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# A 47,XXY female with unusual genitalia

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**Abstract.** A 47,XXY karyotype was found in a 6-year-old girl. The patient had female external genitalia, clitoromegaly, remnants of the ductus mesonephricus, uterus, and gonads in the labia majora which were determined to be testes by histology. Cytogenetic and DNA analyses suggest that the Y chromosome had a normal structure and that both X chromosomes were of maternal origin. The unusual clinical findings in the patient are discussed.

## Introduction

Screenings of unselected newborns have shown that 47,XXY individuals are found with a frequency of 1 in 2000 livebirths (Hook and Hamerton 1977). In contrast, surveys of spontaneous abortions demonstrated 1 in 300 to have a 47,XXY constitution (Hassold and Jacobs 1984). Assuming that spontaneous abortions comprise 15% of all conceptions recognized, the 47,XXY karyotype occurs in 1 in 1000 conceptions, but only 1 in 2000 livebirths (Jacobs et al. 1988).

The overwhelming majority of 47,XXY males present the main clinical features, including the relatively benign phenotype of the Klinefelter syndrome (Jacobs and Strong 1959; Schinzel 1984). To date, only four cases of phenotypic females with a 47,XXY karyotype are described in the literature (German and Vesell 1966; Bartsch-Sandhoff et al. 1976; Gerli et al. 1979; Müller et al. 1990). The testicular feminization syndrome (Morris 1953) has been diagnosed in all of them. This congenital disorder, with an incidence of 1 in 20000 to 1 in 64000 newborns with a 46,XY karyotype (Jagiello and Atwell 1962; German et al. 1973; Pergament et al. 1973) is caused by mutations of the androgen receptor gene. This gene has been mapped to the X long arm at bands Xq11–12 (Brown et al. 1989).

In the present report, a further 47,XXY girl with unusual genitalia is described. Cytogenetic and molecular analyses showed that the Y chromosome is structurally unaltered, and that both X chromosomes are of maternal origin due to non-disjunction during meiosis I.

Dedicated to Professor Ulrich Wolf on the occasion of his 60th birthday

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## Materials and methods

### *Chromosome analyses*

Metaphase chromosomes of the patient and her parents were prepared following standard techniques. QFQ- and CBG-banding, as well as distamycin A/DAPI counterstaining were performed according to Caspersson et al. (1970), Sumner (1972), and Schweizer et al. (1978).

In situ hybridization was carried out with the biotinylated probes pHY2.1 (Cooke 1976) and pXBR (Yang et al. 1982; Willard et al. 1983) simultaneously. Biotin-16-dUTP was incorporated into the probes according to the oligonucleotide technique (Feinberg and Vogelstein 1984). Chromosome preparations were denatured for 1–2 min in 70% formamide/2 × SSC pH 7.0 at 70°C, followed by dehydration through an ice-cold ethanol series. Then 25 µl hybridization mixture (50% formamide/2 × SSC/5% dextran sulfate/5 ng pHY2.1 and pXBR) was denatured for 5 min at 75°C and applied to each slide. Hybridization and detection of the signal with streptavidine-horseradish-peroxidase and DAB was performed as previously described (Schmid et al. 1990).

## DNA analyses

DNA extraction from peripheral lymphocytes of the patient, her sister and their parents, gel electrophoresis, Southern blotting and hybridization with ($^{32}$P)-labeled DNA probes was carried out as described by Schmid et al. (1990). The filters were exposed to X-ray films for 4–20 h.

The following Y- and X chromosomal DNA probes were employed for Southern blot hybridizations: pHY2.1 (Cooke 1976); pJA1143 (Arnemann et al. 1985); Y-190 (Müller et al. 1987); p50f2 (Guellaen et al. (1984); p52d (Bishop et al. 1984); pDP34 (Page et

al. 1984); p47z (Geldwerth et al. 1985); and M27β (Fraser et al. 1987). ($^{32}$P)-dCTP was incorporated into the probes according to Feinberg and Vogelstein (1984).

## Case report

The female patient was the first of two children. The mother was 32 years old at conception, the father 33. Pregnancy only occurred after the mother was treated for sterility. She had received no medication or diagnostic irradiation during pregnancy. The pregnancy, labor and spontaneous delivery (41st week of gestation) were uneventful. The patient's birth weight was 4050 g. A more detailed examination was prompted by an enlarged clitoris and synechia of the labia majora. Following exclusion of adrenogenital syndrome, the first chromosome analysis was performed on 15 QFQ-banded metaphases from peripheral lymphocytes. A 47,XXY karyotype was demonstrated in all cells.

