

# Gonosomal Mosaicism From Deleted Y Chromosomal Nondisjunction

## Case Report

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Structural aberrations of the Y chromosome, such as ring Y, iso- or isodicentric short arm, or large cytogenetically visible deletions of the long arm, are unstable and usually lost during mitosis (Hsu, 1994; Kirsch et al, 2000; Siffroi et al, 2000; Bertini et al, 2005). Therefore, in the clinic, azoospermia factor (AZF) terminal deletion of the long arm was also found to be associated with 45,X cell line. However, the Y chromosome with terminal deletion associated with nondisjunction, leading to mosaicism of 3 cell lines, has not been reported. We present here an azoospermic male with complete masculinization associated with karyotype 45,X/46,Xdel(Y)/ 47,Xdel(Y) del(Y). To precisely define the relationship between the patient's karyotype and the phenotype, testicular pathological and cytogenetic and molecular assay were performed. The complete masculinization might be explained with a lower proportion of 45,X cell line in gonadal tissue than in the blood.

### Case Report

A 30-year-old man attended our center because of an 8-year history of infertility. Physical examination revealed the following: height 168 cm, weight 60 kg, normal male external genitalia, slightly soft and markedly small testes (6 mL bilaterally vs  $19.8 \pm 7.1$  mL for normal adult Chinese). Semen analysis according to WHO guidelines showed no sperm in any of 3 routine tests. Serum hormonal profile was normal for T, E2, luteinizing hormone, and prolactin and high for follicle-stimulating hormone (27 IU/L vs 5–20.0 IU/L for normal adult Chinese). His intelligence and development were nor-

mal. After obtaining patient approval, testicular biopsy was performed, and the pathological result showed the presence of only Sertoli cells and absence of germ cells in all examined seminiferous tubules. No ovarian tissue was found (Figure 1). The Ethics Committee of Jinling Hospital approved this research.

### Materials and Methods

*Cytogenetic and Fluorescence In Situ Hybridization Analysis*—With written approval of the patient, chromosomes were prepared from phytohemagglutinin-stimulated lymphocytes. G-band and C-band staining was done according to standard techniques. One hundred metaphases of G-band and 10 metaphases of C-band were analyzed. Ten metaphases and 50 interphases from the lymphocytes were analyzed by fluorescence in situ hybridization (FISH) with a probe specific for DXZ1 and DYZ3 (CEP X with Spectrum Green and CEP Y with Spectrum Orange, respectively, item 32-111051; Vysis, Downers Grove, Ill). Signals were observed under an Olympus BX51 microscope (Center Valley, Pa) equipped with a cooled charged-coupled device camera and CytoVision 3.0 image analysis

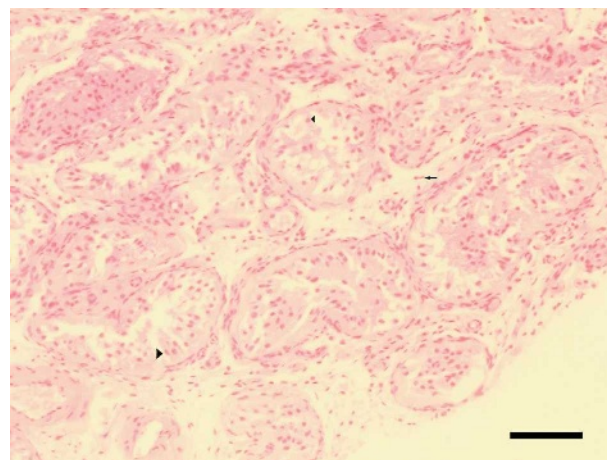


Figure 1. The photo of the testis tissue section tested by microscopy. Sertoli cells, but no germ cells, were visible in all seminiferous tubules. The tubular diameter and lamina propria of the seminiferous tubules, as well as Leydig cells, was normal, but showed a pattern of Sertoli cells-only syndrome. No ovarian tissue was found (hematoxylin and eosin, stain). The arrowhead ( $\blacktriangle$ ) indicates Sertoli cells. The arrow ( $\uparrow$ ) indicates Leydig cells. Scale bar = 40  $\mu$ m.

