding for the red cell surface antigen, Xg, are sometimes lost from the X chromosome in this aberrant exchange. The steroid sulfatase functional gene (*STS*) maps to the distal short arm of the X chromosome proximal to *XG*. We have asked whether *STS* is affected in the aberrant X-Y interchange leading to XX males. DNA extracted from fibroblasts of seven XX males known to contain Y-specific sequences in their genomic DNA was tested for dosage of the *STS* gene by using a specific genomic probe. Densitometry of the autoradiograms showed that these XX males have two copies of the *STS* gene, suggesting that the breakpoint on the X chromosome in the aberrant X-Y interchange is distal to *STS*. To obtain more definitive evidence, cell hybrids were derived from the fusion of mouse cells, deficient in hypoxanthine phosphoribosyltransferase, and fibroblasts of the seven XX males. The X chromosomes in these patients could be distinguished from each other when one of three X-linked restriction-fragment-length polymorphisms was used. Hybrid clones retaining a human X chromosome containing Y-specific sequences in the absence of the normal X chromosome could be identified in six of the seven cases of XX males. All of these clones had *STS* sequences and expressed human steroid sulfatase activity, in keeping with the findings from dosage studies. These results show that *STS* is located proximal to the breakpoint on the X and is not lost from the *TDF*-bearing X chromosomes in these XX males.

Introduction

Human X and Y chromosomes pair at the distal ends of their short arms during male meiosis (Pearson and Bobrow 1970). There is formation of a synaptonemal complex between X and Y chromosomes in this region (Moses et al. 1974; Chandley et al. 1984). Recent molecular studies have shown that there is homology at the DNA level between X and Y chromosomes at their distal short arms, where there is a single obligatory crossing-over between X and Y during meiosis (Cooke et al. 1985; Simmler et al. 1985; Page et al. 1987*a*). As a result, loci mapping in this region do not show strict sex linkage, and hence this homologous segment of the X and Y chromosomes is referred to as the pseudoautosomal region (Burgoyne 1982).

In humans, it is estimated that about 1 in 25,000 newborn males has an XX karyotype (de la Chapelle 1983). Molecular studies have established that the majority of XX males arise as a result of an aberrant exchange between the X and Y chromosomes such that the testis-determining-factor gene (*TDF*), located proximal to the pseudoautosomal region on the Y, is trans-

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ferred from a Y chromatid to an X chromatid (Affara et al. 1986; Andersson et al. 1986; Muller et al. 1986*b*; Page 1986; Vergnaud et al. 1986; Page et al. 1987*b*, 1987*c*; Petit et al. 1987). In humans, the functional gene for the red cell surface antigen, Xg, maps to the distal end of the short arm of the X near but proximal to the pseudoautosomal region (Ferguson-Smith et al. 1982). Evaluation of Xg expression in XX males has shown that XX males do not always express their father's *XG* allele, indicating that the aberrant X-Y interchange leading to the transfer of *TDF* to the paternal X may result in the loss of *XG* from the X chromosome (de la Chapelle 1986; Petit et al. 1987). The functional locus for the steroid sulfatase (STS) gene (*STS*) also maps to the distal short arm of the human X at an estimated genetic distance of 13 cM proximal to *XG* (reviewed in Shapiro 1985; Yates et al. 1987). It is therefore of interest to determine whether *STS* is lost from the *TDF*-bearing X chromosome in XX males.

Earlier studies determined copy number of *STS* in XX males by assaying the activity of the enzyme in cultured fibroblasts (Pierella et al. 1981; Ropers et al. 1981). The results showed that, of a total of 20 XX males evaluated, 17 had STS levels in the female range, indicating that they had two copies of *STS*, while three had activities in the male range, suggesting that only a single copy of *STS* was present in their genome. Results of dosage studies are sometimes difficult to interpret in the case of *STS*, as this gene does not appear to escape X inactivation completely and as the ratio of female- to male-specific activities is less than 2 and averages about 1.6 (reviewed in Shapiro 1985). Recently, cDNA and genomic probes for *STS* have been isolated (Ballabio et al. 1987; Bonifas et al. 1987; Conary et al. 1987; Yen et al. 1987, 1988). A nonprocessed pseudogene for *STS* was identified on the Y chromosome (*STSP*) and maps to the proximal long arm of the Y (Fraser et al. 1987; Yen et al. 1987, 1988). Using a cDNA probe for *STS*, Schempp et al. (1989) have recently reported the presence of two copies of *STS* in 10 of 11 XX males tested by gene dosage. We have used a genomic probe for *STS* to determine the copy number of *STS* in XX males by gene dosage. In addition, the presence of *STS* on the *TDF*-bearing X chromosomes of XX males was evaluated following their isolation in somatic cell hybrids.

