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An XXX Male Resulting from Paternal X-Y Interchange and Maternal X-X Nondisjunction

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SUMMARY

A 2-year-old boy was found to have a 47,XXX karyotype. Restriction-fragment-length-polymorphism analysis showed that, of his three X chromosomes, one is of paternal and two are of maternal origin. The results of Y-DNA hybridization were reminiscent of those in XX males in two respects. First, hybridization to Southern transfers revealed the presence in this XXX male of sequences derived from the Y-chromosomal short arm. Second, in situ hybridization showed that this Y DNA was located on the tip of the X-chromosomal short arm. We conclude that this XXX male resulted from the coincidence of X-X nondisjunction during maternal meiosis and aberrant X-Y interchange either during or prior to paternal meiosis.

INTRODUCTION

The basis of testis differentiation in the absence of a Y chromosome has recently begun to be understood (de la Chapelle 1986; Page 1986). Much of the progress in this field has come from the study of so-called XX males, who are phenotypic males with a 46,XX karyotype (de la Chapelle 1981). The genomes of most XX males contain DNA originating from the Y chromosome (Guellaen et al. 1984; Page et al. 1985; Affara et al. 1986; Müller et al. 1986; Vergnaud et

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al. 1986). In such XX males, the Y-chromosomal DNA is found on the end of the short arm of one of the X chromosomes (Andersson et al. 1986), where it has been transferred through an abnormal X-Y interchange in the meiosis of the father (D. C. Page and A. de la Chapelle, unpublished data), as predicted (Ferguson-Smith 1966; Polani 1982). Testes develop in such XX males because the testis-determining factor (TDF), which has been mapped to distal Yp (Page 1986; Vergnaud et al. 1986), is among the Y-chromosomal DNA sequences that they possess.

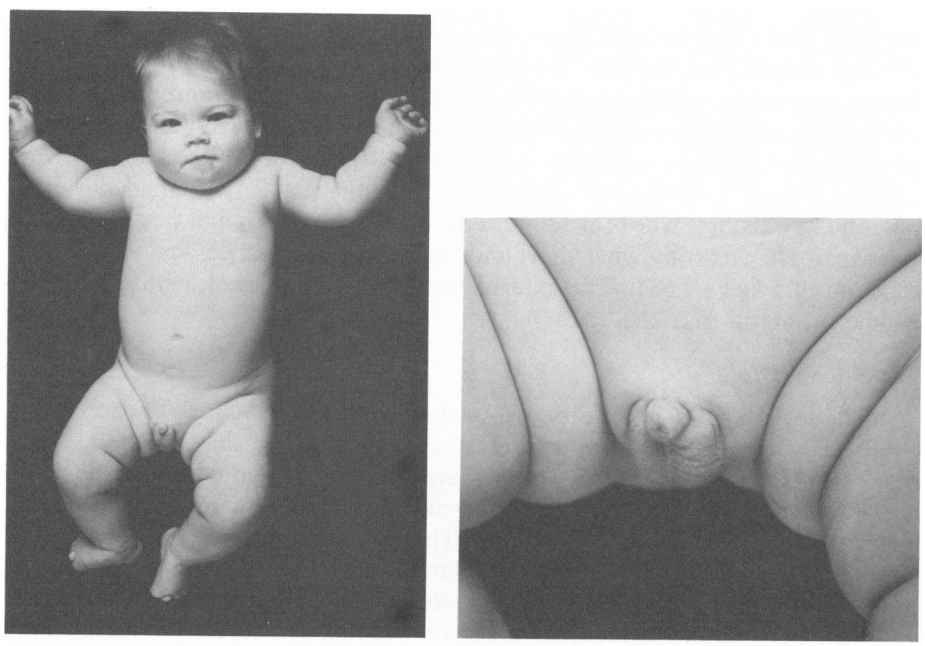
A more uncommon type of male apparently lacking a Y chromosome is the 45,X male. Evidence from a small number of patients indicates that 45,X maleness can be due either to translocation of Y DNA to an autosome (Disteche et al. 1986; Maserati et al. 1986; Gal et al. 1987) or to mosaicism involving an intact Y chromosome (de la Chapelle et al. 1986).

