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A possible common origin of "Y-negative" human XX males and XX true hermaphrodites

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Summary. We have studied nine patients aged 1 month to 16 years with 46, XX karyotypes and testicular tissue. Some of these patients were followed through puberty. Phenotypically, two presented normal and seven abnormal external genitalia (AG). Among this latter group, four showed hypospadias and three true hermaphroditism (TH). The endocrine data were similar in all three groups: testosterone levels were within normal limits during puberty, decreasing in adulthood; gonadotrophin levels were above the control values at mid puberty. Histologies of the two sub groups of AG patients were identical up to 5 years of age and presented differences when compared with controls, regardless of the ovarian part of the ovotestis. However, in patients older than 8 years, germ cells disappeared and dysgenesis became obvious. In one patient, the ovarian zone of the gonad was detected only after complete serial sections of the removed gonad were examined. Southern blot analysis with Y-DNA probes displayed Y-specific material for the classic 46 XX males and a lack of such sequences for all patients with AG and TH. Based on these findings , we postulate that 46, XX males with AG and 46, XX TH may represent altenative manifestations of the same genetic defect. These data together with those concerning familial cases of 46, XX males with AG and 46, XX TH suggest an autosomally (or pseudoautosomally) determined mechanism.

Introduction

About 1 in 20000 males have a 46, XX karyotype. Their maleness results from accidental X-Y interchange during paternal meiosis, so that the paternal X chromosome bears a portion of the Y chromosome (Ferguson-Smith 1966; Affara et al. 1986; Seboun et al. 1986a). Most, if not all, are sporadic cases with no genital ambiguities and a male phenotype as defined by de la Chapelle and coworkers (de la Chapelle et al. 1964, 1965, 1983; de la Chapelle 1987). However some XX patients present genital abnormalities such as vaginal pouch hypospadias and cryptorchidism.

Initially it was thought that the underlying genetic defect of the XX male syndrome with sexual ambiguity was different from that of the XX true hermaphrodities, who present both testicular and ovarian tissue or ovotestis. Surprisingly, no Y DNA material could be detected in any of these cases, raising the question of how testicular tissue can develop without Yp DNA material (Vergnaud et al. 1986; Muller et al. 1986; Naud et al. 1985; Ferguson-Smith and Affara 1988). Key probes tested include GMGY3 (Affara et al. 1986), pDP1007 (Page et al. 1987), and 47b (Bishop et al. 1983), which cover the whole sex-determining locus on the Y chromosome (Page et al. 1987). In addition to these observations, studies carried out by mating male *Mus musculus poschiavinus* (POS A mice) with female C57BL/6J mice have led to the conclusion that autosomal genes (Tda-1, Tda-2) must interact with TDF (testis-determining factor or Tdy in mice) on the Y chromosome to result in correct sex determination (Eicher and Washburn 1986).

We and others have studied familial cases of XX males with genital abnormalities and XX true hermaphrodites and found no evidence of the presence of Y sequences. All these observations seem compatible with the existence of non-Y downstream sex-determining genes that must interact with Y-determining genes (TDF) to achieve primary sex determination in man (Seboun et al. 1986; de la Chapelle 1987; Waibel et al. 1987).

Patients and methods

Nine patients with a 46, XX karyotype and testicular tissue or testes were studied between the ages of 1 month to 16 years. Some of them were followed through puberty. External genitalia were examined and measured; internal genitalia were investigated by

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laparoscopy or by genitography and intravenous pyelography. Ultrasonography was used in some more recent patients. Biopsies, obtained during surgery, were fixed and serial sections were cut and stained by hematoxylin, phloxine, saffran, and periodic acid-Schiff. Chromosome analysis were performed on peripheral blood lymphocytes and fibroblasts derived from testis (in one case: case 8) or skin biopsis. Chromosomes were also studied using Q and R banding. Karyotypes were perfomed on 40–50 metaphases from each patient.

