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True Hermaphroditism in a Phenotypic Male without Ambiguous Genitalia: An Unusual Presentation at Puberty

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Established Facts

- Even though the appearance of external genitalia is not essential to define true hermaphroditism, it is generally assumed that various degrees of genital ambiguity do exist.
- We report the case of an adolescent boy with 46,XX/46,XY true hermaphroditism who presented with normal external genitalia and no sexual ambiguity.
- In patients of Caucasian origin a 46,XX/46,XY karyotype is frequently found, but the mechanism of generation is debated.
- Analysis of DNA polymorphisms allows to distinguish both maternal and paternal contributions to the genotype, and it would allow to explain whether this karyotype is generated by chimerism or by mosaicism.

Novel Insights

- We report a particular patient with 46,XX/46,XY true hermaphroditism without ambiguous genitalia.
- In this patient, we present clinical and pathological findings and markers of gonadal function before and after surgery.
- We performed a polymerase chain reaction mediated analysis using seven microsatellite markers which yielded a genotype that did not confirm chimerism and supported the hypothesis of mosaicism as the mechanism of generation of this karyotype.

Key Words
True hermaphroditism · Ambiguous genitalia · Mosaicism · Chimerism

Abstract
True hermaphroditism usually appears with ambiguous genitalia requiring extensive evaluation during the neonatal period. There have been occasional cases with better differentiation of external genitalia, leading to delays in diagnosis. We report the case of an adolescent boy with true hermaphroditism who presented with normal external genitalia and no sexual ambiguity. He was referred due to progressive gynecomastia and arrest of puberty. He presented at the age of 16 years for gynecomastia of rapid progression with normal penile development and both gonads in scrotum and normal testosterone and increased gonadotropin levels. Gonadal ultrasound scan was compatible with testicular and

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ovarian tissues in scrotum, and the karyotype showed two cellular lines (46,XX/46,XY). Gonadal histology revealed bilateral ovotestes. A genotype polymerase chain reaction mediated analysis using seven microsatellite markers did not confirm chimerism. Clinical findings and mechanism of generation are discussed.

Introduction

True hermaphroditism (TH) is a rare abnormality of gonadal differentiation characterized by the presence of testicular and ovarian tissues within the same individual [1, 2]. Even though the appearance of external genitalia is not essential to define TH, it is generally assumed that various degrees of genital ambiguity do exist. The most common gonadal abnormality found in TH is an ovotestis, but different combinations have been described [2–4]. As in other patients with an abnormal gonadal histology, TH may have a mixture of müllerian and wolffian derivatives due to various degrees of insufficiency in antimüllerian hormone (AMH) and testosterone production by the fetal gonads during embryogenesis. In 65% of the TH patients of Caucasian origin a 46,XX/46,XY mosaicism or chimerism is present [3], while 46,XX is prevalent in patients of African origin.

Here, we report the case of an adolescent boy with TH who presented with progressive gynecomastia, small testes, and no genital ambiguity.

Case Report

A 16-year-old boy was referred due to progressive gynecomastia and arrest of puberty. He had been born by normal delivery after an uneventful pregnancy. The parents were nonconsanguineous and had 3 older and healthy children (1 son and 2 daughters). His height was 166.8 cm (SDS –0.34, target height 177 cm), and his weight was 61,200 kg (50th percentile). There were no other relevant findings. On physical examination, he showed male habits, breast stage Tanner IV with nipple hyperpigmentation, and a normal penis of 6.3 cm in length and 2 cm in width. Both testes were placed in the scrotum; they had a firm consistency, particularly on one pole, where a nodular area was noted bilaterally. The bone age was 15 years. Hormonal values were as follows: luteinizing hormone 7.8 U/l (radioimmunoassay normal range 2–6 U/l), follicle-stimulating hormone 26.1 (normal range 2.2–6) U/l, testosterone 3.9 (normal range 2.8–8.1) ng/ml, estradiol 25 (normal <20) pg/ml, AMH 125 (normal range 10–140) pmol/l, and inhibin B 114.8 (normal range 260–488) pg/ml. The karyotype of the peripheral lymphocytes was 46,XX (80%)/46,XY (20%).