In the present paper we describe the basis of maleness in a third type of male without a Y chromosome, viz., the 47,XXX male.

CASE REPORT

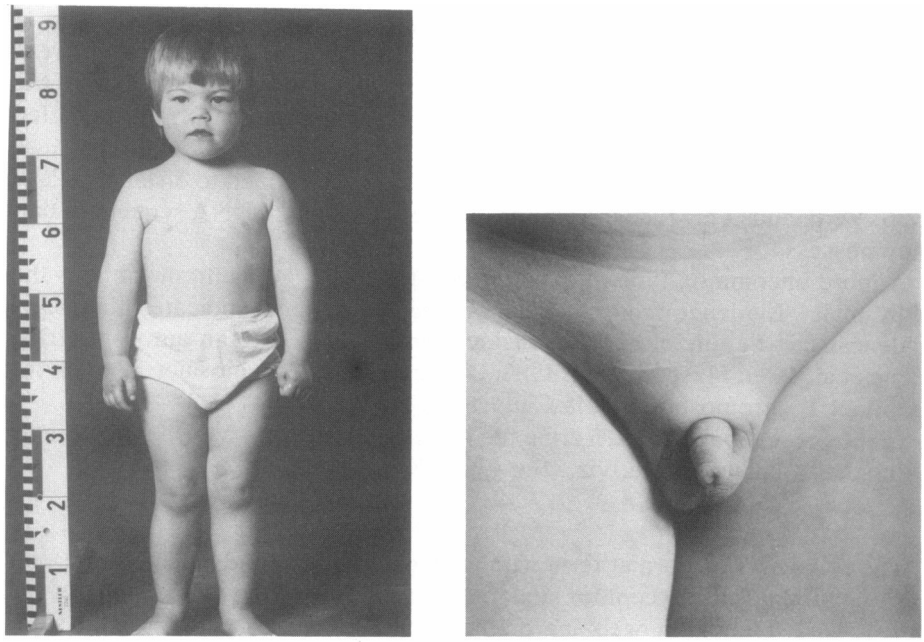
The proband was born at term with a weight of 3.4 kg and a height of 51 cm. He appeared normal except for simian creases of the palms, clinodactyly of the fifth fingers, and slight webbing of the neck with an excess of nuchal skin. Chromosome analysis performed to rule out Down syndrome showed a 47,XXX karyotype.

At age 5 mo the habitus (fig. 1*A*) and psychomotor development were nor-



A B

FIG. 1.—The patient (*A*) and close-up of his external genitalia (*B*) at age 5 mo



A B

FIG. 2.—The patient (*A*) and close-up of his external genitalia (*B*) at age 2 years, 4 mo

mal. The penis was normal; there were two scrotal testes of normal size (fig. 1*B*), and cystoscopy revealed no evidence of vaginal remnants. A cystogram showed low-grade reflux on the left side but was otherwise unremarkable, as was an intravenous pyelogram. Serum luteinizing hormone, follicle-stimulating hormone, and testosterone were in the normal range for a boy of his age.

At age 2 years 4 mo the boy still appeared healthy (fig. 2*A*). He had started to walk and say his first words at 13 mo. Psychomotor development was judged to be normal. He was somewhat short and obese, measuring 87.5 cm (−1 SD) and weighing 14.5 kg (+1 SD). The external genitalia were normal, with a testicular volume of 0.8 ml (fig. 2*B*).

Testicular Histology

Small biopsies (1 × 1 × 3 mm) were taken from the distal pole of each testis, as far away as possible from the rete testis. Portions of the specimens were used to initiate tissue cultures for chromosomal investigation. The remaining tissue was fixed in 5% glutaraldehyde and processed for light and electron microscopy (Läckgren and Ploen 1984). The histology revealed testicular tissue with abundant tubules and normal tubular membranes. An examination of 100 tubular cross sections per testis, using semithin Epon sections, revealed no germ cells; the tubules contained only Sertoli cells. The interstitium contained few cells and no mature Leydig cells (findings normal for the proband's age). No ovarian elements were seen.

The Family

At the patient's birth in 1984 his mother was age 38 years and his father age 39 years. There was no consanguinity. The patient was the fourth child, having a brother born in 1968, a sister born in 1971, and a brother born in 1979. All family members were healthy. However, like the proband, both the father and the older brother had bilateral simian creases and bilateral clinodactyly of the fifth fingers.