Material and Methods

Cell Lines

A total of nine fibroblast cultures were obtained from a number of laboratories and from the Human Genetic Mutant Cell Repository in Camden, NJ. Previous studies have shown that XX male lines GM1889 and 385 have no Y-specific sequences in genomic DNA, whereas XX male cell lines GM 2670, 510, 102, 547, 693, and 775 have Y-specific sequences in genomic DNA (Page et al. 1985; Muller et al. 1986*a*, 1987; Vergnaud et al. 1986). Cell line S.R. had not been tested for Y-specific sequences previously.

Cell Hybridization

Fibroblasts from XX males S.R. (CF128-), GM2670 (CF132-), 547 (CF134-), 102 (CF135-), 510 (CF137-), 693 (CF141-), and 775 (CF142-) were fused with the established mouse cell line, A9, deficient in hypoxanthine phosphoribosyltransferase (HPRT), and multiple independent hybrid clones were isolated in HAT medium according to a method described elsewhere (Mohandas et al. 1986). The prefix for hybrid clones isolated from each of the XX males is indicated in parentheses. Cytogenetic analysis of cell hybrid clones was done with the aid of Q-banding on a minimum of 10 photographed metaphases/individual hybrid clone (Mohandas et al. 1986). Hybrid clones with intact human X chromosome(s) and no detectable rearrangements in the human autosomes retained were used for further study.

Probes and Filter Hybridization

High-molecular-weight DNA was extracted from fibroblast cultures and hybrid cell lines according to a method described elsewhere (Yen et al. 1984). A 1.6-kb genomic fragment, which includes the first exon of the functional *STS* on the X that does not cross-hybridize with the *STSP* sequences, was used as a probe for determining dosage of *STS* in XX males. An autosomally encoded anonymous cDNA probe, λ21 (P. H. Yen, unpublished data) was used as an internal reference for hybridization intensities in dosage studies. To determine the presence of Y-specific sequences, several probes were used. pDP1007 codes for *ZFY* (zinc-finger protein on Y), which is a candidate gene for *TDF* (Page et al. 1987*c*; Page 1988). *ZFY* is located just proximal to the pseudoautosomal region in interval 1A2 of the Y chromosome (Page 1986; Page et al. 1987*c*). Additional probes used for detecting Y-specific fragments were pDP132 (interval 1; Ramsay et al. 1988), pDP61 (interval 2; Ramsay et al. 1988), 50f2/A,B (interval 3; Guellaen et al. 1984), and pDP105/A (interval 3; Ramsay et al. 1988). To distinguish between the two X chromosomes of the XX males isolated in somatic cell

hybrids, RFLPs identified by the following three probes were used: p19B, a genomic probe that specifies the *MIC2* locus which is pseudoautosomal (Goodfellow et al. 1986); DX13 (DXS15), which recognizes a single-copy sequence from the distal end of the long arm of the X chromosome (Drayna et al. 1984); and 113D (DXYS15), which detects an anonymous pseudoautosomal sequence (Simmler et al. 1985). Presence of *STS* sequences on the X chromosomes in XX males was determined using a full-length cDNA probe for *STS* (λ331, Yen et al. 1987). An additional X-specific probe 38j (DXS283; Levilliers et al. 1989), which maps distal to *STS* on the X short arm, was also tested on hybrids retaining X chromosomes from XX males. DNA was digested with restriction enzymes, transferred to nitrocellulose filters, and hybridized to probes according to a method described elsewhere (Yen et al. 1984; Page et al. 1987*c*).

