Sex Vesicle "Entrapment": Translocation or Nonhomologous Recombination of Misaligned Yp and Xp as Alternative Mechanisms for Abnormal Inheritance of the Sex-Determining Region

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Abnormal inheritance of the sex determining region, normally located on Yp, results in about 1 in 20,000 phenotypic males with a 46,XX genotype. Studies to date indicate that many 46,XX males apparently arise due to a balanced, yet abnormal, nonhomologous interchange of Xp and Yp termini. However, 2 of the 5 XX males we report here have 3 copies of the pseudoautosomal locus, MIC2. Thus, they appear to have inherited the sex determining region as a result of Yp sequences being added onto the X pseudoautosomal region. Such an unequal, extremely nonhomologous interchange could alternatively be considered to arise from an unbalanced translocation of Yp to Xp. Our results suggest that very unequal interchange or translocation of Yp sequences onto the X pseudoautosomal region is not as rare a mechanism for XX males as originally thought. We also suggest that sex vesicle "entrapment" favors the association of a Yp fragment to the X pseudo-autosomal region over a translocation to either Xq or an autosome.

KEY WORDS: XX males, pseudoautosomal region, sex chromosomes

INTRODUCTION

Sex determination in humans depends on the presence or absence of a testis determining factor (TDF) located on the Y chromosome. Structural anomalies of the sex chromosomes have provided the means to study genes responsible for sex determination. Approximately 1 in 20,000 phenotypic males has a 46,XX genotype [de la Chapelle, 1981]. Based on studies of 46,XX males by several groups of investigators [Goodfellow et al., 1985; Page, 1986], it has been proposed that TDF is localized to the Y short arm. Some XX males have visible translocations of Y chromosome material to one of the X chromosomes [Evans et al., 1979; Magenis et al., 1982]. High resolution cytogenetic analyses indicate that the Y fragment in these X:Y translocations is probably a derivative of Yp [Magenis et al., 1982]. Although many 46,XX males have no visible translocation, Southern analysis of DNA from these individuals with probes that detect Yp sequences indicates that all but a few individuals have Yp sequences [Goodfellow et al., 1985; Page, 1986] and *in situ* hybridization with Yp probes indicates that the Y material is located on one X chromosome but not the other in several of the 46,XX individuals studied [Andersson et

al., 1986|. Most recently, Page et al. [1987b]
report cloning a region on Yp that appears
to be sex determining and can encode a
protein similar to other proteins known to
have DNA regulatory properties.

During human meiosis homologous
autosomes and X chromosomes pair over
their entire length. However, the
synaptonemal complex of the sex vesicle in
male meiosis only joins the heterologous X
and Y chromosomes by their short arms
(distal Xp and Ycent-Ypter) [Moses et al.,
1975|. Only in a small distal portion of this
pairing region, the pseudoautosomal region,
is there sequence homology between Xp and
Yp [Rouyer et al., 1986a]. It has long been
considered a possibility that 46,XX males
might arise due to a balanced, yet abnormal,
nonhomologous X-Y interchange during
paternal meiosis [Rouyer et al., 1986a].
Recent molecular analysis of the sex
chromosomes has shown that there is an
obligate crossing over and exchange of
sequences between the X and Y
chromosomes in the region of shared
homology during male meiosis [Rouyer et al.,
1986a]. Also localized to distal Yp, TDF
appears to be just proximal to the border of
the pseudoautosomal region [Page et al.,
1987b]. Thus, 46,XX males could arise via
an illegitimate crossing over between X and
Y chromosomes centromeric from the
pseudoautosomal region (Fig. 1).

