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Complete Androgen Insensitivity in a 47,XXY Patient With Uniparental Disomy for the X Chromosome

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We describe a unique patient with complete androgen insensitivity syndrome and a 47,XXY karyotype. Androgen receptor assay using cultured pubic skin fibroblasts showed no androgen-binding capacity. Sequence analysis of the androgen receptor gene demonstrated two nonsense mutations, one in exon D and one in exon E. Microsatellite marker analysis showed that the patient is homozygous for all five Xq loci examined. The results suggest that the long-arms of the two X chromosomes are identical, i.e., uniparental isodisomy at least for Xq, and carry the same mutations in the androgen receptor gene. This explains how complete androgen insensitivity syndrome occurred in this 47,XXY individual. Am. J. Med. Genet. 86:107–111, 1999.

KEY WORDS: androgen insensitivity syndrome; Klinefelter syndrome; microsatellite markers; meiosis II nondisjunction; double mutation; uniparental disomy

INTRODUCTION

Patients with androgen insensitivity syndrome (AIS), an X-linked disorder with a prevalence of about 1 in 65,000 males [Jagiello and Atwell, 1962], have female genitalia and breasts (despite a male karyotype) with a very low grade of pubic hair development, a blindly ending vagina, intra-abdominal or inguinal testes, absence of Wolffian and Müllerian derivatives, and high serum concentrations of testosterone and gonadotropins. These abnormalities are caused by the absence or malfunction of the androgen receptor (AR) resulting from various types of mutations of the AR gene located on Xq12 [Graffin and Wilson, 1989; Sinnecker et al., 1997]. The 47,XXY karyotype causes Klinefelter syndrome, which is characterized by tall stature, a slender build with long extremities, male external genitalia with a small penis and small testes, and infertility due to azoospermia. The prevalence of Klinefelter syndrome is approximately 1 in 1,000 males [Levitan, 1988]. Endocrinologic evaluation in adult patients with 47,XXY typically demonstrates hypergonadotropic hypogonadism, and atrophic changes in the testes.

A combination of AIS and 47,XXY was reported in 5 cases [Bartsch-Sandhoff et al., 1976; Gerli et al., 1979; German and Vessell 1966; Müller et al., 1990; Scully et al., 1990]. The rarity of this concurrence is explained by the random nature of X-inactivation in XXY patients [Disteche, 1995; Lyon, 1961], because overall, a defect resulting from a mutant AR allele on one X chromosome is masked by the effect of the normal allele on the other X chromosome. However, AIS can occur in XXY patients when a mutant allele is preferentially expressed through non-random X-inactivation, or when both X chromosomes carry mutant alleles, i.e., homozygosity.

Here we describe a Japanese patient with complete AIS and 47,XXY chromosome constitution, and discuss the mechanism leading to the manifestation of AIS in 47,XXY individuals.

CLINICAL REPORT

A 30-year-old woman presented with primary amenorrhea and sterility for 4 years. Her height was 165 cm (+1.5 *SD* for the Japanese women), and weight 61.5 kg. She was muscular for a woman and lacked breast development (Tanner grade I). The vulva appeared immature with no pubic hair development (Tanner grade I); she had a narrow vagina lacking the cervix. Pelvic

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examination, ultrasonography, and magnetic resonance imaging showed absence of the uterus. The patient was hypergonadotropic: serum luteinizing hormone was 19.2 mIU/ml (normal, 0.8 to 8.3 mIU/ml for males, 1.4 to 15.3 mIU/ml for follicular-phase females); follicle-stimulating hormone, 46.3 mIU/ml (1.2 to 10.2 mIU/ml for males, 3.2 to 12.2 mIU/ml for follicular-phase females); prolactin, 2.8 ng/ml (3.4 to 16.2 ng/ml for males, 3.7 to 24.8 ng/ml for females); 17β-estradiol, 20.6 pg/ml (<5 to 48 pg/ml for males, 4 to 60 pg/ml for early follicular-phase females); progesterone, 0.99 ng/ml (<2.4 ng/ml for males, <1.82 ng/ml for follicular-phase females); and testosterone, 1.0 ng/ml (2.86 to 11.48 ng/ml for males, <0.1 to 0.9 ng/ml for females). Laparoscopic examination demonstrated small, whitish, testis-like gonads on the walls of the pelvis. No Müllerian or Wolffian derivatives were identified. Bilateral gonadectomy was performed. Histopathological examination demonstrated a few degenerating seminiferous tubles with no spermatogonia and Leydig cell clusters in the stroma with fibrotic replacement; no tumors were seen. Two maternal aunts also had primary amenorrhea and sterility. Chromosome analysis of lymphocytes and fibroblasts showed a non-mosaic 47,XXY karyotype.

