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## Small deletions of the short arm of the Y chromosome in 46,XY females

(sex determination/chromosomal deletion)

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**ABSTRACT** Structural anomalies of the sex chromosomes provide a means to study the location of genes responsible for sex determination. Recently, a type of sex reversal in humans, the 46,XX male, was shown to result in some cases from translocation of Y chromosome material to the X chromosome. In the present report, another type of sex reversal, the 46,XY female, is shown to result, in two cases, from small deletions of the short arm of the Y chromosome. Prometaphase chromosome analysis showed a 46,X,Yp- karyotype. Several Y chromosome-specific DNA probes were found to be deleted in the two female patients. DNA analysis showed that the two deletions were different but included a common overlapping region likely to be essential for male determination.

Genes located on the Y chromosome play an essential role in human male sexual development and the presence of a Y chromosome usually correlates with testis development. However, there are males with an apparently normal 46,XX karyotype and females with an apparently normal 46,XY karyotype. These two conditions may have several causes, including gene mutations and small chromosomal abnormalities. For example, some 46,XX males appear to have a small translocation of Y short arm material to the X chromosome (1-4), which is evidence for the X-Y interchange hypothesis (5). This hypothesis stated that at meiosis in the father, exchanges between the pairing regions of the X and Y chromosomes could produce an X chromosome carrying Y chromosome material. DNA hybridization studies of 46,XX males map the male-determining function of the Y chromosome to a small region of the short arm (4).

Females with a 46,XY karyotype have gonadal dysgenesis and many have features of Turner syndrome, a condition often associated with a 45,X chromosomal constitution. Most 46,XY females with the Turner phenotype are mosaic, with a 45,X cell line in addition to the 46,XY cell line. Two 46,XY patients with features of Turner syndrome and no evidence of mosaicism were shown to have a deletion of the short arm of the Y chromosome (6, 7). This and other more complex chromosomal rearrangements confirm that functions essential to normal male differentiation are found on the short arm of the Y chromosome. In addition, presence of the euchromatic portion of the long arm of the Y chromosome is important for normal male meiosis (8, 9).

This report describes two female patients with features of Turner syndrome and a 46,XY karyotype (by metaphase analysis). Evaluation of these individuals by prometaphase analysis and with seven DNA probes for regions on the short and long arms of the Y chromosome revealed the presence of

different, but overlapping, small deletions of the short arm of the Y chromosome.

### MATERIALS AND METHODS

Patient 1 has some Turner stigmata. She is of normal height at 4 years of age (75th percentile). At birth she was noted to have lymphedema, especially of the feet. Her external genitalia are normal. Her gonads, which were removed at 17 months, were streaks that consisted of dense ovarian stroma with no primordial follicles or testicular tissue. Patient 1 and her mother were both Xg<sup>a</sup>-positive.

Patient 2 has several features of Turner syndrome. At age 15, she has a short neck, wide-spaced nipples, and very small breasts but is of normal height. She had congenital lymphedema. She had primary amenorrhea and developed bilateral gonadoblastoma. Histological examination of the gonads showed gonadoblastoma and streaks with no primordial follicles. Patient 2 and her mother were both Xg<sup>a</sup>-positive; her father was Xg<sup>a</sup>-negative.

Cytogenetic analysis was performed on peripheral blood samples from patients 1 and 2 and on fibroblast cultures from skin and gonadal biopsies of patient 1 and from gonadal biopsies of patient 2. Prometaphase cells were obtained by the ethidium bromide method of Ikeuchi (10). The chromosomes were stained by G-banding, R-banding, C-banding, and Q-banding.

DNA was prepared from blood samples and fibroblast cultures of both patients, their parents, and normal control male and female individuals. The DNAs were digested to completion with the restriction endonuclease *Taq* I (for probe pDP34) or *Eco*RI (for probes 50f2, 52d, 47b, 118, p12f2, and p12f3). The DNA fragments were separated by agarose gel (0.7%) electrophoresis and transferred to membrane filters (Nytran or GeneScreen) for Southern blot hybridization. The probes were labeled with <sup>32</sup>P by nick-translation. Hybridization and washing of the blots were as described (11). The following probes were used. Probe pDP34 (*DXYS1*) detects homologous sequences on the short arm of the Y chromosome and on the long arm of the X chromosome (12). Probe 50f2 (*DYS7*) hybridizes with DNA sequences located on the Y chromosome (long and short arm) and on an autosome. Probe 52d (*DXYS11*) detects sequences on the long arm of the X chromosome and on the short and long arm of the Y chromosome (2, 4, 13). Probe 47b (*DXYS5*) detects Y (short arm), X, and autosomal sequences, whereas probe 118 (*DYS8*) is Y-specific, detecting sequences on the long and

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Abbreviation: kb, kilobase(s).