Early studies attempting to trace such
an illegitimate crossing over using the
inheritance of Xg locus in XX males were
consistent with terminal exchange [de la
Chapelle, 1986]. However, recent studies
using molecular analysis of loci in the
pseudoautosomal region, have verified that
many 46,XX males arise due to an abnormal
XY interchange whereby the entire paternal
X pseudoautosomal region was replaced by a
segment of Yp containing TDF and the Y
pseudoautosomal region [Petit et al., 1987;
Page et al., 1987a]. An apparently less
frequent mode of inheritance of the TDF in
46,XX males is from Yp sequences added
onto the X pseudoautosomal region by an
unequal, extremely nonhomologous XY
interchange (perhaps more analogous to an
unbalanced translocation than "interchange"
would imply; Fig. 1). This would appear to
account for three XX males that inherited
TDF and the Y pseudoautosomal region in
addition to a portion of the paternal X
pseudoautosomal region [Page et al., 1987a;
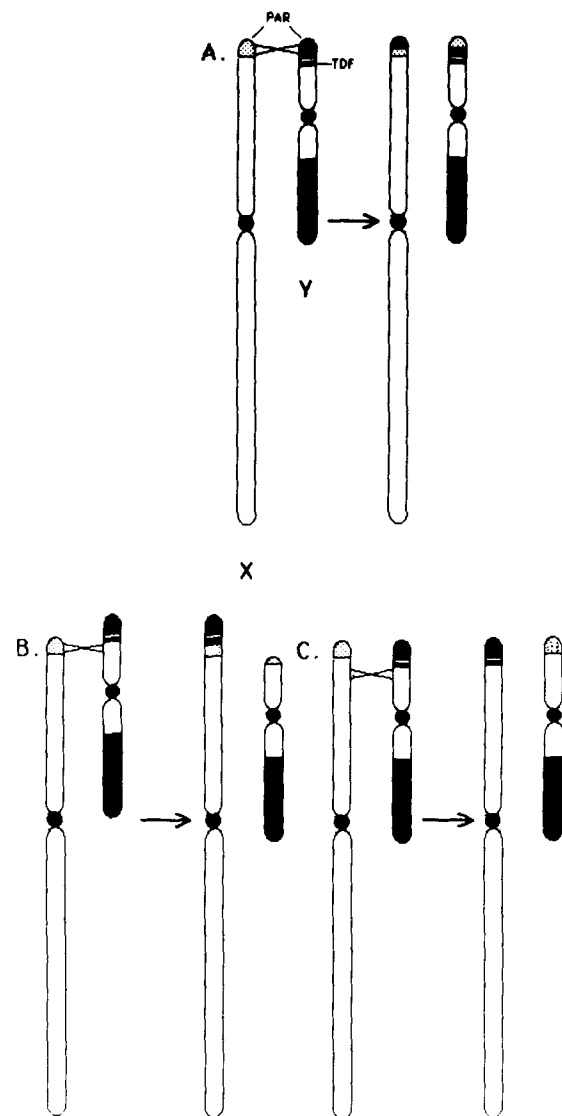Rouyer et al., 1986b; Rouyer et al., 1987].

Figure 1. Hypothetical representation of
the interaction between Xp and Yp in the
pseudoautosomal region (PAR) during meiosis
A) resulting in normal inheritance of the
testis determining factor (TDF) on the Y,
B) resulting in a nonhomologous interchange
moving TDF to the X (family 1), or   C)
resulting in an illegitimate crossing over
moving TDF to the X (family 2).

Based on the 46,XX males reported here, we
would like to suggest that 1) very unequal
XY interchange or translocation is not as
rare as originally thought and   2)
entrapment of distal Yp by the synaptonemal
complex or the sex vesicle could account for
the translocation of Yp sequences to the
paternal X distal short arm rather than to
autosomes.

MATERIALS AND METHODS

The probe p75/79 was subcloned from cosmid Y75 which was derived from a partial human Y library [Wolfe et al., 1984] and detects a Y-specific 2.0 kb Eco RI restriction fragment localized to Yp by somatic cell genetics and in situ hybridization. Sequences from the pseudoautosomal MIC2 locus were detected with the cDNA probe, pSG1, which recognizes allelic restriction fragment length polymorphisms generated by the enzymes Taq I, Msp I, Pst I, and Hind III and a genomic clone, p19B, that recognized allelic restriction fragments generated by Pvu II and Taq I [Darling et al., 1986; Goodfellow et al., 1986]. Cosmid Y97 was originally isolated from the same library as cosmid 75. Cosmid Y97 detects a 5.5 kb Eco RI fragment characteristic of the alphoid centromeric repeat in the human Y chromosome [Wolfe et al., 1985].