METHODS

Whole-Cell Receptor Assay of Androgen Receptor

The method of Nakao et al. [1992] was followed for AR radioreceptor assay. Briefly, pubic skin samples from the patient and from a female control (obtained under informed consent at vaginal hysterectomy performed to treat her uterine carcinoma *in situ*) were minced and cultured in flasks containing 5 ml Eagle's MEM (GIBCO BRL, Rockville, MD) supplemented with 15% FBS until sufficient numbers of fibroblasts were obtained. Two days before the assay the medium was changed to serum-free MEM. Fibroblasts were harvested in Hanks BSS containing 0.01% trypsin and 0.02% EDTA, rinsed with PBS, and suspended in MEM containing 25 mM tricine (Sigma-Aldrich Japan, Tokyo). Duplicate tubes of fibroblasts (7×10^5 cells/ml) were incubated for 1 hr at room temperature with various concentrations (0.16 to 5 nM) of [^{3}H] methyltrienolone ([^{3}H] R1881; New England Nuclear Research Products, Boston, MA) in the presence or absence of a 1000-fold excess of unlabeled R1881. After incubation, cells were washed three times with PBS and then lysed

TABLE I. Primer Sequence Used in the Present Study

Primer pair	Sequences (5′–3′)
AR-Exon B	GCCTGCAGGTTAATGCTGAA
	GTTATTTGATAGGGCCTTGC
AR-Exon C	GTTTGGTGCCATACTCTGTC
	ATGGCCACGTTGCCTATGAA
AR-Exon D	GAGTTTAGAGTCTGTGACCA
	GATCCCCCTTATCTCATGCT
AR-Exon E	AACCCGTCAGTACCCAGACT
	GCTTCACTGTCACCCCATCA
AR-Exon F	GGGCTTATTGGTAAACTTCC
	GTCCAGGAGCTGGCTTTTCC
AR-Exon G	TCAGATCGGATCCAGCTATC
	TCTATCAGGCTGTTCTCCCT
AR-Exon H	GAGGCCACCTCCTTGTCAAC
	AAGGCACTGCAGAGGAGTAG
HUMARA [AGC]n	TCCAGAATCTGTTCCAGAGCGTGC
	GCTGTGAAGGTTGCTGTTCCTCAT
HUMHPRTB [AGAT]n	ATGCCACAGATAATACACATCCCC
	CTCTCCAGAATAGTTAGATGTAGG
DXYS156 [TAAAA]n	GTAGTGGTCTTTTGCCTCC
	CAGATACCAAGGTGAGAATC
DXS731 [CA]n	CCTGATCATCCCTGAGGCTA
	TTAGTCCCAGGGGGGAGAG
DXS738 [CA]n	CCAGCAATAACCATAAGTAAAACC
	AATGTGTTGTTGTATTCACCTTGC

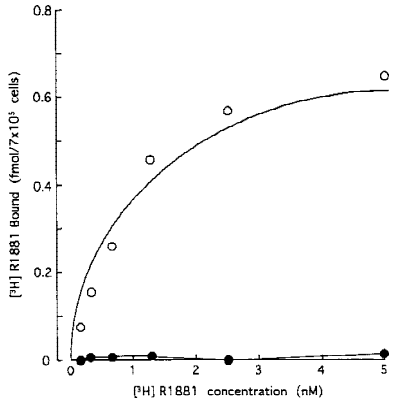

Fig. 1. Saturation curve of specific binding of [3H] R1881 to the androgen receptor on cultured fibroblasts derived from the patient (●) and a control subject (○). No androgen binding was detectable in fibroblasts from the patient.

