CASE REPORT

SRY-negative 46,XX infertile male with Leydig cell hyperplasia: clinical, cytogenetic, and molecular analysis and review of the literature

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Objective: To describe a 46,XX male whose infertility is not accounted for by a translocation of the *SRY* gene to the X chromosome or to the autosomes.
Design: Case report.
Setting: Fertility Center of CHA Gangnam Medical Center, Seoul, South Korea.
Patient(s): A 29-year-old male with normal male phenotype, in whom seminal analysis showed complete azoospermia.
Intervention(s): Laboratory evaluations, radiologic studies, testicular biopsy, G-banding karyotype, in situ fluorescence hybridization, and polymerase chain reaction.
Main Outcome Measure(s): Clinical and laboratory findings.
Result(s): Peripheral blood culture for chromosome studies revealed 46,XX chromosome complement. Cytogenetic and molecular analyses excluded the presence of *SRY* gene. Radiologic studies displayed male structures without Müllerian ducts. Gonadal biopsy showed testicular Leydig cell hyperplasia.
Conclusion(s): This is a very rare case of testicular differentiation in a 46,XX chromosomal constitution without *SRY*. This finding suggests that some unknown genes downstream participate in sex determination. (Fertil Steril® 2010;94:753.e5–e9. ©2010 by American Society for Reproductive Medicine.)

Key Words: 46,XX male, sex reversal, *SRY* gene, Leydig cell hyperplasia

The process of sex determination in humans has not yet been completely elucidated. For the majority of individuals, sex determination is as simple as the presence or absence of a Y chromosome. Individuals with a Y chromosome will develop into males, and those without one will become female. It is clear that this process involves a gene regulatory network in which a Y chromosome-linked master gene, named *SRY* (sex-determining region Y), plays a crucial role (1). *SRY* normally triggers testes formation from bipotential gonadal primordium.

Whereas gonad development is a result of the presence or absence of *SRY*, sex differentiation is determined by the hormonal products produced by the gonads. Whereas testosterone is required for Wolffian duct development, Müllerian inhibiting substance, produced by Sertoli cells, blocks the development of Müllerian ducts, promoting their regression.

However, some individuals will undergo primary sexual reversal, whereby the X and Y chromosomes cross over and exchange genetic materials. This is rare, with a reported incidence of 46,XX maleness of 1 in 9,000 to 1 in 20,000 (2).

Testicular Leydig cell hyperplasia is a rare benign condition that is characterized by small, multifocal, and frequently bilateral testicular nodules (3). In the primary form, male children may present with precocious puberty. However, the secondary form, which more often occurs in adults, most commonly presents as a testicular mass, with approximately 30% of patients developing gynecomastia (4).

The case presented here is a rare *SRY*-negative 46,XX male without ambiguous genitalia, and to our knowledge it is the first case report of the rare disease with histologically confirmed Leydig cell hyperplasia.

CASE REPORT

Clinical Report

A 29-year-old phenotypic male presented with complaints of a painless swollen erythematous lesion in the right inguinal region lasting for 1 week, and difficulty in morning erection began 2 months before the initial evaluation. He also mentioned a weaker grade of hardness and lesser duration of erection than 7 months earlier. His height was

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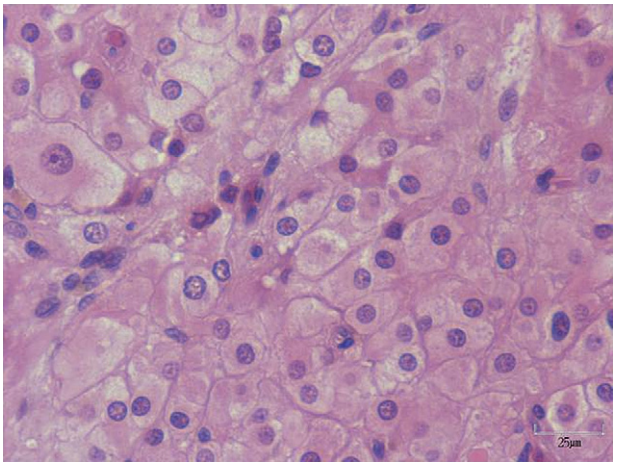
165 cm, and he weighed 62 kg, small for his age group. Thorough physical examination revealed an approximately 0.5 × 0.5-cm soft mass around the right inguinal canal. No other lump was palpable. Both testes were palpable in the scrotum and appeared smaller than average, approximately 3 mL for the right testis and 5 mL for the left testis, according to Prader orchidometer. Both testicles demonstrated abnormal heterogenic architecture with normal vascularity during sonographic imaging, which also demonstrated a right testicular volume of 4.9 cm^3 and left testiclular volume of 5.2 cm^3. The external urethral meatus was in the normal position, and the penis appeared normal and was recorded as 10.7 cm on full erection. Pubic hair was of male type with axillary but no chest hair, as well as no definite sign of gynecomastia. The hormone profile revealed hypergonadotropic hypogonadism, with increased levels of FSH and LH (76.9 mIU/mL and 41.4 mIU/mL, respectively; normal ranges are 1.1–13.5 mIU/mL and 0.4–5.7 mIU/mL). The level of T, 1.79 ng/mL, was slightly below the normal range (2.45–18.36 ng/mL). Otherwise, no hormonal abnormalities were uncovered. At this stage the patient was diagnosed with nonobstructive azoospermia. Transrectal ultrasound showed the prostate gland beneath the bladder base, with no detectable abnormalities and a size of 11.7 cm^3, underdeveloped for the patient's age. Transrectal ultrasound also showed seminal vesicles and ejaculatory duct, which appeared normal and in the correct position. Abdominal and pelvic CT, as well as pelvic ultrasound, showed no abnormalities other than bilateral inguinal lymphadenopathy. Histologic evaluation of the testes revealed totally hyalinized tubules with Leydig cell hyperplasia of testicle with fibrous left epididymis. Histologic evaluation clearly showed spermatogenic failure, increased number of Leydig cells and nucleoli, and less lipofuscin pigment (Fig. 1).

