46,XX MALE: CLINICAL, HORMONAL/GENETIC FINDINGS

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The clinical genetics and hormonal status of the 46,XX male is well determined. This is a rare condition that affects one out 20,000 male births. This study evaluates 5 infertile patients with no abnormalities in sex definition in whom we noted variants in their phenotype, like small penis, hypospadias, cryptorchidism, flat scrotum, and in some of them small testis. Only one patient had gynecomastia; all patients were azoospermics. Otherwise, serum FSH levels were elevated in only 3 patients and LH in 2. Serum levels of testosterone were low in 3 cases. Karyotype was 46,XX without evidence of mosaicism. PCR of genomic DNA studied revealed only the presence of SRY gene. DNA material in the Y chromosome was similar in all patients, but this did not correlate with the phenotype findings and hormonal levels in all of them. Testing new chromosomal markers should be of great value in the definition of clinical difference.

Keywords 46,XX male, azoospermia, male infertility, Y-chromosome sequences

The genetic sex, determined by the presence or absence of the Y chromosome, directs the embryogenic gonads to develop as testes or ovaries and the cascade of events that determine sexual differentiation during embryogenesis [21]. Sexual function and reproduction occur after puberty. In adult males, sterility is a common cause of consultation in andrology services. Some have an apparently normal female karyotype and are commonly referred to as XX males. This rare condition affects 1 out 20,000 male births. They have a normal external male genitalia, development of male sexual ducts, but small and azoospermic testis. Clinical features of Klinefelter syndrome are found, but patients are

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shorter and do not have disproportionate long legs. Their IQ is higher and they have fewer learning disabilities [9].

Analysis of such individuals has permitted identification at the molecular level of some genes involved in sex determination, including SRY (sex determination region of Y) [14]. This gene is the switch to start male sexual differentiation by triggering the Sertoli cell lineage [15]. SRY encodes a transcription factor that binds specific sequences in the DNA. However, other target genes for its action have not yet been identified, because the DNA recognition sequence for SRY is found in many genes [4, 17].

Other genes involved in the process of sexual differentiation have been isolated; SF-1 and WT-1 are required for the early formation of the gonadal primordium [7]. An autosomal gene SOX-9, which is downstream from SRY, can cause sex reversal by direct interaction of this gene with SF-1. These two genes are also involved in the regulation of Sertoli cell specific expression of MIS (anti-Mullerian hormone) and the synthesis of testosterone in the fetal testes [11].

XX sex reversal may be caused by at least 3 different mechanisms: (1) an abnormal X-Y interchange (SRY, positive) [16]; (2) genes other than TDF (testis determining factor) that when mutated can trigger testis differentiation in SRY negative XX males; and (3) mosaicism with a prevalent XX lineage and a hidden or scarce lineage containing a Y chromosome [10].

In about 80% of these individuals, the male differentiation of the gonads can be ascribed to the presence of Y-specific DNA, including the SRY gene, encodes the testis determining factor [25]. The sequences of this gene, by in situ hybridization studies, are usually located on the distal portion of the paternal X chromosome (Xp22.3 to Xpter) [1, 20], resulting from an abnormal interchange or crossing over between the X and Y during paternal meiosis near the pseudoautosomal boundary.

We present here 5 XX adult males caused by the first mechanism described above. Clinical, hormonal, cytogenetic, and molecular data are described.

MATERIAL AND METHODS

Five adult male patients were referred to us because of sterility. None of them had a family history of sterility, and all were sporadic cases. Phenotype, age, height, hair distribution, external and internal genitalia, and testicular volume (measured by Prader orchidometer) were evaluated. Chromosome analysis was performed on peripheral lymphocyte culture using GTG banding. In each patient 100 metaphases were analyzed [24]. Seminal analysis, according to WHO criteria, was performed on 3 occasions [27]. Measurements of baseline levels of FSH and LH were done by IRMA (MAIA clone, Serono), testosterone (T) and estradiol (E2) by Ria (DPC), and prolactine (PRL) by IFMA.