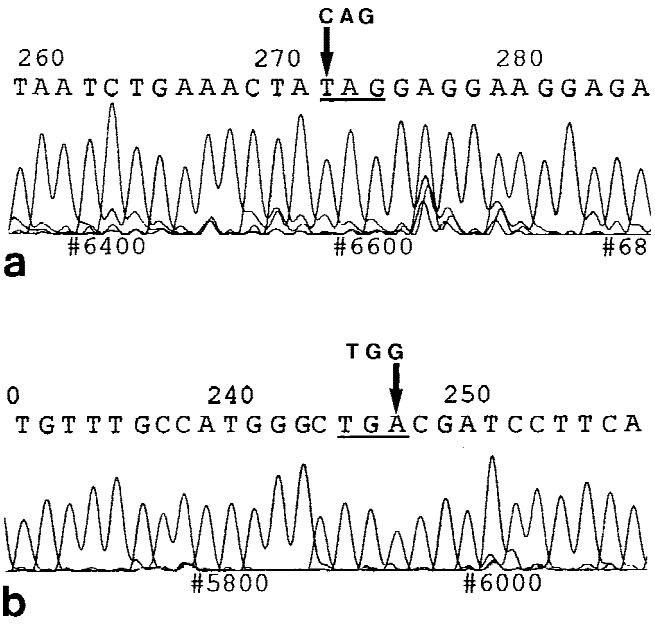

Fig. 2. Sequences for the androgen receptor (AR) gene in the patient. **(a)** A partial sequence of AR-exon D with a C-to-T substitution that changes glutamine (CAG) to a stop codon (TAG) at codon 640. **(b)** A partial sequence of AR-exon E with a G-to-A substitution that changes tryptophan (TGG) to a stop codon (TGA) at codon 749. Arrows indicate each substituted nucleotide, bar depicts the substituted termination codon, and nucleotides above the arrows indicate the wild type.

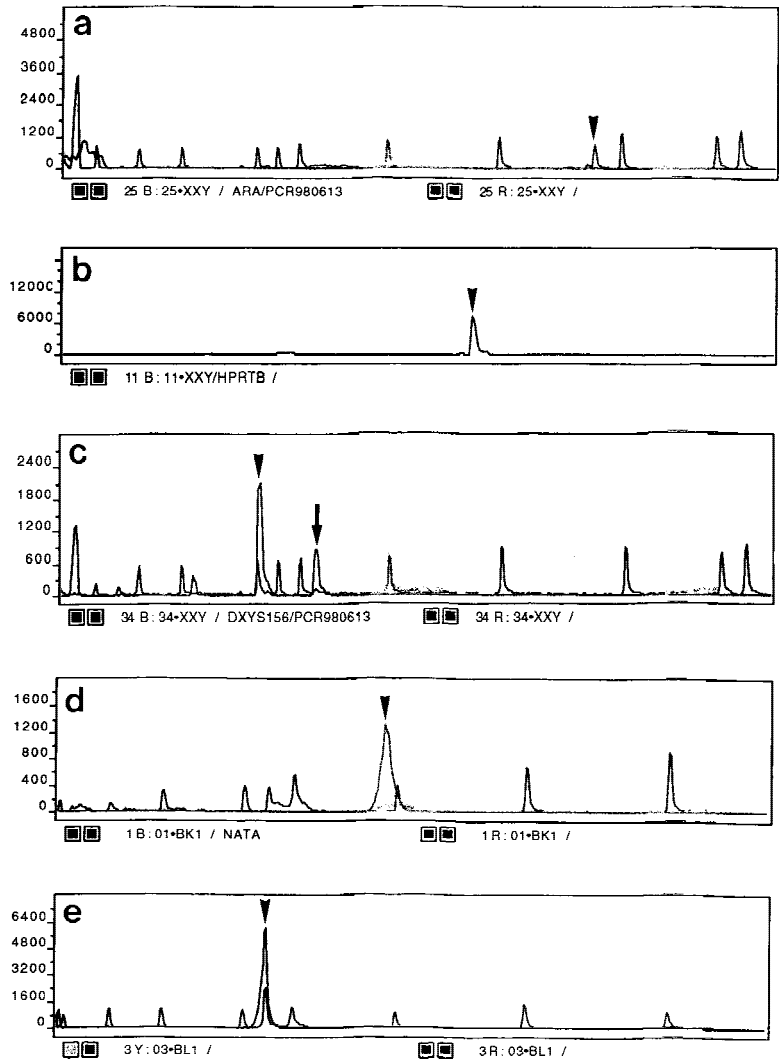

Fig. 3. Microsatellite marker analysis of HUMARA **(a)**, HUMHPRTB **(b)**, DXYS156 **(c)**, DXS730 **(d)**, and DXS738 **(e)**. X chromosome-specific markers are present in a single peak **(arrowhead)**. Product size is 288 bp for HUMARA, 287 bp for HUMHPRTB, 140 bp for DXYS156, 195 bp for DXS730, and 150 bp for DXS738. For DXYS156, a Y-specific marker also is detected **(arrow)**; X-specific and Y-specific alleles are discriminated by their product size. Remaining peaks represent internal size controls.

with 1 ml of absolute ethanol. The aliquot was transferred to vials containing 10 ml of ACS-II cocktail (Amersham, Buckinghamshire, England) for liquid scintillation counting. The results were analyzed by the Scatchard method to quantify specific [^{3}H] R1881 binding.

