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Molecular detection of a translocation (Y;15) in a 45,X male

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Summary. A 45,X male individual was shown to have a translocation of Y-chromosome material to the short arm or proximal long arm of chromosome 15. This translocation was detected by genomic DNA blotting and in situ hybridization with Y-chromosome-specific DNA probes.

Introduction

The presence of a Y chromosome in mammals is usually necessary for the indifferent gonad to develop into a testis. Occasionally testes develop in the absence of an observable Y chromosome, and the existence of males with a 46,XX karyotype is well documented (reviewed by de la Chapelle 1981). Recent studies have shown that a number of 46,XX males carry Y-chromosomal material that can be detected by Y-specific genetic markers (de la Chapelle et al. 1984) and Y-specific DNA probes (Guellaen et al. 1984; Page et al. 1985; Vergnaud et al. 1986).

In the presence of one X chromosome and no Y chromosome, female gonadal differentiation usually occurs. Most humans with a 45,X karyotype have Turner syndrome with gonadal dysgenesis (oocyte-depleted "streak" ovaries) and female external genitalia. Males with a 45,X karyotype are much less common than either 45,X females or 46,XX males (Fraccaro et al. 1966; Lo Curto et al. 1974; Forabosco et al. 1977; Tolksdorf et al. 1980; Seidel et al. 1981; de la Chapelle et al. 1986). In some 45,X males, cytogenetic studies revealed or suggested translocation of Y-chromosomal material to an autosome (Subrt and Blehova 1974; Koo et al. 1977; Turleau et al. 1980; Schempp et al. 1985). In a patient with no cytogenetic evidence of a translocation, hybridization of Y-chromosome-specific DNA probes to genomic DNA revealed low-grade mosaicism 45,X/46,XY (de la Chapelle et al. 1986). We report a phenotypic male who had a 45,X karyotype and showed no detectable Y-chromosomal material by Q-, R-, and G-banding studies. However hybridization of Y-chromosome-specific DNA probes to the patient's DNA and to chromosomes in situ showed a translocation of Y-chromosomal material to chromosome 15.

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Case report

J.A. was a male infant born at 35-weeks gestation to a 21-year-old gravida I para I Guamanian woman. The pregnancy was uncomplicated, with no exposure to drugs, ethanol, or radiation. The parents were unrelated. Delivery was by caesarean section because of fetal distress. There was meconium staining and asphyxia with Apgar scores of 0 at 1 and 5 min. The infant was immediately transferred to a neonatal intensive care unit where he required mechanical ventilatory support for 3 days. Seizures occurred on the first day and were treated with phenobarbitol. Examination in the neonatal period revealed a depressed, premature infant with a weight of 2435 g (50th percentile for 35-week gestation) and head circumference of 31.5 cm (50th percentile). The eyes, ears, and palate were normal, as were the heart, chest, and abdomen. The phallus was normal without hypospadias or chordee; however, the testes were not palpable in the scrotum or inguinal canal. There was a single palmar crease on the left hand, with the rest of the extremities being unremarkable. Neurological examination revealed weak Moro and grasp reflexes.

At follow-up evaluations, he was noted to have hypertonia, spastic quadriplegia, microcephaly, growth retardation, and severe developmental delays. At 35 months of age, his weight was 9.5 kg, head circumference 44.5 cm (50th percentile for 7 months), and he was functioning at a 10-month level. There was no family history of spontaneous abortion, fetal wastage, early infant death, or infertility. The patient's mother had 11 healthy siblings. The patient's father was not available for examination, and his family history was not known.

At 17 months of age, the patient underwent an exploratory laparotomy because of bilateral cryptorchidism. No structures of mullerian origin were observed. The right testis appeared abnormally small and soft and was removed. The left testis appeared normal and was reimplanted in the scrotum. Histological preparations of the right testis showed immature seminiferous tubules lacking lumina, and a moderately increased amount of interstitial fibrous tissue. Biopsy of the left gonad showed numerous immature seminiferous tubules with prominent Sertoli cells. There were no poorly differentiated areas to suggest a streak gonad. There was no evidence of neoplastic transformation or of ovarian stroma in either gonad. The overall appearance of both gonads suggested that they were not dysgenetic.