For PCR amplification, genomic DNA was extracted from peripheral blood leukocytes. DNA (250 ng) from the patients and normal male and female controls were amplified by polymerase chain reaction (PCR). It was performed in 100-mL reactions containing 50 mM KCl, 20 mM Tris–HCl, pH 8.4, 2.5 mM MgCl, 100 µg/mL BSA (BRL), 200 mM each dNTP (dATP, dCTP, dTTP, and dGTP), 50 pM of each amplification primer, and 2 U Taq DNA polymerase (BRL). The samples were overlaid
with 100 μL of mineral oil and were subjected to 35 cycles of amplification as previously described [8]. The cycling reaction was performed in a programmable heat block (PTC-100 Thermal Cycler, MJ Research). The amplified products were separated by electrophoresis in 1.5% agarose gels in TBE buffer and stained with ethidium bromide.

SRY1/SRY2 amplify a 299-bp region that belongs to encoded sequence of SRY gene equated to TDF (testis determining factor). Y1.1/Y1.2 amplify a 154-bp region belonging to tandem repeat HaeIII 3.4-kb sequences. As a member of DYZ1 sequences it constitutes a Y-chromosome heterochromatic region (Yq12-Yqter). AMXY-1/AMXY-2 primers amplify a 977-bp region belonging to amelogenin gene on the X-chromosome short arm (Xp22.1) and a 788-bp belonging to a homologous sequence on the Y-chromosome short arm (interval 4A in Yp11.2). Y1/Y2 amplify a 170-bp region belonging to tandem repeat sequences of Y centromere (interval 4B). All these primers were used as previously described [8].

Patients refused to undergo testicular biopsies. All patients signed the informed consent and the study was approved by the Ethical Committee of the Durand Hospital.

### RESULTS

In the 5 cases a 46,XX karyotype was documented. Clinical findings are shown in Table 1. The mean stature of the patients was 1.70 m. Two men were in the 5 percentile despite the average stature of their parents (50 percentile). The external genitalia was masculine, with no abnormalities in sex definition. One of the patients had a small penis and hypospadia and the other was cryptorchid with a small, flat scrotum; testicular volume in all cases was smaller than 15 mL, except in case 4; only 1 patient had unilateral gynecomastia (case 1).

All patients were azoospermic. Testosterone serum level was normal in 2 cases, in the lower limit of the normal range in the other two, and only one had a low concentration of this hormone (case 3) (Table 2). Serum levels of FSH were above the normal range in 3 patients (cases 1, 3, and 5). Serum LH levels were elevated in cases 1 and 5.

Molecular studies revealed only the presence of sequence of 299 pb of SRY gene (Figure 1).

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>28</td>
<td>35</td>
<td>28</td>
<td>39</td>
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<tr>
<td>Height (cm)</td>
<td>180</td>
<td>170</td>
<td>160</td>
<td>174</td>
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<td>Corporal hair</td>
<td>Normal</td>
<td>Normal</td>
<td>Gynoid</td>
<td>Gynoid</td>
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<td>External genitalia</td>
<td>Testis &lt; 15 mL</td>
<td>Small scrotum</td>
<td>Bilateral cryptorchidism</td>
<td>Hypospadia</td>
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<tr>
<td>Testicular volume</td>
<td>—</td>
<td>—</td>
<td>Testis &lt; 15 mL</td>
<td>Testis &gt; 15 mL</td>
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<tr>
<td>Gynecomastia</td>
<td>Unilateral</td>
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DISCUSSION

All cases reported here were positive for SRY gene, which is the most frequent type of XX male reported. Molecular studies of XX males fit into groups based on their phenotype and the presence or absence of Y specific material; 80% of XX males have Y material including TDF [12]. SRY gene is one of the principal genes for testicular differentiation, while other genes on the Y chromosome are not so important in defining the phenotype. However, studies have demonstrated the absence of a strict correlation phenotype–genotype. Three categories of XX males have been observed: phenotypic males with or without gynecomastia, males with genital ambiguities, and true hermaphrodites. Our series is that of sterile males without genital ambiguities.