Nucleotide Sequencing

Sequence analysis was carried out for exons B to H of the AR gene, using the method of Nakao et al. [1992] and Lubahn et al. [1989] (Exon A was not examined because of the rarity of mutations.) Primer sequences are shown in the Table I. Polymerase chain reaction (PCR) products were purified and concentrated using a QIAEX II apparatus (Qiagen, Tokyo), and subcloned into pGEM-T (plasmid, Promega, Madison, WI). DH-5α (*Escherichia coli*) that was transformed by the plasmid were cultured, thereafter direct colony PCR using both M13 forward and reverse primers was performed to screen for bacterial colonies containing the correct insert. Each PCR product was sequenced with a DSQ-1000L automated DNA sequencer (Shimazu, Tokyo, Japan) using the dideoxynucleotide method. To distinguish mutations from randomly misincorporated nucleotides, sequences were examined in six clones obtained from each exon.

Microsatellite Markers Analysis

Leukocyte genomic DNA from the patient was analyzed for polymorphisms of five microsatellite markers on the long-arm of X chromosome. DNA from her parents was not available because both were deceased. Briefly, DNA sample was amplified by PCR using primers for HUMARA (AR) on Xq12 [Edwards et al. 1992], HUMHPRTB (HPRT) on Xq26.1 [Edwards et al., 1992; Huang et al., 1991], DXYS156 on Xq and Y [Chen et al., 1994], DXS730 (MS21) on Xq26 [Hudson et al., 1992], and DXS738 (E114) on Xq21.1 [Hudson et al.,

1992] (Table I). PCR products were subjected to denaturing polyacrylamide gel electrophoresis. The product sizes were determined on a 377 DNA sequencer (Perkin-Elmer, Foster City, CA) by Genescan 2.0.2 software (Perkin-Elmer).

RESULTS

In contrast to fibroblasts from the control subject, the AR assay gave no detectable androgen binding in the fibroblasts from the patient (Fig. 1). Two mutations in the AR gene were identified in the patient (Fig. 2). The glutamine codon (CAG) at amino acid 640 in exon D was changed to a termination codon (TAG), and the tryptophan codon (TGG) at amino acid 749 in exon E also was changed to a termination codon (TGA). The results were observed consistently in all six clones analyzed. Analysis with microsatellite markers on Xq showed only one allele at each of the 5 loci examined (Fig. 3).

DISCUSSION

The fibroblasts of the patient showed no androgen-binding capacity. This finding, as well as her clinical manifestations such as completely female external genitalia, absent Wolffian derivatives, and lack of pubic hair development, is consistent with complete AIS. The diagnosis of AIS was confirmed by sequence analysis of the AR gene. One (in exon D) of the mutations detected may be responsible for the absent androgen-binding capacity because it results in a truncated AR protein lacking all of the steroid-binding domain [Lubahn et al., 1989]. In addition to AIS, her XXY karyotype and other manifestations, i.e., severely atrophic testes and a low serum testosterone concentration, are all compatible with Klinefelter syndrome. Although our patient had inadequate breast development which is inconsistent with complete AIS, this atypical finding was modified by the coexistent 47,XXY karyotype, and may be due to a relatively low serum 17β-estradiol concentration reflecting the paucity of testosterone from which the estrogen is converted.

The patient has two mutations in the AR gene. Although such a double mutation in the gene has been reported in an AIS patient by Komori et al. [1997], it is rare in Japanese AIS patients. Because two X chromosomes in our patient share the same polymorphic alleles at all 5 microsatellite marker loci examined, the two chromosomes (at least their long-arms) are most likely identical and carry the same mutations. In other words, our patient is homozygous for the AR locus that carries the two mutations, as well as for other loci. This can explain why AIS occurred in our XXY patient. A condition in which genes on homologous chromosomes are homozygous often occurs in uniparental disomy (UPD), where a pair of homologous chromosomes is derived from one parent [Engel, 1980; Ledbetter and Engel, 1995]. When the chromosome pair is derived from one parental homolog, it constitutes uniparental isodisomy, in which all genes are homozygous. Human UPD has been observed for most chromosomes including the X chromosome [Benlian et al., 1996; Gelb et al., 1998; Ledbetter and Engel, 1995], and UPD may lead to a recessive disorder through consequent homozygosity, as complete AIS in our patient. According to Jacobs et al. [1988], a 47,XXY karyotype results from X chromosome nondisjunction at paternal meiosis I ($X^{M}X^{P}Y$) in 53% of patients, maternal meiosis I ($X^{M1}X^{M2}Y$) in 34%, maternal meiosis II ($X^{M1}X^{M1}Y$ or $X^{M2}X^{M2}Y$) in 9%, and at post-zygotic mitosis ($X^{M}X^{M}Y$) in the remaining patients. Therefore, it is most likely that the UPD in our patient resulted from a maternal meiosis II error.

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