STS Activity

Expression of human STS activity in mouse-human cell hybrid clones was determined according to a method described elsewhere (Shapiro et al. 1978; Mohandas et al. 1980).

Results

Presence of Y-specific Sequences in XX Male DNA

The presence of Y-specific sequences in the XX male DNA was reevaluated using pDP1007, a candidate gene for *TDF,* and additional probes from different deletion intervals of the Y chromosome (deletion intervals are as defined by Page [1986], Vergnaud et al. [1986], Page et al. [1987*c*]). Two of nine XX males, 385 and GM1889, did not have any detectable Y-specific fragments, consistent with previous findings in these patients (Muller et al. 1986*b,* 1987). The remaining seven XX males showed the presence of Y-specific fragments, all being positive for pDP1007 and pDP132 (table 1). Some of the XX males were positive for additional Y-specific fragments from deletion intervals 2 and 3. These seven DNA samples were also tested for the presence of seven additional probes from intervals 4A–7 of the Y and were found to be negative (data not shown). Interval 4A of Y represents the proximal short arm, and interval 7 is the distal, fluorescent, heterochromatic region of the long arm of the Y chromosome (Page 1986). These results show that seven of the XX males most likely originated from an aberrant exchange between X and Y chromosomes.

Table 1

Evaluation of Y-specific Restriction Fragments in XX Males

	PROBE (deletion interval[a])				
XX Male	pDP1007 (1A2)	pDP132 (1)	pDP61 (2)	50f2/A,B (3)	pDP105/A (3)
GM1889	–	–	–	–	–
385	–	–	–	–	–
510	+	+	–	–	–
102	+	+	–	–	–
GM2670	+	+	+	–	–
547	+	+	+	+	+
693	+	+	+	+	+
775	+	+	+	+	+
S.R.	+	+	+	+	+

NOTE.—A plus sign (+) indicates presence of Y-specific restriction fragments; a minus sign (–) indicates absence of Y-specific restriction fragments.

[a] Deletion interval on the Y, as defined by Page (1986), Vergnaud et al. (1986), and Page et al. (1987*c*).

Dosage Studies of STS in XX Males

We estimated the dosage of *STS* in the XX males who have gained Y-specific sequences using a genomic probe (1.6 kb) that includes the first exon of *STS* and does not cross-hybridize with *STS* sequences on the Y chromosome. In *Eco*RI-digested human genomic DNA it hybridizes to a single fragment of 9.2 kb. Approximately 5 μg DNA from nine XX males as well as from male and female controls was digested with *Eco*RI and hybridized to the *STS* probe. As control, the DNA samples were also hybridized to probe λ21, which is an anonymous cDNA probe that detects an autosomal locus and hybridizes to a single 7.4-kb fragment in *Eco*RI-digested human genomic DNA (P. H. Yen, unpublished data) (fig. 1). The relative intensity of hybridization of the two probes in these XX males is comparable to that in normal females and different from that in normal males, indicating that the XX males have two copies of *STS* in their DNA (fig. 1). The relative intensity of the two bands was quantitated by densitometry of the autoradiograms, and the results are presented in table 2. The values ranged from 1.59 to 2.89 in these XX males, compared with 0.9 and 2.0 in the male and female controls, respectively. Although there is variability in the intensity measurements, the values are consistent with the XX males having two copies of *STS.* These results suggest that the *TDF*-bearing X chromosome in the XX males have retained at least the 5′ sequences of *STS.*

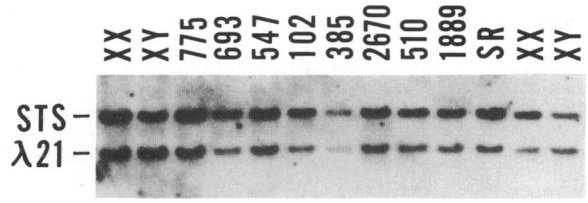

Figure 1 Dosage of *STS* in XX males. DNA samples were digested with *Eco*RI. A 1.6-kb genomic fragment of *STS* that does not cross-hybridize with *STSP* on the Y chromosome was used as a probe; it hybridizes to a 9.2-kb band. λ21 is an autosomally encoded anonymous cDNA probe, which detects a 7.4-kb fragment. XX and XY are female and male controls, respectively. Lanes 775, 693, 547, 102, 385, 2670 (GM2670), 510, 1889 (GM1889), and S.R. contain DNA from XX males. The relative intensity of the signal for *STS* in all nine XX males are comparable with that in XX and different from that in XY controls.