Materials and methods

Cytogenetics

Chromosome preparations were obtained from peripheral blood lymphocytes and from a small skin biopsy grown in culture. The cells were treated with a hypotonic solution (0.075 *M* KCl) and fixed in a 3:1 mixture of methanol:acetic acid. Staining included Q-banding (QFQ), G-banding (GTG), R-banding (RHG), and NOR staining. DAPI-distamycin staining was performed according to the method of Schweizer et al. (1978).

In situ hybridization

In situ hybridization to metaphase cells using probe pDP105 (see below) was performed as described previously (Disteche et al. 1985).

DNA studies

DNA was prepared from peripheral leukocytes or cultured skin fibroblasts by published methods (Kunkel et al. 1977). Restriction digestion, electrophoresis, transfer, and hybridization of DNA were performed as previously described (Page and de la Chapelle 1984; Disteche et al. 1985). As specified below, each hybridization probe was used at either "reduced" or "high" stringency. "Reduced" stringency implies that hybridizations were carried out at 42°C and that the final wash was in 0.1 × SSC, 0.1% sodium dodecyl sulfate (SDS) at 55°C. "High" stringency implies that hybridizations were carried out at 47°C and/or the final wash was in 0.1 × SSC, 0.1% SDS at 65°C.

DNA hybridization probes

Probe 47a detects highly homologous sequences on the X and Y chromosomes (locus *DXYS5*). At high stringency, 47a detects a Y-specific TaqI fragment of 4.3 kb, a marker for deletion interval 1 on the short arm of the Y chromosome (Vergnaud et al. 1986).

Probe pDP61 (D.C. Page, unpublished work) is a subclone derived from plasmid 115 (Geldwerth et al. 1985), and it detects highly homologous sequences on the X and Y chromosomes *(DXYS8)*. At high stringency, pDP61 detects a Y-specific TaqI fragment of 2.1 or 2.6 kb, a marker for interval 2 on the short arm of the Y chromosome (Vergnaud et al. 1986).

Probe pDP105 (D.C. Page, unpublished work) defines multiple Y-specific loci. At low stringency, pDP105 detects many Y-specific TaqI fragments. The presence or absence of TaqI fragments of 2.5 kb (pDP105/A) and of 5.2 kb (pDP105/B) was scored. Fragment pDP105/A is a marker for interval 3 (short arm), while pDP105/B is a marker for interval 6 (long arm) of the Y chromosome (D.C. Page, unpublished work).

Probe pDP34 detects highly homologous sequences on the X and Y chromosomes *(DXYS1)* (Page et al. 1982, 1984). At high stringency, pDP34 detects a Y-specific TaqI fragment of 15 kb, a marker for interval 4, which includes the proximal short arm and centromere (Vergnaud et al. 1986).

Probe pDP97 is a subclone derived from cosmid Y97 (Wolfe et al. 1985). At high stringency, it detects a repeated Y-specific EcoRI fragment of 5.5 kb *(DXZ3)*, a marker for the centromere of the Y chromosome (and for interval 4; D.C. Page, unpublished work).

Probe 12f detects sequences on autosomes and the X, as well as on the Y chromosome (Bishop et al. 1984). At high stringency, 12f detects two or three Y-specific TaqI fragments. The presence or absence of an 8-kb, Y-specific TaqI fragment, a marker for interval 5 on the long arm of the Y chromosome was scored (Vergnaud et al. 1986).

Probe pY431-HinfA, derived from the 2.1-kb HaeIII male repeat *(DYZ2)* (K. Smith, personal communication), detects highly repeated, Y-specific sequences. At low stringency, it detects a Y-specific smear in either EcoRI or TaqI digests, a marker for interval 7 (heterochromatic region) on the long arm of the Y chromosome (Vergnaud et al. 1986). It can also be detected as a prominent 2.3-kb MboI fragment.