Human genomic DNA was prepared by standard procedures as previously reported [Stalvey and Erickson, 1987]. Aliquots of DNA were incubated with the appropriate restriction enzyme at 4 units/μg overnight at the appropriate temperature (as suggested by Bethesda Research Laboratories, Gaithersburg, MD). Fragments were separated by electrophoresis in 0.9% agarose gels containing 0.0002% ethidium bromide except when large fragments were of interest and then 0.6% gels were used. Samples were transferred to Gene Screen Plus (New England Nuclear, Boston, MA). The filters were prehybridized and hybridized at 42°C in 50% formamide, 1.0M NaCl, 1.0% SDS. 10% dextran sulfate, or in 1M NaCl, 1.0% SDS, and 10% dextran sulfate at 65°C. Inserts were isolated from the plasmid probes and labeled by the random primer method [Feinberg and Vogelstein, 1984] to a specific activity of 5 x 10^9 cpm/μg. Cosmid Y97 was nick translated to a specific activity of 5 x 10^8 cpm/μg. Filters were washed to a final stringency of 0.5X or 0.2X SSC at 65°C and exposed for 1 to 7 days to Kodak XAR film at -70°C with intensifying screens (Dupont Cornex Lightening-Plus).

Scanning laser densitometry was performed as previously described [Stalvey and Erickson, 1987] to determine relative band intensity.

RESULTS

Human 46,XX male DNA was restricted with Eco RI and probed with p75/79. Normal male DNA is positive for the Y-specific 2.0 kb Eco RI fragment but the normal female DNA is not [Wolfe et al., 1984]. All 46,XX males studied here were positive for p75/79 (data not shown). Additionally, the 5 sex reversed individuals were negative for the Y centromeric probe Y97, consistent with the absence of a complete Y chromosome. The ability of Y97 to detect extremely small amounts of Y centromeric material [Stalvey and Erickson, 1987] lessens the possibility that the 46,XX males have a minor cell line that contains a complete Y chromosome.

Parents and sibs of two 46,XX males were available for pedigree analysis of the pseudoautosomal locus MIC2 which appears to be localized to the proximal border of the pseudoautosomal region [Goodfellow et al., 1986]. Analysis of family 1 showed informative allelic restriction fragments with Taq I/p19B, Msp I/pSG1, and Pst I/pSG1 (Fig. 2). Figure 3 illustrates the allelic pattern of the family with Taq I/p19B. The father (lane 2) is homozygous for the 3.2 kb Taq I fragment and the mother (lane 4) is homozygous for the 2.5 kb Taq I fragment. The brother (lane 1) and sister (lane 5) are heterozygous being positive for both restriction fragments. The XX male (lane 3) is also heterozygous, but the intensity of the signal from the two fragments is not equal. Dosage analysis by scanning laser densitometry is summarized in Table I. The relative intensity of the signals from the brother and sister are 1.0:1.1 and 1.0:1.2, respectively. However, for the XX male the signal from the 3.2 kb Taq I fragment is 2.3 fold more intense than that from the 2.5 kb Taq I fragment. This is consistent with the XX male inheriting two copies of MIC2 from his father, one each from the paternal X and Y (two 3.2 kb Taq I fragments), and one locus from the mother (the 2.5 kb Taq I fragments).

Southern analysis of family 2 demonstrated that the father, mother, and sister were heterozygous for the Taq I/p19B allele (3.2/2.5 kb: as summarized in pedigree, Fig. 4). However, the 46,XX male was homozygous for the TaqI/p19B allele (2.5/2.5 kb). This is consistent with the XX male inheriting only one maternal and one paternal pseudoautosomal region.