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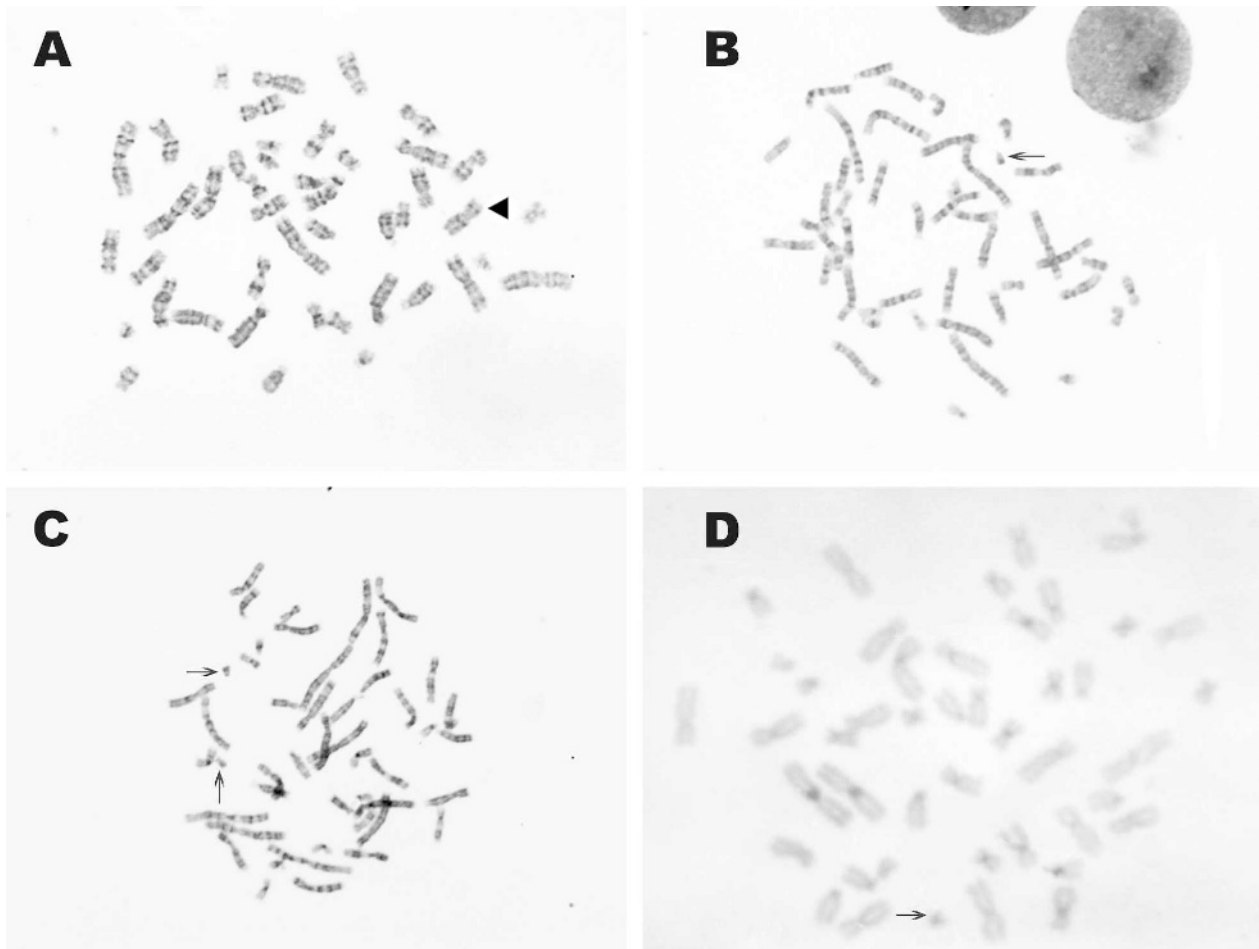