Probe pY3.4, derived from the 3.4-kb HaeIII male repeat *(DYZ1)* (Lau et al. 1984), also detects highly repeated Y-specific DNA sequences located in the heterochromatic region of the long arm (interval 7). On an MboI digest, a prominent 2-kb band appears.

Results

Cytogenetic studies

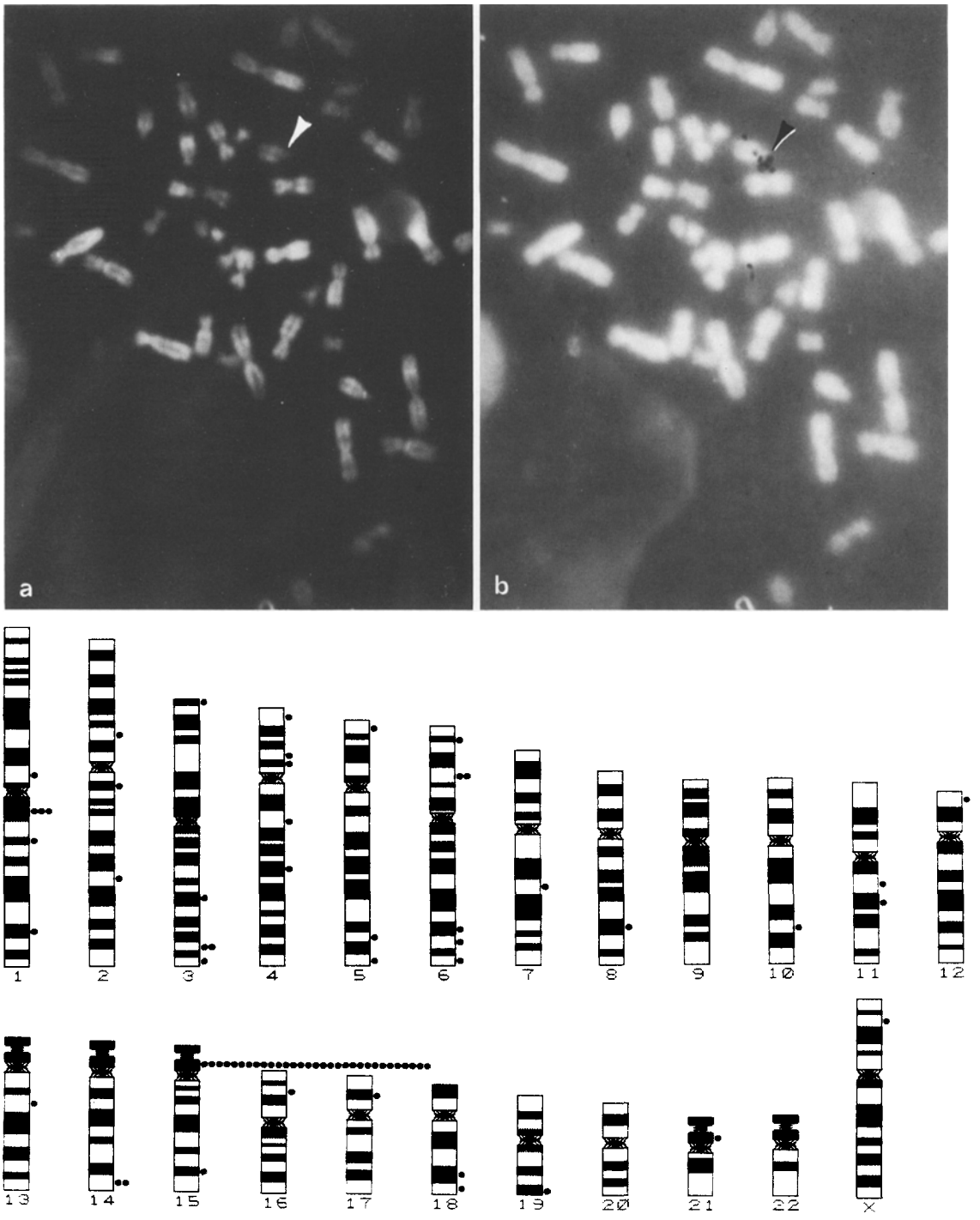
Fifty-two metaphase cells from a peripheral blood lymphocyte culture had a consistent chromosome number of 45 with apparently normal autosomes and a single X chromosome. By G-banding, R-banding, or Q-banding, no translocation of Y-chromosomal material could be demonstrated to any of the autosomes or to the X chromosome. In addition, no Y body was found using quinacrine staining in 100 cells. Similarly, a skin fibroblast culture showed a karyotype of 45,X in all 50 metaphases examined after R-banding, and no Y body was seen after quinacrine staining.