Family 1
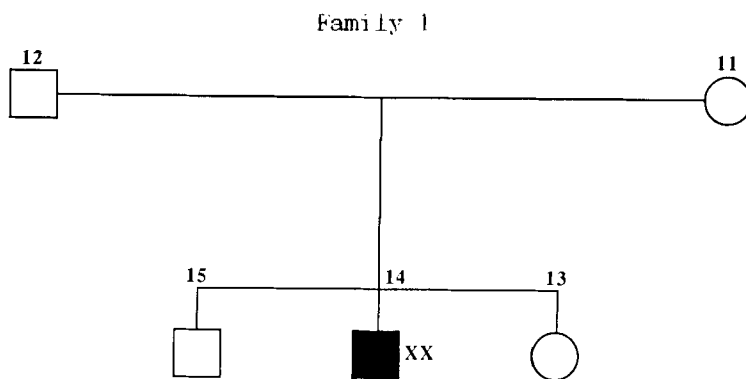
Taq I/p19B*	3.2/3.2	2.5/3.2	2.5/3.2/3.2*	2.5/3.2	2.5/2.5
Pvu II/p19B	2.5/2.5	2.5/2.5	2.5/2.5	2.5/2.5	2.5/2.5
Taq I/pSG1	1.2/1.2	1.2/1.2	1.2/1.2	1.2/1.2	1.2/1.2
Pst I/pSG1	5.1/5.1	5.6/5.1	5.1/5.1	5.6/5.1	5.1/5.6
Hind III/pSG1	12/12	12/12	12/12	12/12	12/12

*See Table I for dosage analysis

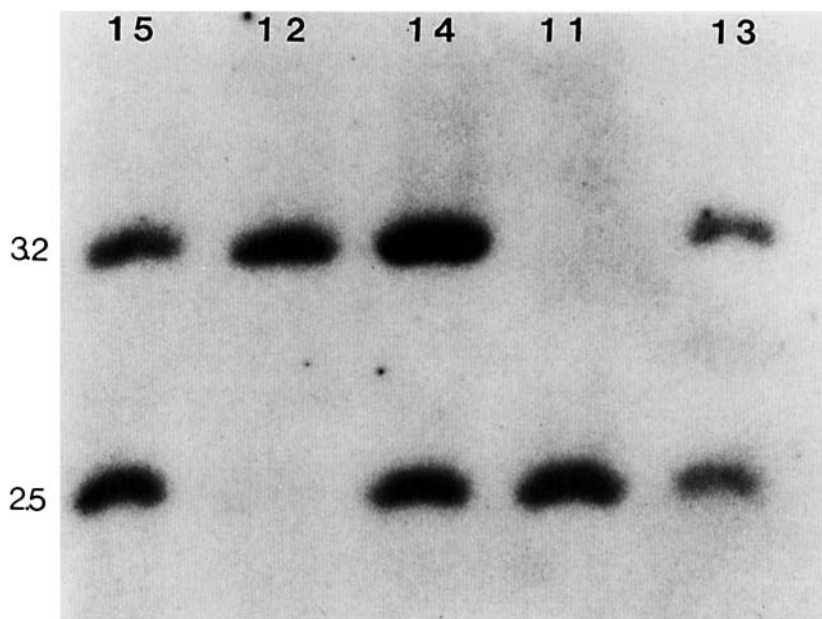Figure 2.   Summary of pedigree and MIC2 RFLP data on family 1.

Figure 3.   Southern analysis of Taq I digested DNA from 46,XX male family 1 probed with p19B.   Each lane contains 10 μg DNA: lane 1 (15), brother; lane 2 (12), father; lane 3 (14), XX male, lane 4 (11), mother; lane 5 (13), sister. The probe, p19B, was oligo-labeled to approximately 5 x 10⁹ cpm/μg and hybridiation was performed at 65°C.   Sizes in kb.