METHODS

Cytogenetic Studies

Chromosome studies of the patient were performed using cells from peripheral blood, skin, and testis cultured by means of standard techniques. Blood cells from the parents and sibs were studied. Our method for G-banding has been previously described elsewhere (Borsgård et al. 1974).

Molecular Studies

DNA extraction and gel-transfer hybridization.—DNA was prepared from blood leukocytes or cultured skin fibroblasts by means of published methods (Kunkel et al. 1977). Restriction digestion, electrophoresis, transfer, and hybridization of DNA were performed according to methods described elsewhere (Page and de la Chapelle 1984).

DNA probes detecting Y-specific restriction fragments are listed in table 1. Probe St14-1 (*DXS52;* Oberle et al. 1985) detects, at high stringency, an X-linked *Taq*I restriction-fragment-length polymorphism (RFLP).

In situ hybridization.—Metaphases for use in in situ hybridization were obtained from Epstein-Barr virus–transformed lymphoblastoid cell cultures. Air-dried chromosome preparations were hybridized with ^{3}H-labeled DNA probe according to standard techniques (Chandler and Yunis 1978; Harper and Saunders 1981) described elsewhere (Andersson et al. 1986). Probe pDP105, ^{3}H-labeled to a specific activity of 9×10^{6} cpm/μg, was used at a concentration of 30–40 ng/ml.

RESULTS

Cytogenetic Studies

The patient's karyotype (fig. 3) was 47,XXX as judged by the study of 100 metaphases from each of the following: blood lymphocytes (on two occasions), skin fibroblasts, testis fibroblasts, and Epstein-Barr virus–transformed lymphoblasts. There was no indication of any structural abnormality of any of the three X chromosomes. Blood cultures from the parents and sibs had normal karyotypes.

Molecular Studies

Parental derivation of the X chromosomes.—To determine the parental origin of the proband's X chromosomes, the proband and parents were typed for X-linked RFLPs. Among these, the polymorphism detected by probe St14-1

TABLE 1

PRESENCE (+) AND ABSENCE (−) OF Y CHROMOSOME–SPECIFIC DNA

Y-CHROMOSOME INTERVAL[a]	DNA PROBE DATA				Y CHROMOSOME–SPECIFIC FRAGMENT				
	Probe/Band	Restriction Enzyme	Stringency[b]	Reference	Patient	Father	Mother	Normal Males	Normal Females
1	47a	*Taq*I	High	Vergnaud et al. 1986	+	+	−	+	−
2	pDP61	*Taq*I	High	D. C. Page, unpublished data[c]	+	NT	NT	+	−
3	50f2/A,B	*Eco*RI	Reduced	Guellaen et al. 1984	+	+	−	+	−
3	pDP105/A	*Taq*I	Reduced	D. C. Page, unpublished data	+	NT	NT	+	−
4A	pDP34	*Taq*I	High	Page et al. 1982, 1984	−	+	−	+	−
4B	pDP97	*Eco*RI	High	D. C. Page, unpublished data[d]	−	+	−	+	−
4B	50f2/D	*Eco*RI	Reduced	Guellaen et al. 1984	−	+	−	+	−
5	12f	*Taq*I	High	Bishop et al. 1984	−	NT	NT	+	−
6	pDP105/B	*Taq*I	Reduced	D. C. Page, unpublished data	−	NT	NT	+	−
6	50f2/C,E	*Eco*RI	Reduced	Guellaen et al. 1984	−	+	−	+	−
7	pY431-HinfA	*Taq*I	Reduced	K. Smith, personal communication	−	NT	NT	+	−

NOTE.—NT = not tested.

[a] Sources: Page 1986; Vergnaud et al. 1986.

[b] Each hybridization probe was used at either "reduced" or "high" stringency. "Reduced" stringency implies that hybridizations were carried out at 42 C and that the final wash was in 0.1 × SSC, 0.1% SDS at 55 C. "High" stringency implies that hybridizations were carried out at 47 C and that the final wash was in 0.1 × SSC, 0.1% SDS at 65 C.