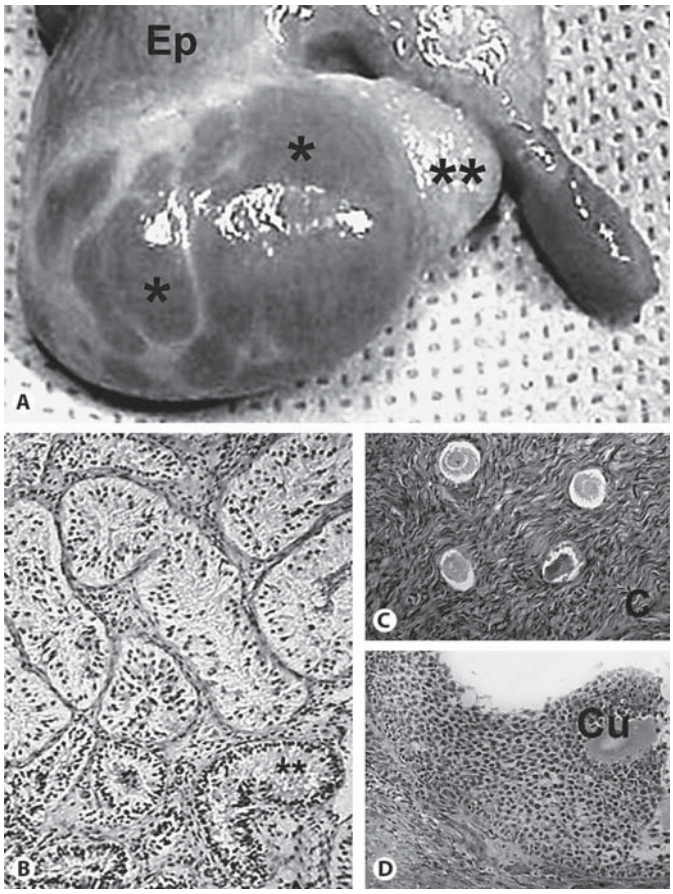

Fig. 1. A Right gonad. The testis occupies more than three quarters of the gonad. Various tan-yellowish irregular areas correspond to infiltration of the tunica by cortical seminiferous tubules (asterisks). Clearly demarcated from the testis, a smaller ovarian sector is seen to the right (double asterisk). Ep = Epididymis. **B** Histological appearance of the testicular biopsy specimen. Seminiferous tubules are populated only by Sertoli cells, some of them immature (double asterisk). **C** Ovarian sector. Four primary follicles are seen embedded in a thick fusocellular ovarian stroma. **D** Antral follicle. Note the cumulus oophorus (Cu).

Testicular ultrasound showed a homogeneous parenchyma with multiple microcysts on each pole and a 13 × 11-mm dominant left cyst. The testicular volumes were 3.4 and 3.3 ml, respectively.

On exploratory surgery, both gonads showed two well-demarcated areas. The testicular component was clearly predominant and appeared as an ovoid-shaped gonad with a thin tunica and numerous tan-yellowish spots of irregular shape representing groups of seminiferous tubules invading the tunica albuginea (fig. 1A). The ovarian component was limited to a small section of each pole of the gonads. There was a rudimentary fallopian tube on each side. An uterus was not found. The ovarian component was completely removed, and a testicular biopsy specimen was obtained. Gonadal histology revealed seminiferous tubules with severe germ cell depletion, foci of immature tubules, peritu-

Table 1. Longitudinal variations of hormone levels and ultrasound findings after surgery

Age, years	16	16.7	17.5	18.4	19
Postoperative time, months	preoperative	4	11	24	32
Testosterone, ng/ml	3.4	2.5	3.0	1.5	3.3
Testosterone after human chorionic gonadotropin, ng/ml		5.1			
Estradiol, pg/ml	25	41.5	<28	57	<28
Estradiol after human chorionic gonadotropin, pg/ml		62			
Luteinizing hormone, mU/l	4.1	5.6		4.8	9.3
Follice-stimulating hormone, mU/l	24.4	31		4.1	21.5
Antimüllerian hormone, pmol/l	125				
Inhibin B, pg/ml	114.8	149.4			
Testicle (T) size by ultrasound, mm	right 35 × 13 × 15 left 28 × 12 × 20 cyst with thick wall in left T	right 28 × 10 × 16 left 29 × 11 × 16 homogeneous	right 25 × 11 × 15 left 27 × 16 × 13 homogeneous	cyst in left T of 8 mm	cyst disappearance

bular fibrosis, and different degrees of hyalinization (fig. 1B). The ovarian component had follicles of all stages of development, including antral follicles embedded in a thick fusocellular cortical stroma (fig. 1C, D). Fluorescence in situ hybridization using X and Y centromere probes was carried out on histological sections of the testicular tissue and showed a XX/XY ratio of 8:2, similar to that seen in the karyotype of the peripheral lymphocytes.

Six months after surgery, gynecomastia had not regressed which prompted bilateral mastectomy. The postsurgical hormonal values during the follow-up period are shown in table 1.