TABLE I.   Dosage Analysis by Densitometry
            of RFLPs for Which the 46,XX
            Males Were Heterozygous

Family 1

Taq I - p19B (3.2/2.5)

    15   1.0:1.1 + 0.1
    14ᵃ   2.3:1.0 + 0.2

Family 2

Pvu II - P19B (2.5/2.3)

    22   1.1:1.0 + 0.1
    24ᵃ   1.0:1.3 + 0.1

Isolated XX males

Taq I - p19B (3.2/2.5)

    I-1ᵃ   1.0:2.2 + 0.2
    I-2ᵃ   1.0:1.3 + 0.1
    I-3ᵃ   1.0:1.2 + 0.1

ᵃ 46,XX males

Further support for this conclusion is gained from dosage analysis of an allele for which the XX male in family 2 was heterozygous (Table I).   Scanning laser densitomerty indicated that for the two Pvu II/p19B restriction fragments (2.5/2.3 kb), the relative intensities were 1:1:1.0 for the father and 1.0:1.3 for the XX male.
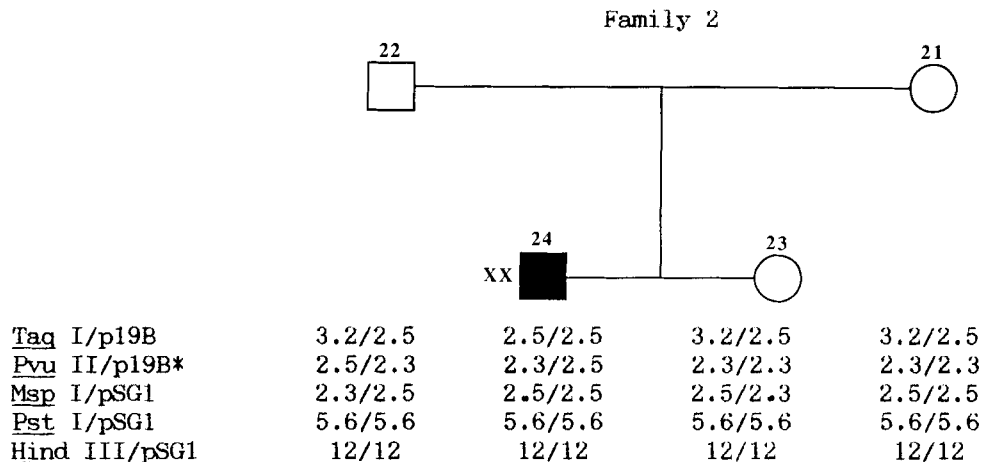
Although we were unable to obtain DNA from relatives of three other 46,XX males, we were able to study the relative intensity of signals from allelic fragments in these isolated XX males because they were heterozygous for Taq I/p19B (Fig. 5). The relative intensity of the two restriction fragments for Taq I/p19B (3.2/2.5 kb) were 1:0:1.3 and 1.0:1.2 for isolated XX males, I-2 and I-3, respectively (Table I). For XX male I-1, the relative intensity of the 3.2 kb and 2.5 kb fragments was 1.0:2.2, indicating that this individual may have inherited pseudoautosomal regions from both paternal X and Y chromosomes similar to the XX male from family 1. Although caution must be exercised because pedigree analysis cannot be done to confirm this interpretation, we have conducted Southern analysis with p19B on a series of normal males and females (Fig. 6).   Densitometry was conducted on a light and dark exposure and the ratio of band intensity in heterozygotes was 1:0:1.1 + 0.1 for Pvu II/p19B and 1.0:1.0 + 0.2 for Taq I/p19B.

DISCUSSION

Pedigree analyses of 2 families with 46,XX males studied for the inheritance of the MIC2 locus from the pseudoautosomal region, indicate that each XX male arose due to an abnormal XY interchange in which a segment of Yp containing TDF and the pseudoautosomal region was transferred to distal Yp. In family 2, the XX male appears to have inherited TDF when an abnormal XY

Family 2

Taq I/p19B	3.2/2.5	2.5/2.5	3.2/2.5	3.2/2.5
Pvu II/p19B*	2.5/2.3	2.3/2.5	2.3/2.3	2.3/2.3
Msp I/pSG1	2.3/2.5	2.5/2.5	2.5/2.3	2.5/2.5
Pst I/pSG1	5.6/5.6	5.6/5.6	5.6/5.6	5.6/5.6
Hind III/pSG1	12/12	12/12	12/12	12/12