Endocrinologic evaluation

Testosterone levels, before and after hCG (1500 U every 3rd day) and gonadotropin levels, before and after LH-RH infusion tests (0.1 mg/m^2) were determined by radio immuno-assays (Job et al. 1981) and compared with values obtained from controls of the same pubertal stage.

DNA analysis

High molecular weight DNA prepared from human lymphocytes was digested with *Eco*R1 and 15-µg samples were electrophoresed on 0.8% agarose gels for 14–16 h at 50 V. The gels were depurinated in 0.3 *M* HCl for 7 min and rinsed twice in distilled water before denaturation in 1.5 *M* NaCl, 0.5 *M* NaOH for 45 min. The gels were rinsed twice in distilled water and finally neutralized in 0.2 *M* TRIS (pH 7.2), 0.1 × SSC, 1 m*M* EDTA (pH 8) for 45 min. The DNA was then transferred onto Hybond-N membranes using 20 × SSC, overnight. After being washed in 2 × SSC, the filters were dried and fixed by UV irradiation for 4 min. Prehybridizations and hybridizations were carried out for 24 h at 42°C in a solution containing 50% formamide, 5 × Denhardt's, 5 × SSC, 0.5% SDS, 50 m*M* phosphate buffer, and 100 µg/ml salmon sperm DNA (sonicated). Filters were probed using DNA labeled with ^{32}P by nick-translation to a specific activity of approximately 1×10^8 cpm/µg DNA. Filters were washed, either under non-stringed conditions (2 × SSC + 0.1% SDS) or under stringent conditions (0.1 × SSC + 0.1% SDS), and exposed using Kodak XAR 5 films at −70°C for 24–48 h (Vergnaud and Al 1986).

Human DNA probes

Several human Y-derived sequences were used to analyze the DNA of the XX males. Probes 47b, 118, 52d, 48d, 50f, and GMGY3 have been described previously (Bishop et al. 1983; Affara et al. 1986; Seboun et al. 1986a). The pDP1007 probe recognizing the sex-determining region (TDF) of the human Y chromosome was a gift of Dr. D. Page. The genetic organization of the sex-determining region corresponds to the following map of the short arm of the Y chromosome: telomere-preudoautosomal region-GMGY3-ZFY-47b-118-52d-50f-entromere.

Table 1. Description of the nine XX males or true hermaphrodites (TH) and the two familial cases (A.R. and A.H.). ND, Not determined

Cases	Age	Penile length	Hypo-spadias	Testicular size or crypt-orchidism	Internal genitalia	Biopsy of testis	Karyotype	Phenotype
1	1 month	1.5 cm	Anterior	1.5 × 0.8 cm, unilateral	Vaginal pouch	Spermatogonia, Leydig cells, Sertoli cell hyperplasia, normal for age	46,XX	Male with ambiguities
2	1 year, 11 months	4 cm	Anterior	1.5 × 1 cm, unilateral	Normal male	ND	46,XX	Male with ambiguities
3	2 years, 9 months	2.3 cm	Anterior	Bilateral	Vaginal pouch	Leydig cells, no spermatogonia, Sertoli cells immature, normal for age	46,XX	Male with ambiguities
4	4 years, 9 months	3 cm	Anterior	Bilateral	Normal male	Leydig cells, spermatogonia, Sertoli cells, normal for age	46,XX	Male with ambiguities
5	9 years, 3 months	1.7 cm	None	1.5 × 0.8 cm	Normal male	ND	46,XX	No ambiguities
6	15 years	9 cm	None	3 × 1.8 cm	Normal male	ND	46,XX	No ambiguities
7	20 days	1.7 cm	Posterior	1.5 × 1.0 cm and 1 ovary	Vaginal pouch, uterus	Immature tubules, spermatogonia sparse, normal for age, stroma and follicles	46,XX	T.H.
8	8 years	3 cm	Posterior	Bilateral	Vaginal pouch	Leydig cell hyperplasia, no spermatogonia, Sertoli cell hyperplasia, dysgenetic, stroma and follicles	46,XX	T.H.
9	18 years	3 cm	Posterior	2.5 × 1.0 cm	Uterus	Leydig cell hyperplasia, no spermatogonia, Sertoli cell hyperplasia, stroma and follicles	46,XX	T.H.
A.R.	21 years	Small, curved	Anterior	3 × 2 cm	ND	Testes with no germ cells	46,XX	Male with ambiguities
A.H.	14 years	2.5 cm	Posterior	Small, ectopic	ND	Immature testes	46,XX	Male with ambiguities