In patients with normal male external genitalia the amount of Y material was greater than in those with hypospadias or genital ambiguities [18]. But there does not seem to be a single cause/effect relationship between the SRY gene and clinical findings [6]. A normal male phenotype may develop without the SRY gene, indicating that this gene does not act alone in this process.

This was observed in our cases in which the amount of Y-specific material was similar in all patients. The clinical appearance was in the spectrum of the classic XX males, but we noted some variation in the phenotype, such as the cases with a small, flat scrotum, cryptorchidism, hypospadias, and micropenis; that may be due to the synthesis and regulation of testosterone in fetal testes by genes involved in the process of sexual differentiation [11]. Besides, none of our cases showed Y centromeric sequences, arguing against a hidden mosaicism for an XY cell line. Two patients were shorter in stature than normal XY males. That may be due to the absence of Yq chromosome material, which are genes responsible for the final height [19], and not to familial stature, which were in all cases in the 50 percentile ± 1SD.

Our patients had spontaneous virilization and not all of them had reduced testicular volume. As expected, testosterone serum levels correlated with the gonadotropin concentrations. All patients were azoospermic, but only three of them had high levels of FSH. The mechanism of FSH regulation remains controversial. It is accepted that inhibin B produced by the Sertoli cell is the principal inhibitory factor of FSH secretion at pituitary level, but also testosterone acts per se or by its central aromatization in estradiol. Serum inhibin B levels are highly correlated with sperm concentration; based on this evidence inhibin B has been also proposed as a reliable marker of spermatogenesis [22]. However, the clinical usefulness of circulating inhibin B levels in predicting the presence of mature

<table>
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<th>Table 2. Serum levels of testosterone, estradiol, prolactin, LH, and FSH in the 5 XX males</th>
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<tr>
<td>Normal values</td>
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<tr>
<td>Testosterone (ng/mL)</td>
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<tr>
<td>Estradiol (pg/mL)</td>
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<tr>
<td>Prolactin (ng/mL)</td>
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<td>LH (mIU/mL)</td>
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<td>FSH (mIU/mL)</td>
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Figure 1. Molecular studies. (A–D) Banding patterns of one XX male as an example (P) and normal female (F) and male (M) controls. Control (D) without DNA (water sample). Molecular weight marker, 123-bp ladder (W). (A) the 299-bp band, belonging to SRY was present in the patient and male control, and absent in the female control. (B) Results obtained using primers for the (AMGL) gene: the 977-bp band belonging to Xp22.3 was present in the patient and in both male and female controls. The 788-bp band belonging to Yp11.2 was absent in the patient and in the female control, and present in the male control. (C) The 170-bp region belonging to tandem repeat sequences of the Y chromosome (DYZ3) was absent in the patient and the female control and present in the male control. (D) The 154-bp band corresponding to Y heterochromatin sequences (DYZ1) was present in the patient and in the male control, and absent in the female control.
sperm in the testis has been recently challenged [13, 26] and the hypothesis of the existence of inhibin B synthesis in the absence of FSH and sperm has been raised [2]. In experimental models mRNA levels for the inhibin subunits and immunoreactive inhibin secreted by the seminiferous tubules vary according to the stage of the seminiferous cycle [5]. Germ cells are also involved in the modulation of inhibin synthesis [23] and it seems that human inhibin is a joint product of Sertoli and germ cells [3]. It is possible that in our cases, the hormonal variability may be due to the different impact on the Sertoli cell and/or on spermatogenesis, but this cannot be confirmed because patients refused to undergo testicular biopsy.

In conclusion, the amount of DNA material in the Y chromosome was similar in our patients but this was not correlated with phenotype findings and hormonal levels in all of them. Testing new chromosomal markers in 46,XX male will establish correlations with the clinical findings, identifying gene regions either in the Y or other autosomal chromosomes implicated in the definition of the phenotype.

REFERENCES

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