*See Table I for dosage analysis

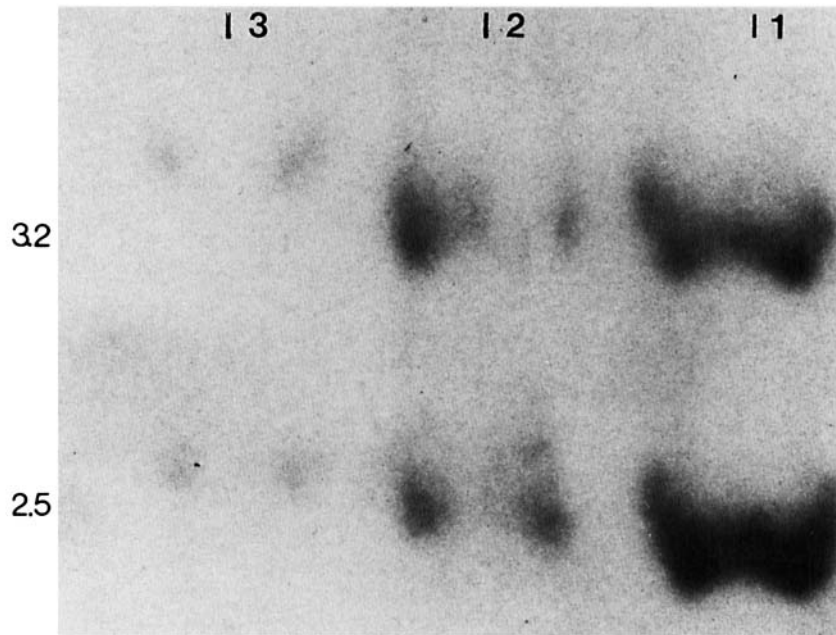Figure 4.   Summary of pedigree and MIC2 RFLP data on family 2.

Figure 5.   Southern analysis of DNA from three isolated 46,XX males. DNA was restricted with Taq I and probed with p19B. Each lane contains 10μg DNA: lane 1 (1-3); lane 2 (I-2; lane 2 (I-1). The probe, p19B, was oligo-labeled to approximately 5 x 10⁹ cpm/μg and hybridization was performed at 65°C. Sizes in kb.
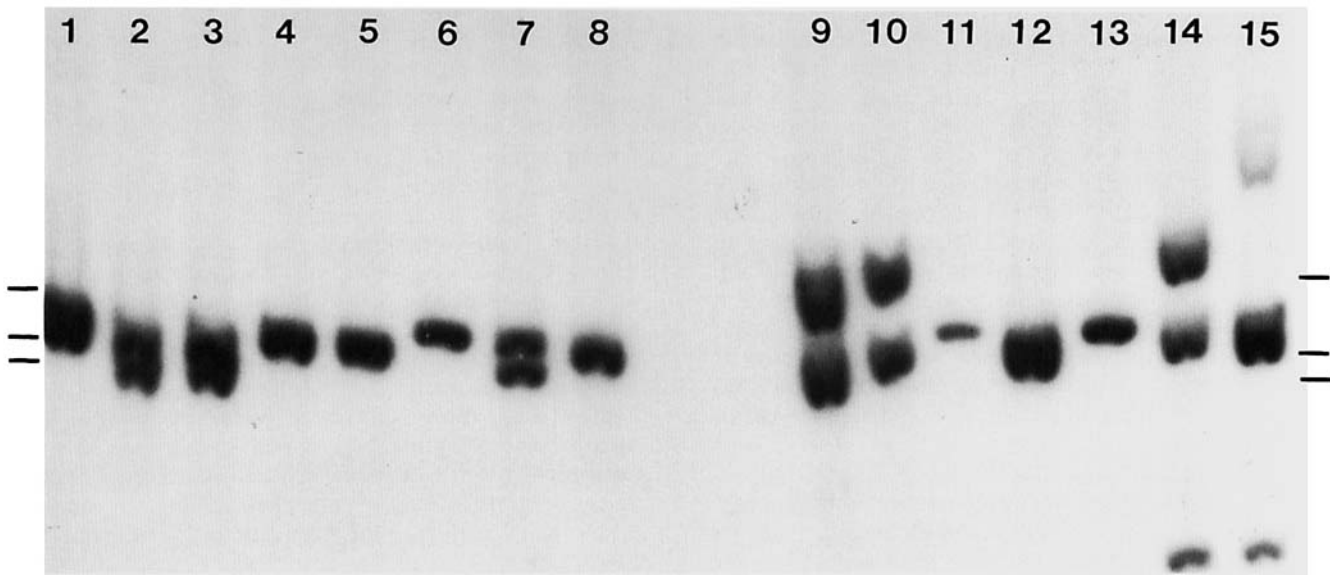