Molecular hybridization studies

DNA from the patient was examined for the presence of Y-chromosomal material using the Y-specific DNA probes listed

Table 1. Y-specific DNA studies. DNA hybridization probes were used to test these individuals for the presence (+) or absence (−) of the indicated Y-specific restriction fragments. As described in Materials and methods, some of the probes detect multiple Y loci (e.g., 50f2 detects loci A, B, C, D, and E)

DNA probe/ locus	Individual			
	45,X male	Normal males	Normal females	Y-chromosome interval
47a	+	+	−	1
pDP61	+	+	−	2
50f2/A,B	+	+	−	3
pDP105/A	+	+	−	3
pDP34	+	+	−	4
pDP97	+	+	−	4
50f2/D	+	+	−	4
12f	+	+	−	5
pDP105/B	−	+	−	6
50f2/C,E	−	+	−	6
pY431-HinfA	−	+	−	7
pY3.4	−	+	−	7



C

Fig. 1a–c. In situ hybridization of probe pDP105 to metaphase chromosomes from the 45,X male patient. **a** Example of a metaphase stained with Q-banding and **b** the same metaphase photographed after increasing the background light for grain visualization. **c** Diagram of the human chromosomes indicating the position of grains recorded in 53 cells. A prominent site is seen on the short arm of chromosome 15

in the methods section. Those probes were chosen to cover the seven intervals of the Y chromosome defined by Vergnaud et al. (1986). Table 1 summarizes the data obtained with the nine different DNA probes hybridized to DNA from the patient and from control males and females. The DNA studies indicate that the patient carries intervals 1, 2, 3, 4, and 5 of the Y chromosome. That is, he appears to carry the entire short arm (including the male-determining region), the centromere, and the proximal long arm of the Y chromosome. In contrast, DNA from the patient did not hybridize to probes for interval 6 and 7 of the Y chromosome, indicating that the distal long arm is absent in him. Interval 7 corresponds to the heterochromatic region of the Y chromosome that the patient lacks.

In conclusion, the 45,X male reported here retained at least some DNA sequences from the euchromatic region of the Y chromosome including sequences located in the short

and proximal long arm, but lacked sequences from the distal long arm of the Y chromosome.

In situ hybridization

Fifty-three metaphase cells were examined after hybridization with probe pDP105. This probe detects moderately repeated DNA sequences on the short arm and long arm of the Y chromosome. Forty-eight of 53 cells showed positive hybridization. Thirty-one of the 76 sites of hybridization, or 41% of the sites, were located at the short arm of one chromosome 15 of the patient (Fig. 1). Thus this patient has a translocation of Y-chromosomal material to chromosome 15. This translocation was not apparent on the examination of the R-, G- and Q-banded chromosome preparations (Fig. 2).