Somatic Cell Genetic Studies

To obtain more definitive evidence for the presence of *STS* on both X chromosomes in XX males, somatic cell hybrids were generated from the fusion of mouse A9 cells and fibroblasts of the seven XX males who showed the presence of Y-specific sequences in their DNA. Hybrids were selected in HAT medium for the retention of an active X chromosome. Multiple independent hybrid clones were isolated from each of the fusions. Cytogenetic analyses were done on at least 10 Q-banded, photographed metaphases in each case to ensure that there was an intact human X chromosome(s) and that there were no detectable in vitro rearrangements among the human autosomes retained. Heterozygosity for an X-linked RFLP was identified in each of the seven cases of XX males by using probe p19B, DX13, or 113D. DNA isolated from the hybrid clones was screened for the presence of the relevant RFLP, and clones retaining each of the X chromosomes in the absence of the other were identified. The clones were also screened for the presence of *STS* sequences, by using a cDNA probe, and for the presence of Y-specific fragments, by using probe pDP132. Results obtained from two different XX males are shown in figure 2. XX male S.R. was heterozygous for a 3.5-kb/2.7-kb RFLP detected by probe p19B following *Taq*I digestion. Four hybrid clones derived from the fusion of S.R. fibroblasts and mouse A9 cells retaining a single human X chromosome were analyzed. Clones CF128-2 and CF128-13 retained the X chromosome carrying the 3.5-kb allele of p19B, whereas clones CF128-4 and CF128-5 retained the X chromosome carrying the 2.7-kb allele. Probe pDP132 detects a 4.4-kb Y-specific band in *Taq*I-digested human DNA. It also detects X-specific bands of size 3.2 kb or 3.2 kb and 5.7 kb. Hybridization of

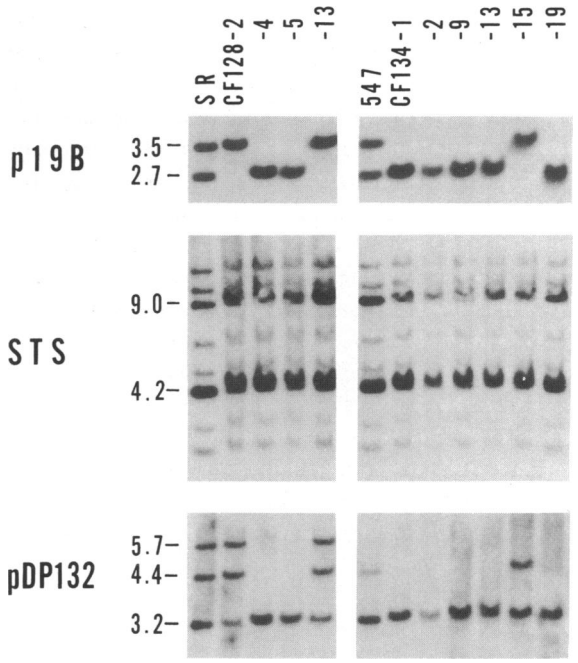

Figure 2 Southern analysis of DNA from fibroblasts of XX males S.R. and 547 and mouse-human cell hybrids (CF128s and CF134s, respectively) derived from them. DNA was digested with *Taq*I and hybridized to the pseudoautosomal probe p19B (*MIC2*) or to pDP132, which detects a Y-specific fragment of 4.4 kb. DNA was digested with *Eco*RI for hybridization to the *STS* cDNA probe. Patients S.R. and 547 are heterozygous for the RFLP detected by p19B, and the hybrid clones have one or the other X chromosome. All clones have STS sequences; however, only clones retaining the 3.5-kb fragment for p19B have Y-specific sequences. pDP132 detects X-specific fragments of 3.2 kb or 3.2 kb and 5.7 kb.