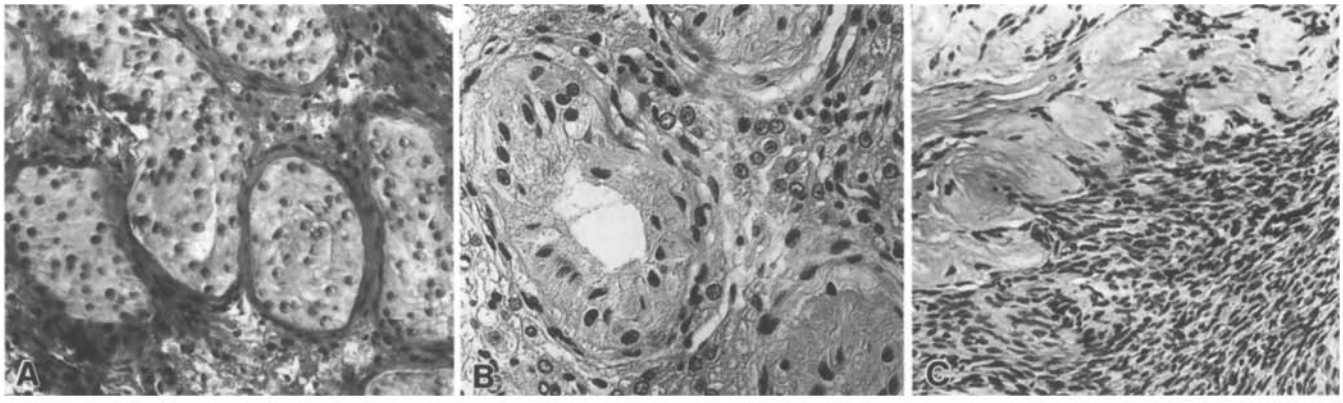

Fig. 1A–C. Biopsy of right gonad of patient 8: **A** at age 8, showing only testicular tissue; **B** serial cut of the same gonad at age 22 showing typical testicular tissue coexisting with **C** a corpus albicans

Results

Analysis of sporadic cases

Patients were divided into three groups according to their phenotypes: "classic" XX males (patiens 5 and 6), XX males with genital ambiguities (patients 1, 2, 3, and 4), and XX true hermaphrodites (patients 7, 8, and 9) (Table 1). The phenotypes of the two classic 46, XX males differed from that described in the literature in that one had short stature (157.5 cm) and the other, a small penis for his age (Job and Canlorbe 1981).

In the four XX males with genital abnormalities, penis size and testis size, measured directly or after surgery in the case of cryptochid patients, were below the normal values for the patient age (Job and Canlorbe 1981; Zachmann et al. 1974). Genitography revealed a vaginal pouch in two of these four patients. Penis and testis sizes were similar in the XX true hermaphrodites. Two of these patients had a uterus in addition to a vaginal pouch. Gonadal biopsies were performed in three XX males with genital abnormalities between the ages of 1 month and 5 years. They were normal for their ages with regard to spermatogonia, although in one case (patient 3), the testicular tissue showed lack of germ cell maturation.

In the two oldest patients, the testicular tissue was dysgenetic, without any spermatogonia and with Sertoli and Leydig cell hyperplasia. Patient 8 underwent biopsies at 8.9 and 22 years, one on the left side and three on the right. Each time, only testicular tissue was found. (Fig. 1A). After gonadectomy (Fig. 1B, C), the gonads were cut into sections and a small ovarian zone with corpus albicans was discovered. The diagnosis was therefore subsequently changed from XX male to true hermaphrodite. In all biopsies the ovarian tissue was normal.