[c] Probe derived from plasmid 115 (Geldwerth et al. 1985).

[d] Probe derived from cosmid Y97 (Wolfe et al. 1985).

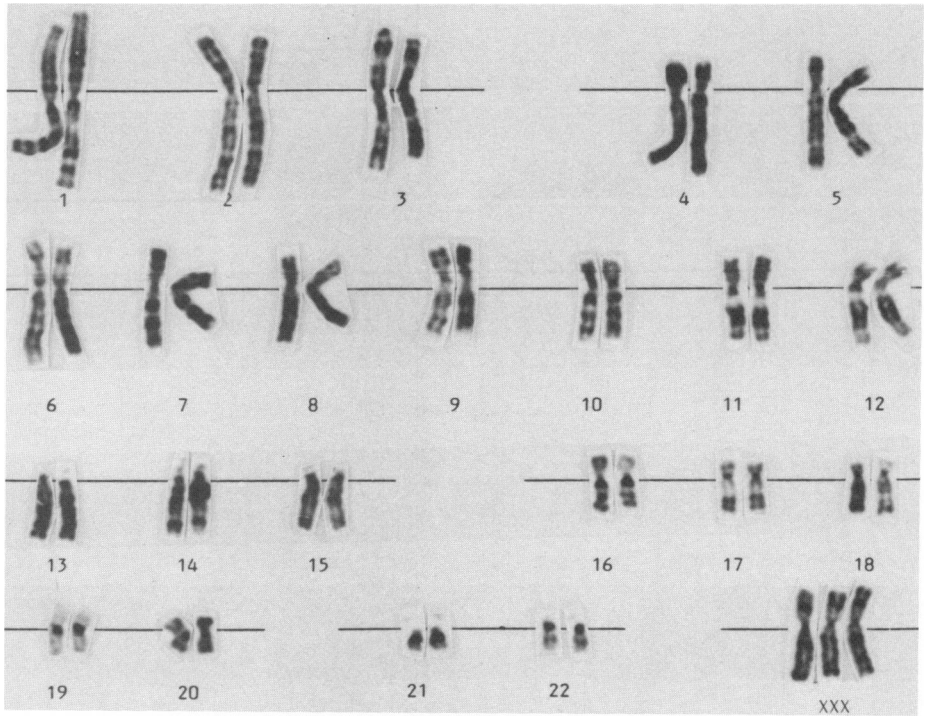


FIG. 3.—A trypsin-Giemsa-banded karyotype from a blood culture of the proband showing 47,XXX.

was informative. When hybridized to *Taq*I digests of genomic DNAs, probe St14-1 detected the following allelic fragments: in the father, a 4.5-kb fragment; in the mother, a 4.8-kb fragment (for which she is homozygous); and in the 47,XXX son, 4.5- and 4.8-kb fragments, the 4.8-kb fragment being twice as intense as the 4.5-kb fragment (fig. 4). Thus, the proband has one paternal and two maternal X chromosomes.

Presence of Y-chromosomal DNA.—By means of a panel of probes detecting different parts of the normal Y chromosome (Page 1986; Vergnaud et al. 1986), the genome of the proband was examined for the presence of DNA derived from the Y chromosome. These studies indicated that the 47,XXX male carries deletion intervals 1–3 of the Y chromosome (table 1). Thus, he appears to carry the same portion of the short arm as is found in class 3 XX males (Vergnaud et al. 1986). The father appears to have an intact Y chromosome, and there is no evidence of Y DNA in the mother.

Localization of Y chromosome–specific DNA.—To determine the chromosomal localization of the Y-specific DNA, probe pDP105 was hybridized in situ to metaphase chromosomes from the proband. It had previously been shown that, by means of in situ hybridization on normal 46,XY males, pDP105 intensely labels the Y chromosome but does not significantly label any other