FIGURE 1

Histologic evaluation of testicular biopsy shows an increase in the number and size of Leydig cells, which have multiple nucleoli in an intertubular growth pattern.



Kim. SRY*-negative 46,XX infertile male. Fertil Steril 2010.*

Cytogenetic Analyses

Karyotype analysis of the patient showed apparently 46,XX chromosome complement (Fig. 2A) and no evidence of mosaicism in peripheral blood cells.

To elucidate whether *SRY* gene was present, fluorescence in situ hybridization (FISH) analysis was performed in lymphocytes culture. Two signals for X chromosome but no *SRY* signal was detected, ruling out the presence of an *SRY*-containing cell line (Fig. 2B). The same analyses were performed on paraffin sections of testicular tissue to verify for mosaicism, but again no *SRY* signal was detected (Fig. 2C).

Molecular Analyses

To confirm the absence of Y-chromosome-derived sequences, we amplified the Y chromosome sequences by polymerase chain reaction. No amplification of *SRY* (Fig. 2D) and *DAZ* gene and other sequences in the azoospermic factor (AZF) regions (AZFb, AZFc: *sY117*, *sY127*, *sY143*, *sY134*, *sY138*, *sY152*, *sY153*, *sY147*, *sY149*, *sY269*, *sY157*, and *sY158*) was identified.

Ethical Considerations

This study was approved by the institutional review board of CHA Gangnam Medical Center, and written informed consent was obtained from the patient.

DISCUSSION

Two classes of sex reversal syndromes are known in humans. One is 46,XY complete gonadal dysgenesis, in which testis development fails despite the presence of a Y chromosome. The other is XX sex reversal, in which testicular tissue develops in the apparent absence of Y chromosome (5). Generally, 46,XX sex reversal cases can be classified clinically into two distinct groups. One group includes individuals known as 46,XX true hermaphrodites. It is defined by the presence of both testicular and ovarian tissue in the gonads of the same individual, either separately or together (5). Ambiguous external genitalia are usually present, along with Wolffian and/or Müllerian derivatives (6). The other group includes individuals known as 46,XX males. They are characterized by a full development of both gonads as testes without any evidence of ovarian tissue. The phenotype varies from a fully normal male to ambiguous genitalia. At the molecular level, XX males can be classified as Y-DNA positive or Y-DNA negative, according to the presence or absence of Y-specific sequences, such as *SRY* gene (7).

Analysis of 20 metaphases showed only 46,XX cell populations without any numeric or structural chromosomal aberrations. Peripheral blood DNA was negative for *SRY* gene and other Y-chromosome sequences, and FISH analysis using an *SRY* probe showed no *SRY* hybridization in all cells of peripheral blood and gonadal tissues. Azoospermia may be related to the absence of the Y chromosome long arm, which is known to contain AZF regions, related to spermatogenesis (8).

To explain the causes of the induction of testicular tissue in *SRY*-negative patients, several hypotheses have been put forward. These hypotheses have been previously reviewed by Rajender et al. (9): the hidden gonadal mosaicism for *SRY* gene that has been identified in a few cases as the reason for the development of testicular tissue and male phenotype; a regulatory autosomal recessive gene, termed "Z" gene, whose product inhibits *SRY* that normally inhibits the male pathway; and X-linked locus dosage-sensitive sex reversal that acts as a repressor of male pathway. Furthermore, the *DAX1* and *SOX9* genes might function downstream to the *SRY* gene in the sex-determination pathway (10, 11), and a mutation leading to increased expression of either of these genes could lead to 46,XX female-to-male sex reversal (9, 12). However, we did not perform *SOX9* and *DAX1* analysis in the setting of the patient's 46,XX, *SRY*-negative karyotype, according to the hospital protocol.

We reviewed the literature regarding 46,XX sex reversal in the absence of *SRY* (Table 1). Twenty-five cases were reviewed, and