Family histories

Our patients were of French origin in four cases: patients 4, 5, 6, and 8; Algerian in one case: patient 3; mixed Algerian and French in one case: patient 2; and of black African origin in two cases: patients 7 and 9. All of them were unrelated. No family cases of genital ambiguity were found when parents and siblings were examined; all had normal genitalia. All patients had normal XX karyotypes without any structural anomaly of the X chromosomes. On Q banding, no Y fluorescence was observed on the 40–50 metaphase analyses.

Hormonal data

In the neonatal period, testosterone levels in one XX male with genital ambiguities and in one case of true hermaphrodism were comparable with control levels. The prepuberal gonadotropin and testosterone levels were within the normal ranges. At the beginning of puberty Tanner stage PII (Tanner 1969), the testosterone level was normal, but the peak of gonadotropins after LH-RH was elevated. After stage PIII, basal and peak values of gonadotropins were above control values. Testosterone levels were still normal in stage PV and declined at adulthood except in two cases; patients 3 and 6. After hCG treatment, the rise of testosterone was variable regardless of age. In all adults, Leydig cells were responsive to stimulation. Consequently, the hormonal values do not distinguish the two groups of XX males with AG and 46,XX TH.

DNA results

All six XX males and the three XX true hermaphrodites were analyzed with seven Yp DNA probes (see Patients and methods). Among the six XX males, cases 1–4 were negative for all the Y DNA sequences tested (Table 2, Fig. 2). In contrast, cases 5 and 6 were positive for all the probes. Cases 7, 8, and 9, the true hermaphrodites, had identical patterns to those of cases 1–4, suggesting that XX males without Yp DNA sequences and with external sexual ambiguities might originate by a similar mechanism to that of XX true hermaphrodites.

Familial cases

We also analyzed two familial cases of XX males with genital ambiguity (patients A.R. and A.H.) in an Algerian family. Their parents were clinically normal as were

Table 2. Molecular analysis of the nine sporadic cases and the two familial cases (A.R. and A.H.) with Y-specific DNA probes. The probes have been ordered as they map on the Yp chromosome

Case		Y DNA probes					
		GMGY3	pDP1007	47b	118	52d	50f
1	XX male, ambiguities	−	−	−	−	−	−
2	XX male, ambiguities	−	−	−	−	−	−
3	XX male, ambiguities	−	−	−	−	−	−
4	XX male, ambiguities	−	−	−	−	−	−
5	XX male, normal	+	+	+	+	+	+
6	XX male, normal	+	+	+	+	+	+
7	TH	−	−	−	−	−	−
8	TH	−	−	−	−	−	−
9	TH	−	−	−	−	−	−
A.R.	XX male, ambiguities	−	−	−	−	−	−
A.H.	XX male, ambiguities	−	−	−	−	−	−

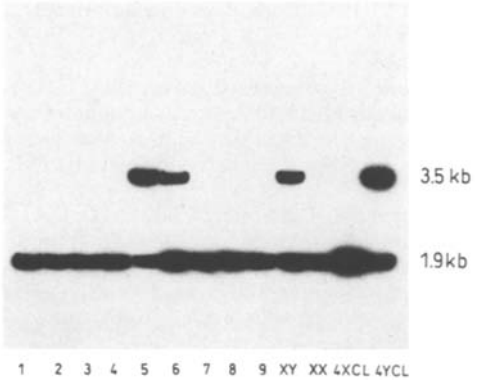

Fig. 2. Southern blot analysis of the nine patients was performed on genomic DNA digested with *Eco*RI, fractionated on 0.8% agarose gel, and hybridized with the pDP1007 probe. The ZFX *1.9-kb band* is an X-specific band; the ZFY *3.5-kb band* is a Y-specific band. The *lanes* for the XX cases are numbered from *1* to *9* and are as follows: *lanes 1–4* XX males with genital ambiguities; *lanes 5, 6* classic XX males; *lanes 7–9* XX true hermaphrodites. The controls are: *XY* normal male; *XY* normal female; *4XCL* a 4-X cell line; *4YCL* a 4-Y cell line

their two brothers and five sisters. Their phenotypes correspond to those of the four sporadic cases of XX males with genital ambiguities (Table 1), and both A.R. and A.H. were also negative for all the Yp DNA sequences tested.