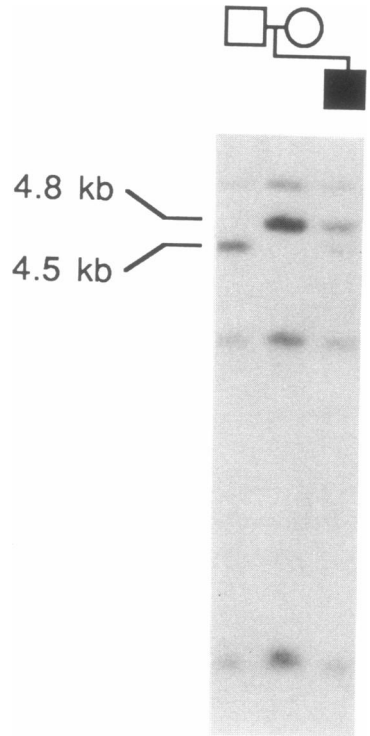


FIG. 4.—Hybridization of *Taq*I-digested DNAs from the parents and 47,XXX male proband with St14. The sizes of the allelic restriction fragments are indicated. The other fragments are not polymorphic.

chromosome (Andersson et al. 1986). Thirty-nine metaphase cells from the XXX male proband were scored. The tip of the short arm of one X chromosome (Xp22) was labeled in 34 (87%) of the 39 metaphases (fig. 5). Of a total of 176 chromosomal grains, 40 (23%) were on one Xp22. Several clusters consisting of 2–4 grains were observed on Xp22; these were counted as one grain. No other site was preferentially labeled, as shown on the histogram (fig. 6). These results demonstrate that the Yp material present in the proband has been transferred onto the distal short arm of an X chromosome.

DISCUSSION

Our patient is the second 47,XXX male reported in the literature, the first being the 18-year-old patient described by Bigozzi et al. (1980). Detailed clinical comparisons of the two patients are difficult because of the difference in age. Common features are a male habitus, male external genitalia, and a male psychosexual orientation. Since the patient of Bigozzi et al. (1980) was 177 cm in height and ours was 1 SD below normal, increased height would not seem to be a feature of XXX maleness. Minor dysmorphic features in our patient led to the first chromosome study; however, since these features also occurred in the

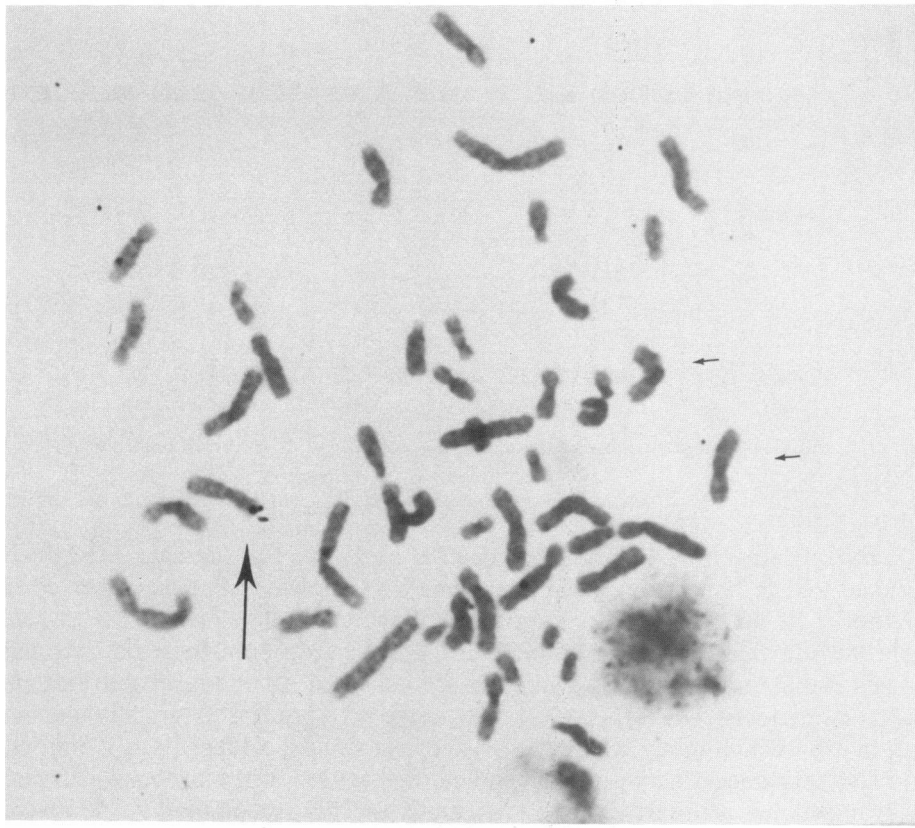


FIG. 5.—A lymphoblast mitosis after in situ hybridization with probe pDP105, showing a cluster of grains on the terminal portion of the short arm of one X chromosome (large arrow). The small arrows indicate the other two X chromosomes, which are unlabeled.

