Maternal meiosis II nondisjunction in a case of 47,XXY testicular feminization

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Summary. An 11-year-old patient with incomplete testicular feminization and a 47,XXY karyotype is described. The patient had female external genitalia, clitoromegaly, and some features of Klinefelter's syndrome, including speech delay and delayed intellectual development. DNA analysis using X chromosomal DNA sequences suggests that the supernumerary X chromosome in the patient resulted from maternal nondisjunction during meiosis II. The M II error thereby provides the basis for homozygosity of a mutation in the androgen receptor locus.

Introduction

The testicular feminization syndrome (Morris 1953) describes phenotypic females with a male karyotype. The main features of the syndrome include normal external female genitalia with blind ending vagina, intraabdominal or inguinal testes and absence of Wolffian and Mullerian derivatives. Patients may come to clinical attention because of inguinal hernias and/or primary amenorrhea (Griffin and Wilson 1989). A variant of this condition, constituting about 10% of cases, is incomplete testicular feminization. Patients with incomplete testicular feminization may have clitoromegaly and selected Wolffian duct derivatives including hypoplastic seminal vesicles and ejaculatory ducts (Griffin and Wilson 1989). Both disorders are caused by androgen resistance because of the absence or malfunction of the androgen receptor (for review, see Griffin and Wilson 1989). Genomic and cDNA sequences coding for this receptor were recently cloned (Chang et al. 1988; Lubahn et al. 1988; Trapman et al. 1988) and assigned to Xq11-12 (Brown et al. 1989). Mutations of the X chromosomal androgen receptor gene thus explain the long recognized X-linked recessive inheritance of the disorder (Meyer et al. 1975; Wilson and Griffin 1985).

The incidence of testicular feminization is 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). Sex chromosome mosaics are rarely detected (Forberg et al. 1965; Uozumi et al. 1967; Gordon et al. 1969). In three instances, a 47,XXY karyotype without evidence of mosaicism was reported (German and Vesell 1966; Bartsch-Sandhoff et al. 1976; Gerli et al. 1979). Here, we describe another individual with a 47,XXY karyotype and testicular feminization. Using polymorphic DNA sequences, we show that the X chromosomes carrying the mutation most probably resulted from maternal non-disjunction during meiosis II.

Materials and methods

Cytogenetics

Chromosomes from both lymphocytes and fibroblasts were prepared and trypsin-Giemsa banded according to Seabright (1971).

Hybridization probes

The X chromosomal DNA probes p8 (locus DXS1, Aldridge et al. 1984) and M27β (locus DXS255, Fraser et al. 1987) were used in this investigation. The probe p8 detects a TaqI (Aldridge et al. 1984) and a HindIII (Fadda et al. 1987) restriction fragment length polymorphism in the proximal long arm (Xq11-Qq13), and M27β hybridizes with highly polymorphic DNA sequences in the proximal short arm (Xp11.3→cen) of the X chromosome. The latter polymorphism is revealed by all restriction enzymes tested (Fraser et al. 1987). An autosomal probe, 16-32, previously assigned to 16p (Harris et al. 1987) was applied as an internal standard for equal DNA loading.

DNA analysis

For restriction fragment length polymorphism (RFLP) analysis, genomic DNA was extracted from peripheral blood leukocytes from the patient and her parents as previously described (Aldridge et al. 1984) and digested with restriction endonucleases according to manufacturers' recommendations. Gel electrophoresis (2μg DNA per lane), Southern transfer to nylon membranes (Hybond, Amersham), and hybridization with 32P-radiolabeled DNA probes were carried out as previously described (Müller et al. 1986). After hybridization with X chromosomal DNA probe M27β, the same filter was hybridized with the autosomal probe 16-32 to confirm equal
DNA loading in each lane. Hybridization with probe p8 of HindIII-cleaved DNA results in two constant X chromosomal bands of 1.9 kb and 5.7 kb in addition to the polymorphic bands. The hybridization intensity of the constant restriction fragments served as an internal control for equal DNA loading. Densitometry was performed as described previously (Kupke and Müller 1989). In brief, the DNA content in the lanes of blots hybridized with M27B was determined based on the intensity of hybridization with the autosomal probe, 16–32. The amount of the mother’s and patient’s DNA was calculated relative to the father’s DNA, which was assigned a value of 1.00. DNA content in lanes of blots hybridized with p8 was determined based on the intensity of hybridization of the 1.9 kb constant band. Hybridization intensity of this band with the mother’s DNA was assigned a value of 1.00, and the hybridization intensities of this band in father and child were calculated relative to the mother’s DNA. Given that p8 detects X chromosomal DNA fragments, values of 1.00 in the mother’s and child’s DNA, but of 0.50 in the father’s DNA, reflect equal DNA loading. Doses of informative alleles for probes p8 and M27B were calculated by determining the mother:child ratio of hybridization intensities taking into account differences in the amount of DNA loaded.