Figure 2. Photos of 45,X/46,Xdel(Y)(q11)/47,Xdel(Y)(q11.23) del(Y)(q11.23) analyzed by C- and G-band staining. (A) Karyotype 45,X analyzed by G-band. (B) Karyotype 46,Xdel(Y)(q11) by G-band staining. (C) Karyotype 47,Xdel(Y)(q11) del(Y)(q11) by G-band staining. (D) Karyotype 46,Xdel(Y)(q11) by C-band staining. The arrowhead ( $\blacktriangle$ ) indicates the X chromosome in panel A. The arrow ( $\uparrow$ ) indicates del(Y) in panels B, C, and D.

software (San Jose, Calif). Karyotyping of both parents was also performed.

*Analysis of the Azoospermia Factor Regions by Sequence-Tagged Site Polymerase Chain Reaction—*Genomic DNA was extracted from peripheral blood of the patient. Multiplex polymerase chain reaction (PCR) was performed for *SRY* and *ZFX/ZFY* genes as the internal control and 6 sequence-tagged site (STS) loci along the Y chromosome (sY86, sY84, sY127, sY134, sY254, and sY255 from azoospermia factor region a [AZFa], AZFb, and AZFc). Primers and the amplification protocol were according to the European Academy of Andrology/European Molecular Genetics Quality Network best practice guidelines for molecular diagnosis of Y chromosomal microdeletions (Simoni et al, 2004), except the primer *ZFX/ZFY* (forward: 5'-ACTGCCCGCCTGTCACTGC-3', reverse: 5'-ACGTCGGTAGTCAGAGGAT-3', 690-bp PCR product). To assess the Y chromosomal break-

point, an additional 6 loci—sY87, sY88, sY95, sY105, sY117, and sY160—were further detected by simple PCR. The STS primers and PCR conditions have been published in the Genome Database (<http://www.ncbi.nlm.nih.gov>). PCR products were separated on 2% agarose gel. The father and a normal fertile man were used as controls, and their blood samples were simultaneously detected by multiplex PCR.

### Results

The karyotype 45,X/46,Xdel(Y)/47,Xdel(Y) del(Y) was observed in the lymphocytes of peripheral blood at a ratio of 27:68:5 (Figure 2A through C). The result of FISH was consistent with that of cytogenetics. The breakpoint of the deleted Y chromosome was located at q11, and the C-band showed the loss of the heterochromatin region (Figure 2D). Multiplex PCR showed the presence of AZFa (sY86, sY84) and the absence of AZFb (sY127, sY134) and AZFc (sY254, sY255). The