Table 2

Densitometric Quantitation of *STS* Copy Numbers in XX Males

STS Signal Intensity/λ21 Signal Intensity Ratio										
XY	XX	775	693	547	102	385	GM2670	510	GM1889	S.R.
.90	2.00	1.97	2.89	1.75	2.58	1.89	1.59	1.8	2.86	2.21

the blot to pDP132 shows that only clones CF128-2 and CF128-13 contain the 4.4-kb Y-specific fragment (fig. 2). These results indicate that in S.R. the X chromosome containing the 3.5-kb allele for p19B is the one that has undergone an aberrant exchange with the Y chromosome. Hybridization of the same DNA samples to *STS* cDNA shows that all of the hybrid clones, regardless of the X chromosome retained, have functional *STS* sequences. These clones were also positive for the expression of human STS activity. These results thus show that functional *STS* sequences are not lost from the *TDF*-bearing X chromosome in patient S.R. Similarly, XX male 547 was also heterozygous for the 3.5-kb/2.7-kb RFLP detected by p19B (fig. 2). Southern analysis of six hybrid clones derived from this XX male showed that clones CF134-1, -2, -9, -13, and -19 retained the X chromosome with the 2.7-kb allele, while one clone, CF134-15, retained the X chromosome with the 3.5-kb allele. This latter clone was positive for the Y-specific 4.4-kb fragment detected by pDP132, indicating that it contains the X chromosome that has undergone the aberrant exchange with the Y. Again, all of these clones were positive for *STS* sequences (fig. 2) and human STS activity.

Similar analyses were carried out in the remaining five XX males, and we were able to isolate the X chromosome that has undergone an aberrant exchange with the Y in four of the five cases. The results from these four cases are summarized in table 3. The fifth case, XX male 693, was found to be heterozygous for the RFLP detected by probe p19B. Analysis of seven independent hybrid clones showed that two of them retained a single X chromosome and that five had two human X chromosomes. Clones with a single human X did not show the presence of Y-specific DNA. Clones with two X chromosomes had Y-specific fragments. Thus we were unable to determine the presence of *STS* sequences on the X chromosome that had undergone an exchange with the Y in case 693 by using somatic cell hybrids. In case GM2670, analysis of six clones showed that each of them retained a human X that contained Y-specific sequences (table 3). Thus, in this case we did not identify a cell hybrid retaining the normal X chromosome.

Table 3

Evaluation of *STS* on the X Chromosomes from XX Males Isolated in Somatic Cell Hybrids

XX Male	RFLP (kb)	pDP132 (kb)	*STS* Sequences	STS Activity[a]
102:	3.5/2.7[b]	4.4/3.2[c]	+	ND
Hybrid clones:[d]				
CF135-5	2.7	3.2	+	+
CF135-20	2.7	3.2	+	+
CF135-15	3.5	4.4/3.2	+	+
CF135-29	3.5	4.4/3.2	+	ND
775:	3.5/2.7[b]	4.4/3.2	+	ND
Hybrid clones:				
CF142-3	3.5	4.4/3.2	+	+
CF142-16	2.7	3.2	+	+
510:	2.8/2.5[e]	4.4/3.2	+	ND
Hybrid clones:				
CF137-18	2.8	3.2	+	+
CF137-21	2.5	4.4/3.2	+	+
GM2670:	6.5/3.3[f]	4.4/3.2	+	ND
Hybrid clones:				
CF132-9, -16, -18, -19, -24, and -26	6.5	4.4/3.2	+	+

[a] ND = not determined.
[b] Detected by probe p19B, after *Taq*I digestion.
[c] pDP132 detects a 4.4-kb Y-specific fragment in *Taq*I-digested human DNA which is derived from interval 1 of the Y chromosome (see text).
[d] Each of the hybrid clones analyzed had a single intact human X chromosome.
[e] Detected by probe 113D after *Taq*I digestion.
[f] Detected by probe DX13 after *Bgl*II digestion.