Results and discussion

The clinical features of the patient are most compatible with incomplete testicular feminization, which is often associated with clitoromegaly (Griffin and Wilson 1989). It is recognized, however, that the genetic defect in both complete and incomplete testicular feminization affects the androgen receptor (Griffin and Wilson 1989). Unlike most cases of testicular feminization, a 47,XXY karyotype was detected in the present case (Fig. 1). Metaphases from 50 peripheral lymphocytes and from 50 skin fibroblasts were examined, and no evidence of mosaicism was obtained. The parents’ karyotypes were normal.

The parental origin of the supernumerary X chromosome in the patient was determined using “probes” that hybridize with polymorphic DNA sequences in the proximal long and

Case report

A black female (11 years, 10 months old) was referred for evaluation of clitoromegaly. She was the 7 lb 2 oz product of a full-term uncomplicated pregnancy. The mother discontinued birth control pills one month before conception. She had not received medication during pregnancy. Parental ages at conception were 26 years (mother) and 30 years (father). Labor and delivery were uneventful. In the neonatal period, a small umbilical hernia was noted. At three months of age, bilateral inguinal hernias were diagnosed and clinically followed during childhood. Two months prior to evaluation, the hernias worsened. The patient’s past medical history was benign with the exception of two episodes of pneumonia at ages 2 months and 1 year. She required remedial summer classes to maintain appropriate grade levels in school. Language delay was also noted and required speech therapy. Gross and fine motor milestones were advanced. The family history revealed no consanguinity, ambiguous genitalia, inguinal hernias in females, or reproductive abnormalities. Two older female siblings had undergone normal puberty and menarche.

On physical examination, the patient was a passive and immature prepubertal female with a normal facial appearance. The height was 157 cm (80th percentile), weight 34 kg (20th percentile), head circumference 53 cm (50th percentile) and armspan was 144 cm. Bilateral inguinal hernias were present containing masses defined as testes by magnetic resonance imaging scan. The clitoris was enlarged, measuring 2 cm, but the labia majora and minora were normal. Breast and pubic hair development was prepupal (Tanner stage I). Clinical laboratory studies revealed normal levels of serum 17-hydroxyprogesterone, 11-deoxycortisol, and androstenedione. The serum testosterone was elevated on one occasion at 107 ng/dl (prepubertal range 0–15 ng/dl), but almost normal at 26 ng/dl with a dihydrotestosterone of 11 ng/dl (normal range 6–33 ng/dl) when repeated two weeks later. Pelvic sonography revealed a normal vagina but absent uterus and ovaries. The family deferred the recommendation for bilateral gonadectomy and herniorrhaphy.

![Image](Fig. 1) GTG banded sex chromosomes of the patient

![Image](Fig. 2A, B) Differential hybridization of probes M27B (A) and p8 (B) to EcoRI-cleaved (A) and HindIII-cleaved (B) DNA from mother (lane 1), father (lane 2), and the 47,XXY testicular feminization patient (lane 3). Size standards are given in kb. Hybridization results using autosomal probe 16–32 (p16–32) obtained with the same blot are given below each lane in A. The relative amount of DNA loaded, standardized to 1.00 for the father’s lane, is given below the p16–32 hybridizations. Note that the 5.0 kb EcoRI fragment detected by M27B hybridizes at double intensity in the patient as in the mother. In B, the DNA content in the lanes was calculated based on the hybridization intensity of p8 with the 1.9 kb (here 2.0 kb) X chromosomal constant band. Amounts of DNA loaded were standardized relative to the DNA content in the mother’s lane; this was assigned a value of 1.00. The value of 0.5 in the father’s lane reflects the presence of one X chromosome as compared with the two X chromosomes in mother and patient. The value of 0.76 in the patient’s lane reflects slight DNA underloading as compared with the mother’s and the father’s lanes. Note the double intensity of the hybridization of the 7.5 kb HindIII fragment detected by p8 in the patient’s DNA as compared with the mother’s DNA. Both probes show the absence of paternal polymorphic X chromosomal alleles in the child, thus demonstrating maternal origin of both X chromosomes in the patient.