Discussion

After the first description of classic XX males by de la Chapelle et al. (1964), many other cases were reported in young adults. On the other hand, XX males with genital abnormalities have been reported mainly in pediatric patients (Roe and Alfi 1977; Takayasu et al. 1973; Shah et al. 1961; Schweikert et al. 1982; Cleveland and Chang 1965), and these cases seem clinically close to true hermaphroditism. Indeed, it has been very difficult to draw a clear line between these two conditions in our patients. The genital abnormalities (external and internal) are identical, and the penile size and cryptorchidism are similar. Only after surgery or biopsy can the gonad be identified as a testis, an ovary, or an ovotestis. However, case 8 demonstrates that biopsy may be insufficient to exclude a limited ovotestis, and complete gonadal examination may be needed to demonstrate it. The histologic evolution of testicular tissue is identical in XX males and in true hermaphrodites, i.e., normal spermatogonia are found in young patients, and dysgenetic tissue without spermatogonia, in patients aged over 8 years. The absence of germ cells in adult testes from XX males can be interpreted as due to disappearance of these cells during puberty, as demonstrated by the series of three biopsies performed in patient 8. These results may be related to similar events observed in Sxr mice (Eicher and Washburn 1986).

No endocrinologic criteria could be found to be characteristic of any group of patients. Although in classic XX males testosterone levels remain normal in some adult patients (Kuhlwein et al. 1981), this is not always the case (Lafranchi et al. 1980). Other clinical evidence for XX males with genital abnormalities and true hermaphroditism having the same genetic defect is that these two phenotypes may be encountered in the same pedigree. Berger et al. (1970) reported a family in which one brother of a true hermaphrodite was an XX male with genital abnormalities. Kasdan et al. (1973) found the same characteristics in two brothers, while an uncle with an XX karyotype presented a normal phenotype. Skordis et al. (1987) reported a family in which two XX males with genital abnormalities had a first cousin and an uncle who were XX true hermaphrodites. All the XX males from these three families underwent testicular biopsy. So far, our molecular analysis has shown no evidence of Y-DNA sequences in XX males with genital ambiguities.

The only two cases bearing Y sequences presented here (patients 5 and 6) can be considered as classic XX males as described by de la Chapelle et al. (1964) and explained by the hypothesis of an accidental X/Y interchange in the paternal meiosis (Ferguson-Smith 1966). Several hypotheses can be proposed to explain the presence of testicular tissue without any detectable Y DNA sequences:

1. Mutation of an autosomal (or pseudoautosomal) gene. This hypothesis is compatible with several human familial cases previously described. This mutated gene could be a downstream sex-determining gene. Such autosomal mutations have been identified in the mouse. The Tda-1 and Tda-2 mutations result in the production of XY female or true hermaphrodite males (Eicher and Washburn 1986). Perhaps a constitutive mutation in an autosomal gene downstream of the primary sex-determining gene is responsible for Y-negative XX males and XX true hermaphrodites. To test such hypotheses, we are presently analyzing several familial cases with at least two affected sibs or twins. Such genetic studies will allow us to map the genetic defect on an autosome or on the X chromosome. Also we are presently analyzing two unrelated families with two affected boys: one XX male with AG, the other an XX true hermaphrodite.

2. Hidden mosaicism limited to the gonads. Since DNA analysis could not always be performed on testis biopsies, we could not completely exclude an undetected mosaicism with Y DNA material present among a few testicular cells such as Sertoli cells. To exclude very limited mosaicism in the gonads, we are presently analyzing the presence of Y DNA material in testis biopsies using a polymerase chain reaction (PCR) with Y-specific DNA probes.

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