These results clearly show that, in the six cases of XX males studied using somatic cell hybrids, the X chromosome containing Y-specific sequences retain *STS* sequences. In addition, these hybrid clones were also positive for the presence of sequences hybridizing with an X-specific probe, 38j (DXS283; data not shown). Using a somatic cell hybrid clone that contains an X/Y translocation with a break on the X between *STS* and *MIC2* (Geller et al. 1986), we determined that sequences hybridizing with 38j map distal to *STS* (data not shown). This is consistent with the findings of Levilliers et al. (1989), who also map 38j distal to *STS*. Thus the presence of 38j in the hybrid clones retaining *TDF*-bearing X chromosomes further suggests that the point of exchange is located distal to *STS* on the X.

Discussion

Seven XX males containing Y-specific DNA in their genome were evaluated for copy number of *STS* by gene dosage by using a genomic probe for *STS*. The results were consistent with the presence of two copies of *STS* in these XX males, suggesting that the *TDF*-bearing X chromosomes in these XX males have retained *STS*. To obtain more definitive evidence, we isolated the *TDF*-bearing X chromosome from six of these seven cases in somatic cell hybrids. Southern analyses and enzymatic studies of these cell hybrids showed that these X chromosomes retained functional *STS* sequences. These results show that the breakpoint of the X in the aberrant X-Y interchange producing these males is distal to the *STS* locus. These findings are consistent with previous observations in XX males based on quantitation of STS activities in fibroblasts. Of 20 XX males tested, three had values in the male range, indicating that they may have only a single copy of *STS* (Pierella et al. 1981; Ropers et al. 1981; Wieacker et al. 1983). One of these patients reported by Pierella et al. (1981) is GM2670 (A. de la Chapelle, personal communication). Our studies on GM2670 clearly show that the *TDF*-bearing X in this case has retained the *STS* sequences; thus the low level of STS activity in this case must be attributed to reasons other than loss of *STS* sequences. A second patient with STS activity in the male range was studied using somatic cell hybrids (Wieacker et al. 1983). Hybrid clones were isolated in HAT medium, and only a few of them expressed human STS activity. Clones without human STS activity expressed human glucose-6-phosphate dehydrogenase and phosphoglycerate kinase, indicating retention of an intact human X (Wieacker et al. 1983). These results suggest that *STS* sequences may be lost from one of the X chromosomes in this XX male. Our results are also consistent with those reported recently by Schempp et al. (1989), who showed the presence of two copies of *STS* in 10 of 11 X-Y-interchange XX males tested by gene dosage by using a cDNA probe for *STS*. The exceptional XX male with a single copy of *STS* was the patient reported by Wieacker et al. (1983). Taken together, these studies indicate that *STS* sequences are not lost from the *TDF*-bearing X chromosome in the majority of XX males.

In contrast, there are several reported cases of XX males who have failed to express their paternal *XG* allele, presumably as a result of its loss in the aberrant X-Y interchange (de la Chapelle 1986). These findings are consistent with the mapping data which place *XG* more distal to *STS*, at an estimated genetic distance of 13 cM (Yates et al. 1987). In addition, *DXS283* (detected by probe 38j) is retained on the *TDF*-bearing X chromosomes isolated in somatic cell hybrids in the present study. Two copies of *DXS283* have been detected in all XX males tested, including an XX male who failed to express his paternal *XG* allele (J. Weissenbach, personal communication). Since *DXS283* is located distal to STS, these findings further support the premise that the loss of expression of the paternal *XG* allele in XX males is due to its more distal location compared with *DXS283* and *STS*.

Schempp et al. (1988) recently demonstrated random inactivation of an X chromosome in the somatic cells of the XX males evaluated by them, with one possible exception. Our observations in somatic cell hybrids provide further evidence for random inactivation in five of seven XX males tested, as either the normal X or the *TDF*-bearing X was retained in the hybrid clones isolated in HAT medium.

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