From the in situ hybridization and Q-banding analyses, the translocated chromosome 15 was identified as the homologue with quinacrine-dull material on its short arm, while the other homologue had a slightly brighter short arm. NOR staining was negative on both chromosomes 15. DAPI-distamycin staining, which stains the short arm of chromosome 15 in about 92% of cases (Schweizer et al. 1978; Okamoto et al. 1981), stained only one chromosome 15 of the patient (Fig. 3). The DAPI-distamycin-negative chromosome 15 was identified by Q-banding as the translocated one. Therefore, the translocated chromosome 15 appeared to lack the DAPI-distamycin positive region characteristic of most short arms of chromosomes 15.

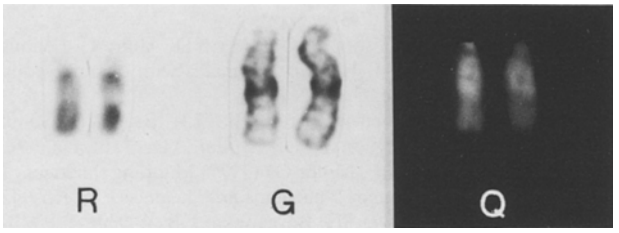


Fig. 2. Panel of chromosomes 15 from the 45,X male. *R,* R-banding; *G,* G-banding; *Q,* Q-banding. The translocated chromosome is on the right in the Q-banding panel. It was not identified in G- or R-banding

Discussion

A phenotypic male with a 45,X karyotype and histologically normal but cryptorchidic testes was found to have a translocation of a euchromatic portion of the Y chromosome to one chromosome 15. DNA probes for the short arm, centromere, and proximal long arm of the Y chromosome (intervals 1 to 5 of the deletion map described by Vergnaud et al. 1986) detected homologous sequences in this individual by genomic DNA (Southern) blot hybridization. The testis-determining factor has been mapped to interval 1 of the Y chromosome (Vergnaud et al. 1986). The presence of interval 1 in this individual accounts for his male phenotype.

Although Q-, R-, and G-banding studies revealed no translocation in the patient, in situ hybridization with one of the Y-chromosome specific probes demonstrated a translocation of Y-chromosome material to chromosome 15. The abnormal chromosome 15 in this individual may be a derivative Y chromosome with breakpoints on the proximal long arms of chromosomes Y and 15. The breakpoint on the Y chromosome appears to be either in interval 5 or 6. Alternatively, the abnormal chromosome may be a dicentric chromosome with the centromeres of both chromosome 15 and the Y chromosome. Although we cannot exclude that the patient may have inherited a DAPI-distamycin negative chromosome 15 (Okamoto et al. 1981), the absence of this staining on the abnormal chromosome is suggestive of a breakpoint very close to the centromere of chromosome 15, either on the short or long arm. The patient did not have features of Prader-Willi syndrome that is associated with chromosome abnormalities in the proximal long arm of chromosome 15.

Other cases of 45,X males with no cytogenetic evidence of a Y chromosome have been reported (Fraccaro et al. 1966; Lo Curto et al. 1974; Forabosco et al. 1977; Tolksdorf et al. 1980; Seidel et al. 1981; de la Chapelle et al. 1986). Additional cases of 45,X males have been shown or suspected to have Y-auto-