father and a brother, who were otherwise entirely normal, they may be unrelated to the 47,XXX karyotype. A detailed comparison of the testicular histologies is not meaningful owing to the difference in age between the patients. It seems likely that our XXX male will be infertile. First, he lacks Yq, which may carry a gene or genes essential to spermatogenesis (Tiepolo and Zuffardi 1976). Second, the sterility of XXY, XXXY, and XXXXY males, all of whom appear to have intact Y chromosomes, suggests that having more than one X chromosome is incompatible with male fertility. We cannot exclude the possibility that one or both of the patients is or will become mildly mentally subnormal. Thus, testicular dysgenesis is the only clinical feature common to these two 47,XXX males, who are otherwise normal or near normal. This is not unexpected in view of the fact that neither 46,XX males (de la Chapelle 1981) nor 47,XXX females (Robinson et al. 1982) have invariable somatic or mental abnormalities.

Our proband is male because his genome contains the testis-determining portion of the Y chromosome. The Southern hybridization results indicate that the Y sequences that he possesses correspond to intervals 1–3 on Yp, as is

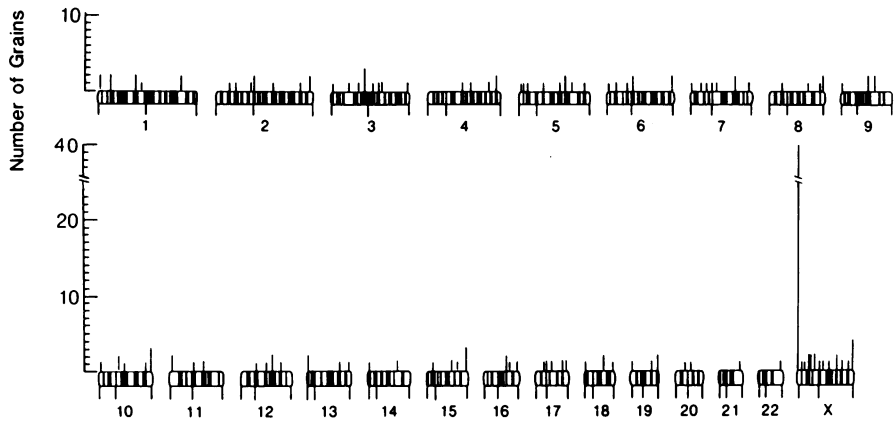


FIG. 6.—Histogram showing the distribution of 176 grains on the chromosomes

commonly seen in XX males (Vergnaud et al. 1986). The segregation of the X-linked RFLP detected by means of probe St14 shows that he has received his father's X, and the in situ hybridization indicates that one of his three X chromosomes is the carrier of the Y DNA. Taken together, these data strongly suggest that an abnormal X-Y interchange occurred either during or prior to his father's meiosis. Thus, this XXX male arose by a process essentially identical to that by which many XX males arise (de la Chapelle 1986; Page 1986).

The segregation of probe St14 indicates that two of the proband's three X chromosomes are derived from his mother. This is commonly the case in 47,XXX females and 47,XXY males (Summitt 1981) and is usually attributed to meiotic nondisjunction of the X chromosomes in the mother's meiosis. Such nondisjunction is age dependent, its frequency rising with increasing maternal age (Hassold and Chiu 1985). The relatively high age of the mother (38 years) at the birth of our proband is compatible with this notion. According to this hypothesis, the proband was formed by the fertilization of a 24,XX ovum by a 23,X sperm in which the X chromosome had acquired a testis-determining portion of Yp at paternal meiosis. If maternal X-X nondisjunction occurs in approximately one meiosis in 1,000 (Summitt 1981) and XX males have an incidence of approximately one in 20,000 newborn males (de la Chapelle 1981), then the coincidence of these events, resulting in an XXX male, might be expected approximately once in 20 million male births.

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