Figure 6. Southern analysis of DNA from normal control males and females. DNA was restricted with Pvu II (lanes 1-8) or Taq I (lanes 9-15) and probed with p19B. Each lane contains 10μg DNA: lanes 1-4 and 6, 9-11, and 13 are males and lanes 5, 7, 8, 12, 14, and 15 are females. The probe, p19B, was oligo-labeled to approximately 5 x 10⁹ cpm/μg and hybridization was performed at 65°C. From top to bottom, the side bars, mark 3.2, 2.5, and 2.3 kb.

interchange resulted in the paternal X pseudoautosomal region being completely replaced by a segment of Yp containing TDF and the Y pseudoautosomal region (Fig. 1). This is evidenced by the observation that the father is heterozygous but the XX male is homozygous for the allele defined by Taq I/p19B. If the Y pseudoautosomal region had been added to the paternal X pseudo-autosomal region, then the XX male would have inherited both paternal X and Y alleles and appeared heterozygous. Furthermore, with Pvu II/p19B, for which the father and XX male are both heterozygous, the signal intensity for the two allelic restriction fragments is approximately 1 to 1 for both individuals. Thus, the mode of inheritance of TDF in family 2, is consistent with the terminal-interchange model [Ferguson-Smith, 1966] recently verified by Petit et al. [1987] and Page et al. [1987a].

In family 1, the XX male probably arose by an unbalanced exchange in which TDF and the Y pseudoautosomal region were added onto the X pseudoautosomal region and as a result, this XX male inherited three copies of the proximal pseudo-autosomal region (Fig. 1). Similarly, the isolated 46,XX male, I-1, also appears to have inherited 3 copies of the proximal pseudoautosomal region. Combined with three previous reports of unbalanced exchange in 45,XX males [Page et al., 1987a; Rouyer et al., 1986b; Rouyer et al., 1987], there are now 5 patients reported to have undergone sex reversal as a result of unequal exchange, indicating that inheritance of TDF by adding Yp to the distal portion of the paternal X pseudoautosomal region is not as rare as originally thought [de la Chapelle, 1986]. If the mode of inheritance is to be viewed as an unequal exchange versus translocation, what characteristics of XY pairing could allow a misalignment to occur and still attach Yp to Xp instead of to an autosome?

Variable amounts of sequence homology between X and Y chromosomes are found in the pairing region. In the pseudoautosomal region there is presumed sequence homology between the X and Y chromosomes [Rouyer et al., 1986a; Rouyer et al., 1986b]. However, just proximal to the Y pseudo-autosomal region is a region which contains sequences found only on Yp (recognized by probe 27a [Pritchard et al., 1987]). These sequences may well play a role in defining the "boundary" of the pseudoautosomal

region and normally prevent crossing over from occurring outside the pseudoautosomal region. Proximal to TDF there is another region containing sequences found on both the X and Y chromosomes. However, the sequences that map to Yp in this region, map to Xq and other sequences that map to Xp, map to Yq [Goodfellow et al., 1985; Page, 1986], probably as the result of a pericentric inversion during evolution of the human Y [Page et al., 1984]. Recently, Chandley [1986] has proposed that initial pairing of chromosomes is organized around functional homologies on chromosomes as opposed to nucleotide sequence homologies (however, the latter are necessary to allow "effective" pairing and crossing over to occur). She suggested that similarities in chromatin configuration found in early replicating regions of chromosomes could serve as organizers of pairing [Chandley, 1986]. It has been reported that, within the XY pairing region, there appear to be two regions on each of the X and Y chromosomes that undergo synchronous, early replication and these areas are within the XY pairing region [Muller and Schempp, 1982; Schempp and Meer, 1983] and are DNase I hypersensitive [Chandley and McBeath, 1987].