A polymerase chain reaction mediated analysis using seven microsatellite markers (Gene Print STR Systems; Promega, Madison, Wisc., USA) yielded a genotype that did not confirm chimerism and supported the hypothesis of mosaicism as the mechanism of generation of this karyotype.

Discussion

TH is a rare disorder of gonadal differentiation characterized by the presence of testicular and ovarian tissues in the same individual [1]. Though it usually appears with ambiguous genitalia prompting extensive evaluation in the neonatal period, there have been occasional cases in whom the diagnosis was delayed due to near-normal external genitalia [3, 5].

Our patient consulted at the age of 16 years for gynecomastia of rapid progression and had normal male external genitalia with both gonads in the scrotum. Serum testosterone was normal, and the gonadotropin levels were increased. Gonadal ultrasound was compatible with testicular and ovarian tissues combined in the same gonad, and peripheral karyotyping showed two cell lines (46,XX/46,XY). His height was below standard for the genetic height and similar to that of his sisters, reflecting probably the low proportion of 46,XY cells.

It is likely that the endocrine function of the testicular interstitium was satisfactory in the fetal testes, as demonstrated by the appropriate virilization of wolffian derivatives and external genitalia. The levels of testosterone achieved during puberty were in the low-normal range, but allowed an appropriate virilization of secondary sexual characters. As shown in table 1, the gonadotropin and testosterone levels decreased when the estradiol levels were increased. It is not clear whether this decrease in testosterone is derived from local paracrine inhibition due to high intragonadal levels of estradiol or from systemic suppression of pituitary gonadotropins (hypogonadotropic hypogonadism). The AMH production during prepuberty is a good indicator of Sertoli cell function [6]. The persistence of fallopian tubes with absence of an uterus suggests only a mild deficiency of the AMH production during early fetal life. The usefulness of inhibin B levels as markers of tubular function is limited, because the inhibin secretion can originate in testicular Sertoli cells or in ovarian granulosa cells. Another unusual feature of our patient was the scrotal position of his testes. It has been demonstrated that insulin-like peptide 3, a product of fetal Leydig cells, together with testosterone regulates testicular descent [7]. The presence of normally masculinized gonaducts and external genitalia indicates normal Leydig cell function during the fetal period which explains the absence of cryptorchidism.

The fertility is usually reduced in males with TH, complete spermatogenesis having been reported in only 2 cases [3, 8, 9]. Up to now, no spermogram has been carried out in the patient presented here, but the histological study revealed severe depletion of spermatogenic cells and different degrees of testicular hyalinization which indicate a seriously compromised spermatogenic potential.

Although no chromosomal study was carried out in the gonad, fluorescence in situ hybridization performed with probes directed to X and Y centromere regions showed a sex pair proportion similar to that found in blood lymphocytes. The role of Y gene transcripts in gonadal differentiation and spermatogenic development has been demonstrated [10]. Since there is a low proportion of 46,XY cells in the karyotype, we presume that their deficiency can explain the spermatogenic regression of this patient, as has been demonstrated in murine models of sex reversal [10].

Approximately 20% of the patients with TH possess a 46,XX/46,XY karyotype [3]. Different hypotheses have been proposed to explain whether it is generated by chimerism (two cellular lines from different zygotes) or by mosaicism (different cell lines derived from the same zygote) [11–13]. At present, the analysis of DNA polymorphisms allows to distinguish both maternal and paternal contributions to the genotype [11, 12, 14]. In the present case, we analyzed seven scoreboards (6 of them in autosomes and 1 in the X chromosome). Among the autosomic scoreboards, two (HUMVWFA31 and D13S317) offer complete information, because both parents are different heterozygotes. The presence in our patient of only two alleles, one from each parent, suggests the formation of a digametic zygote in opposition to the mechanism of chimerism, where more than one allele from a progenitor could be expected. The scoreboard belonging to the X chromosome (HUMPRTB) demonstrates the presence of two alleles, one of which is present in the paternal genotype. We hypothesize that a XY spermatozoon (disomic for the sex pair) fertilized an oocyte, resulting in the formation of a 47,XXY zygote. Subsequent disorders in the mitotic disjunction may have given rise to a triple 47,XXY/46,XX/46,XY mosaic and subsequent loss of the 47,XXY line during early embryogenesis. Niu et al. [15] have demonstrated that this was the case in a patient with similar karyotype and genital ambiguity.

In summary, we reported a patient with TH, male phenotype, and no genital ambiguity, bearing a karyotype with two cell lines probably as a result of nondisjunction of sexual chromosomes after the formation of an aneuploid zygote. Though it is a rare form of presentation, TH should be considered, when there is clinical evidence of an exaggerated estrogenic activity in a male with gynecomastia and small testes.

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