Vaginoscopy was performed at the age of 1 year 10 months. The perineum was high, and the clitoris was enlarged (length 2 cm, diameter 0.6 cm). The sinus urogenitalis separated into ureter and vagina after 5 mm. The meatus urethrae was split dorsally as in hypospadias. The vagina was 7 cm long with a typically infantile portio. There was a noticeable ridge to the left, possibly a remnant of the ductus mesonephricus. Palpable masses in both labia majora were interpreted to be gonads. Portio epithelia, structured in correspondence to the child's age, were demonstrated cytologically. Rectal examination showed the presence of a mass the size of a hazelnut, which was considered to be the uterus. There were no pathological changes demonstrated in the pelvic cavity. At the age of 2 years 1 month biopsy showed normal vaginal mucosa. One month later the gonads in the labia majora were prepared, removed, and histologically defined as testicles. These testicles showed an atrophy of the tubuli and interstitial fibrosis. The epidydimes were hypotrophic and the spermatic cords showed focal lymphangiectasis. There was a varicocele on the left and the spermatic ducts were unremarkable.

At the ages of 2 and 4 years further cytogenetic examinations and DNA analyses on peripheral lymphocytes were performed which confirmed the 47,XXY karyotype of the patient. The patient is now 6 years old, and her physical and psychomotor development to date has been normal.

Eight months after the birth of the patient, the mother became pregnant again. The couple attended for genetic counselling. The family history revealed no consanguinity, no miscarriages and no ambiguous genitalia in females. The karyotypes of the parents were normal. A prenatal chromosome diagnosis revealed an inconspicuous 46,XX karyotype. After a full-term uncomplicated pregnancy a normal female baby was born.

## Chromosome analysis

The cytogenetic analyses of 200 metaphases from peripheral lymphocytes of the patient revealed a 47,XXY karyotype. No evidence for mosaicism was found. After QFQ-banding, distamycin A/DAPI counterstaining, and CBG-banding, the XY chromosomes showed normal structure (Fig. 1a–c). There was no visible evidence for a deletion in the short arm of the Y chromosome. All autosomes and both X chromosomes exhibited unaltered banding patterns.

Simultaneous in situ hybridization with the biotinylated probes pHY2.1 and pXBR specifically labeled the constitutive heterochromatin in the long arm of the Y and in the centromeric region of both X (Fig. 1d). In 200 lymphocyte nuclei analysed, there were always three hybridization signals present (Fig. 1e).

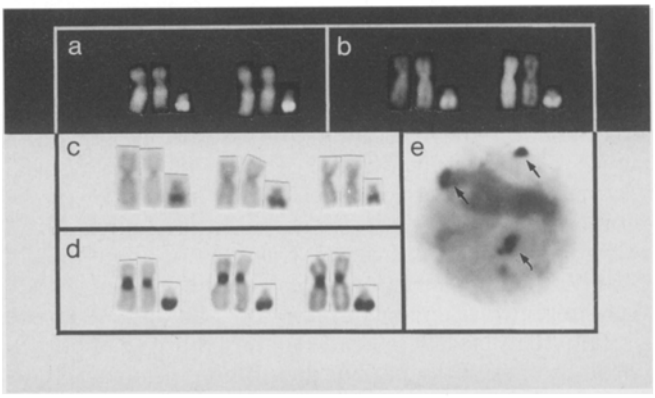


**Fig. 1a–e.** Selected XXY chromosomes of the patient from (**a**) QFQ-banded, (**b**) distamycin A/DAPI-counterstained, and (**c**) CBG-banded metaphases. (**d**) Selected XXY chromosomes and (**e**) lymphocyte nucleus after simultaneous in situ hybridization with biotinylated probes pHY2.1 and pXBR. Note the three distinct hybridization signals in the nucleus *(arrows)*

## DNA analyses

Southern blot hybridization patterns of probes pDP34, Y-190, pHY2.1, pJA1143, p47z, p52d and p50f2 with the DNAs from a normal 46,XY male, the 47,XXY female patient and a normal 46,XX female show that the Y fragments from the short arm, centromeric region, and long arm in the DNAs of the normal male control and the 47,XXY female are in complete agreement in all hybridizations. It can be concluded from these experiments that all 13 loci detected by the probes are present in the apparently structurally normal Y chromosome of the patient (data not shown).

In order to determine the origin of the two X chromosomes, Southern blot hybridization of the probe M27β