If pairing, organized by functional homology does not provide accurate nucleotide pairing during some meioses, misaligned, nonhomologous regions on the X and Y chromosomes may be entrapped by the formation of the synaptonemal complex or the sex vesicle. The pseudoautosomal region has a frequency of crossing over during male meiosis that is 10-20 fold greater than the rest of the genome [Rouyer et al., 1986a]. Juxtaposition of like sequences such as Alu repeats, within otherwise nonhomologous regions, may provide enough homology to create an "effective" pairing in a "hot spot" region and allow crossing over to occur. This is consistent with the findings for one of the three previously reported 46,XX males. Molecular analysis of the breakpoints in the DNA of CON101 indicates homologous recombination between Alu sequences which are surrounded by otherwise nonhomologous regions [Rouyer et al., 1987]. It is not known whether similar "local homology" provides a basis for crossing over in the other four XX male cases due to unequal exchange. If the mode of inheritance of some XX males with Yp added to Xp is to be viewed as due to translocation versus

unequal exchange, what characteristics of XY pairing could account for perferential translocation of Yp to Xp as opposed to Yp to autosomes?

Fryns et al. [1985] reviewed the literature on Y:autosome translocations from the early 1970's to their time of writing; they found approximately 40 cases which they divided into two general types. The first class included translocations with familial transmission, most of which were detected accidentally since there were few phenotypic effects. Of these, the most common type involved chromosome 15 (10/17 families) and chromosome 22 (3/17 families). The other group of translocations were de novo Y:autosome translocations which had more serious phenotypic effects since all patients were sterile.

The physical isolation provided by the sex vesicle might be expected to largely prevent translocation of broken fragments of Yp to autosomes and, instead, enhance the possibility of their attachment to Xp. If one compares the Y:autosome translocation frequency with the frequency of XX males, the putative role of the sex vesicle in enhancing X:Y exchanges seems clearer. As mentioned earlier (see Introduction), the frequency of XX males is approximately 1 in 20,000 phenotypic males. Therefore, assuming a birth rate of approximately 10 million/year for the years and countries covered in the review by Fryns et al. [1985], and with a sex ratio of about 50%, in any one year there would be approximately 5 million males born, of which 250 might be expected to be XX males. That means that for any one year, 6 times more XX males should be present than the total number of Y:autosome translocations reported in the over 10 year period [Fryns et al., 1985]. Of course, reporting of patients probably is not complete and additional Y:autosome translocations with no phenotypic effect probably go undetected. However, there does seem to be at least an order of magnitude difference which argues for a role of the sex vesicle in maintaining X:Y proximity during meiosis. Such proximity could lead to preferential translocation of Yp to Xp during male meiosis and thus increase the incidence of XX males compared with other Y:X or Y:autosome translocations.

Further investigation of the nature and characteristics of the breakpoints involved with unequal XY exchange (or translocation) promises to provide much important information about the nature of XY pairing during meiosis and molecular mechanisms regulating crossing over (and normally limiting XY exchange to the pseudoautosomal region). It also may be informative to study the breakpoints in XX males that arose via terminal exchange. Although apparently equal exchange has occurred in most XX males, perhaps smaller misalignments have produced "micro" unequal exchange. Thus, all XX males (with Yp sequences) may be due to faulty pairing of the X and Y chromosomes, with some resulting from gross misalignment, and some resulting from micro-misalignment. The use of pulsefield gel electrophoresis to study distal Yp [Pritchard et al., 1987] should provide the resolution necessary to investigate this possibility further.

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