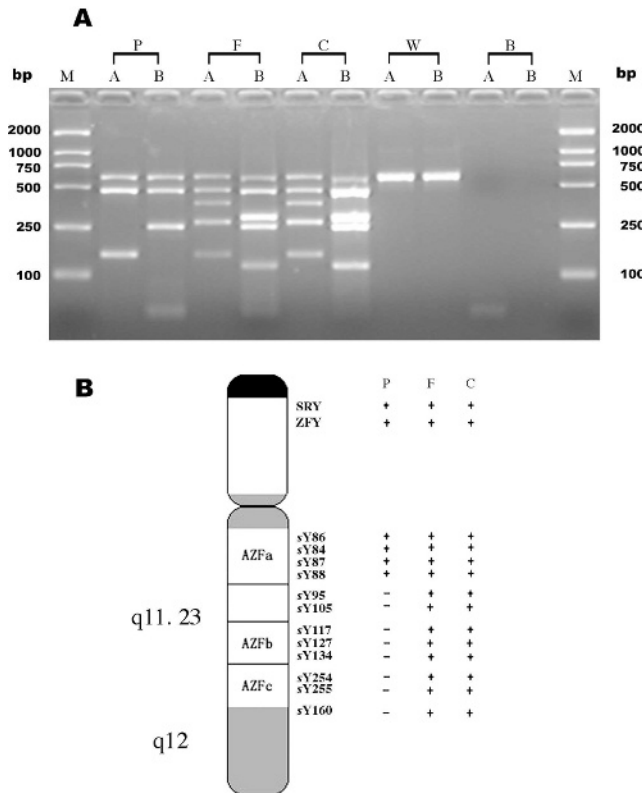


Figure 3. Result of multiplex polymerase chain reaction (PCR). **(A)** Multiplex A: *ZFX/ZFY* (690 bp), *SRY* (472 bp), *sY254* (400 bp), *sY84* (320 bp), *sY127* (274 bp). Multiplex B: *ZFX/ZFY* (690 bp), *SRY* (472 bp), *sY86* (326 bp), *sY134* (301 bp), *sY255* (126 bp). M indicates DL2000 marker; P, patient; F, patient's father; C, a DNA sample from a normal fertile man as a positive control; W, a DNA sample from a woman as a negative control; and B, a blank (water) control. **(B)** Scheme of the regions/loci of the Y chromosome analyzed by PCR. A minus (-) indicates absence; +, presence.

results of simple PCR showed positive *sY87* and *sY88*, whereas *sY95*, *sY105*, *sY117*, and *sY160* were negative (data not shown). The breakpoint was between *sY88* and *sY95* (Figure 3A and B). Taken together, these data showed that the deleted segment was from interval 5H to qter, and the karyotype was 45,X/46,Xdel(Y)(q11.23)/47,Xdel(Y)(q11.23) del(Y)(q11.23). Both parental chromosomes were normal. Multiplex PCR results of the father and the normal fertile man showed presence of all loci tested.

### Discussion

The predominant karyotype in this male with 46,Xdel(Y) coexisting with 45,X and 47,Xdel(Y) del(Y) cell lines suggested that the de novo independent events were involved in the processes of meiosis and mitosis, respectively. First, the deleted Y chromosome was proposed to arise by a break event of the Yq11 region, followed by the loss of a fragment without a centromere during the paternal meiosis stage. Second, the

deleted Y chromosomal nondisjunction event occurred in the postzygotic mitosis stage. The karyotype 45,X/46,Xdel(Y)/47,Xdel(Y) del(Y) was reported for the first time. The mechanism causing the nondisjunction is poorly understood. We suggested a hypothesis that the genes in AZFb and AZFc were not only associated with spermatogenesis but also with Y chromosomal stability. Y chromosomal loss or its nondisjunction might further aggravate the failure of spermatogenesis.

Patients with a karyotype 45,X/46,Xdel(Y)(q11) can present a wide spectrum of sex phenotypes, including complete masculinization, ambiguous genitalia, or Turner syndrome (Robinson et al, 1999; Papadimas et al, 2001; Werner et al, 2005). These phenotypic differences are related not only to the presence or absence of the *SRY* gene on Yp, but also to the proportion of 45,X line in gonadal tissue. As is known, a sufficient *SRY* transcript level is necessary to trigger testes formation. Once testes differentiate, male endocrine function is responsible for the rest of the events involving male phenotypic sexual differentiation. However, if the 45,X line was predominant in gonadal tissue, the phenotype of Turner syndrome would appear. Our patient showed a higher percentage of 45,X cell line (27%) in the peripheral blood, but we speculate that the proportion in gonadal tissue is lower than that in the blood. This is supported by the patient's normal male phenotype.

The case is associated with deletions of *sY117* and *sY127* for *RBMY1* in the AZFb region with the loss of *sY254* and *sY255* for *DAZ* in the AZFc region. These gene deletions should be responsible for the failure of spermatogenesis and male infertility. On the basis of the testicular pathologic pattern with Sertoli cell-only syndrome, the partner of the patient should be inseminated with donor sperm.

We offered an example of del(Y) associated with nondisjunction during mitosis division, which has not been reported previously.

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