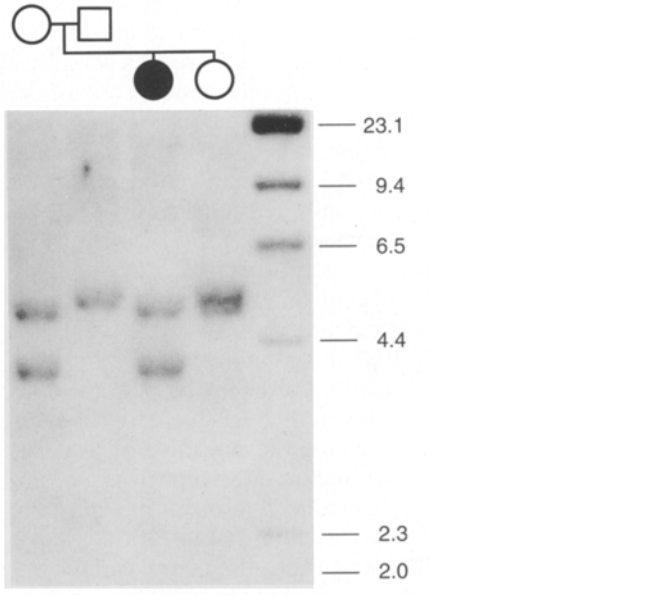


**Fig. 2.** Hybridization patterns of probe M27β to *Eco*RI-cleaved DNAs of the 47,XXY female patient *(black circle)*, her normal 46,XX sister, and their parents. *Hin*dIII-digested phage lambda DNA *(right lane)* was used as molecular weight standard

was performed with the DNAs of the patient and her family. M27β hybridizes to highly polymorphic DNA sequences in the proximal short arm of the X chromosome (Xp11.2 → cen). This polymorphism can be revealed by all restriction enzymes applied (Fraser et al. 1987). As shown in Fig. 2, probe M27β (DXS255) detects two *Eco*RI restriction fragments of about 5.0 kb and 4.0 kb in the mother and her 47,XXY daughter. In the father, M27β recognizes a single restriction fragment of about 5.3 kb. Finally, in the restriction digest of the normal 46,XX daughter, two fragments of about 5.3 kb and 5.0 kb are present. This clearly indicates maternal origin of both X chromosomes in the patient due to non-disjunction during meiosis I.

## Discussion

All methods used show that the sex chromosome constitution of the phenotypically female patient is XXY. The Y chromosome is cytogenetically unremarkable and contains all 13 loci which were also demonstrated in 46,XY control probands with the 7 DNA probes employed.

Of the previously published four cases of 47,XXY females, only one was examined with molecular techniques (Müller et al. 1990). This case was an 11-year-old patient with incomplete testicular feminization and some features of Klinefelter's syndrome. The DNA analysis with probes M27β and p8, which detect restriction fragment length polymorphisms (RFLP) in the proximal X short and long arm, demonstrated non-disjunction during maternal meiosis II. This event resulted in two X chromosomes carrying the presumptive mutation at the androgen receptor locus. Such homozygosity at the testosterone receptor gene is unlikely for the present case, but cannot be entirely excluded. Thus, DNA analysis using X chromosomal DNA probe M27β showed maternal meiosis I non-disjunction to be the cause of the 47,XXY chromosome constitution. Provided there was actually a mutation in the testosterone receptor gene of one of the maternal X chromosomes, and that no crossover occurred between the locus detected by M27β (DXS255) and the receptor gene, meiosis I non-disjunction inevitably leads to a 47,XXY zygote with heterozygosity at the androgen receptor gene. Because the M27β locus (Xp11.3 → cen) and the androgen receptor locus (Xq11 → 12) are in close proximity to the centromere, recombination frequency between both loci are thought to be very low (Mahtani and Willard 1988; Pearson et al. 1987). Furthermore, some of the clinical features in the patient leave some justified doubt as to whether this is a case of testicular feminization at all. Thus, the presence of a uterus and the remnants of the ductus mesonephricus are not known features in the testicular feminization syndrome or its variant, incomplete testicular feminization.

Due to random X inactivation, the cytoplasmatic androgen receptor is not expressed in about 50% of the undifferentiated gonadal cells of a 47,XXY embryo heterozygous for a mutation in the androgen receptor gene. However, it is conceivable that the X carrying the normal androgen receptor gene is inactivated in significantly more than 50% of the embryological gonadal cells due to successive "stochastic fixation events" in early embryogenesis. In such a case, the concentration of androgen receptor could be below the critical threshold value and create a symptom pattern close to that of testicular feminization.

The clinical findings in the present 47,XXY patient exclude the possibility of other known syndromes associated with sex reversal, like Swyer syndrome, Camptomelic dysplasia and Smith-Lemli-Opitz syndrome. There is of course the possibility of a mutation in the sex determining region Y gene (SRY), a gene that has been equated with the testis determining factor, (TDF; Sinclair et al. 1990). In forty 46,XY females analyzed, 4 had de novo mutations in SRY which clearly inactivate the protein (Berta et al. 1990; Jäger et al. 1990; Hawkins et al. 1992). However, these were apparently classic 46,XY females with gonadal dysgenesis. In these patients the ovaries degenerate, losing oocytes at an early stage, and are found in the adults as streaks of connective tissue (streak gonads). This is not the case in the 47,XXY female examined here. Since histological examination revealed the presence of testicular cells in the 47,XXY patient, it is more likely that the cause for the sex reversal is a mutation in the testis-differentiation pathway rather than in the testis-determining pathway.

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