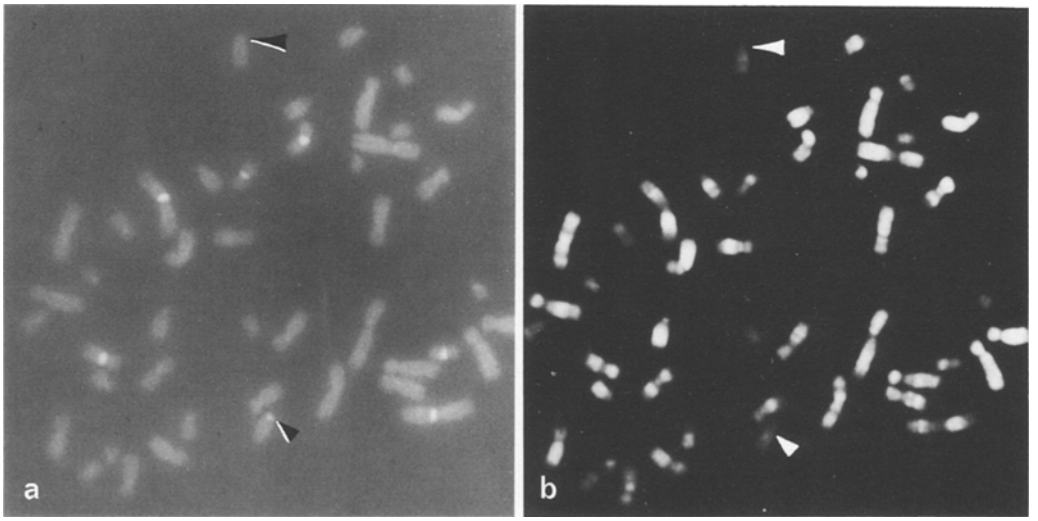


Fig. 3a, b. Example of a metaphase cell stained with **a** DAPI-distamycin and **b** Q-banding. The abnormal and normal chromosomes 15 are indicated by a *large* and *small arrow,* respectively

some translocations (Subrt and Blehova 1974; Koo et al. 1977; Turleau et al. 1980; Schempp et al. 1985) or low-grade mosaicism for a 46,XY cell line (de la Chapelle et al. 1986). The 45,X males reported usually have a hypoplastic phallus, cryptorchidism, and azoospermia, and may have developmental and growth delays. In our patient it was not clear if his developmental delays were the result of his aneuploidy, or of complications of his prematurity and neonatal asphyxia. There are no other distinguishing features common to 45,X males, although some display minor findings of the Turner syndrome.

Males with a 45,X karyotype are much rarer than males with a 46,XX karyotype. A large proportion of 46,XX males have been shown to carry Y-chromosomal material by genetic marker and DNA hybridization studies (de la Chapelle et al. 1984; Guellaen et al. 1984; Page et al. 1985; Vergnaud et al. 1986). In situ hybridization with a Y-specific DNA probe has shown that the Y-chromosomal material in three such 46,XX males tested had been translocated to the short arm of an X chromosome (Andersson et al. 1986). Such translocations could be the result of exchanges between the short arms of the X and Y chromosomes at meiotic pairing (Ferguson-Smith 1966).

In 45,X males, such exchanges resulting in translocation would be between the Y chromosome and an autosome. Although few cases have been reported, preferential autosomal sites may be involved. In two of the 45,X males with evidence of a translocation, the short arm of chromosome 15 and the Y chromosome were involved, as in the present case (Subrt and Blehova 1974; Schempp et al. 1985). In one case, the derivative chromosome appeared to be a dicentric chromosome (Schempp et al. 1985).

Translocations between the short arm of chromosome 15 and the Y chromosome have also been reported in normal individuals with large quinacrine-bright "satellites" on one chromosome 15. Such "satellites" on acrocentric chromosomes have been shown to contain heterochromatin from the distal long arm of the Y chromosome (Cooke and Noel 1979). The presence of repetitive DNA sequences in the heterochromatic region of the Y chromosome and the short arm of acrocentric chromosomes may favor such exchanges. Another region of the autosomal genome that may be prone to translocation with the Y chromosome is the short arm of chromosome 5. Two cases of 45,X males with the "cri du chat" ($5p^-$ syndrome) have been reported (Tolksdorf et al. 1980; Seidel et al. 1981).

In conclusion, DNA hybridization studies have shown that the male phenotype of the 45,X patient reported here can be accounted for by the presence of interval 1 of the Y chromosome, which contains the testis-determining factor (Vergnaud et al. 1986). The Y-chromosome material was mapped to one chromosome 15 of the patient by in situ hybridization. It appears to be a single contiguous portion of the Y chromosome, including the short arm and proximal long arm, consistent with a simple translocation.

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