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short arm (2, 4, 13). Two additional probes, p12f2 (*DYS11*) and p12f3, detect sequences on the long arm of the Y chromosome (13). When hybridized to Southern blots, the probes listed above detect restriction fragments that have been mapped to specific chromosomal locations (2, 4, 13). The blots were washed in 75 mM NaCl/7.5 mM sodium citrate/1% NaDodSO<sub>4</sub> at room temperature, 50°C, and 65°C and autoradiographed for 7 days with an enhancing screen.

## RESULTS

Two female patients with a 46,XY karyotype were reevaluated by cytogenetics using prometaphase banding and by molecular analysis using Y chromosome-specific DNA probes. A small deletion of the short arm of the Y chromosome was identified in both patients, as shown in Fig. 1, in which the Y chromosomes of patient 1 and patient 2 are compared to a normal Y chromosome after G- and Q-bandings. The deletions were barely detectable on metaphase chromosomes. Although it appeared that the deletions might be interstitial rather than terminal, it was not possible to determine their nature with certainty or whether they differed in the two patients. The long arm of the Y chromosome of both patients appeared of normal size, including a Q-bright heterochromatic region of average size. The Y chromosome of the father of each patient was normal. No other karyotypic abnormalities were identified in either patient and no evidence of mosaicism was found in the tissues analyzed: in patient 1, 105 cells, 50 cells, 50 cells, and 51 cells were examined in the blood, skin, and right gonadal and left gonadal samples, respectively, whereas in patient 2, 120 cells, 54 cells, and 58 cells were examined in the blood and bilateral gonadal samples, respectively.

To characterize the deletions seen in the 46,XY female patients, seven <sup>32</sup>P-labeled DNA probes that detect Y chromosome-specific restriction fragments were hybridized to patient and control genomic DNA blots.

Fig. 2 shows the results of the hybridization of probe pDP34 to patient and control DNAs digested with *Taq* I. Lanes 1 and 4 contain normal male and female control DNAs, respectively. A 15-kb band characteristic of the male (Fig. 2, lane 1) is the Y chromosome-specific band, whereas a polymorphic band at 11 or 12 kb is X chromosome-specific (14). Patient 1 (Fig. 2, lane 2) showed a hybridization pattern characteristic of a normal male with bands at 11 and 15 kb, indicating that the Y chromosome-linked locus of *DXYS1* was not deleted in this case. Patient 2, however (Fig. 2, lane 3), was missing the band at 15 kb, indicating that the Y chromosome-specific DNA homologous to *DXYS1* had been deleted in that patient. The father of the latter patient showed

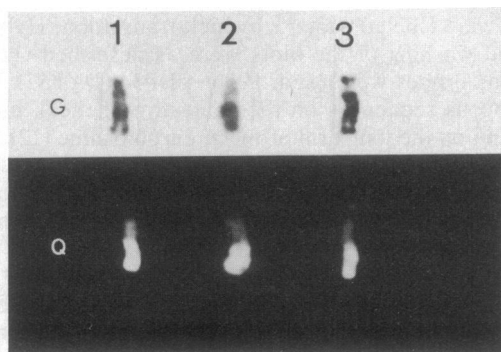


FIG. 1. Examples of Y chromosomes from patient 1 (no. 1), from patient 2 (no. 2), and from a normal male control (no. 3). The lower row shows Y chromosomes stained by Q-banding; staining in the upper row was by G-banding.

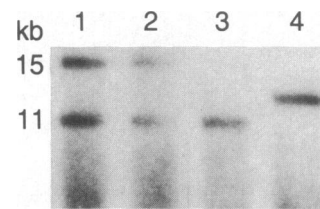


FIG. 2. Autoradiograph of a Southern blot with *Taq* I digests of DNA from a normal male (lane 1), a normal female (lane 4), patient 1 (lane 2), and patient 2 (lane 3). Five micrograms of DNA was loaded on each lane of a 0.7% agarose gel. After transfer to GeneScreenPlus filters, <sup>32</sup>P-labeled probe pDP34 was hybridized to the blots at 47°C. kb, Kilobases.

normal Y and X chromosome-specific bands at 15 and 12 kb, respectively (data not shown).

Fig. 3 shows the hybridization of <sup>32</sup>P-labeled probes 118 (Fig. 3a), 52d (Fig. 3b), 50f2 (Fig. 3c), and 47b (Fig. 3d) to *Eco*RI-digested DNAs from the patients and their parents. With all four probes, patient 1 (lane 3) showed deletions of a total of seven of the male-specific bands (7-, 2.6-, and 3.2-kb bands in Fig. 3a, 1.8-kb band in Fig. 3b, 10- and 8-kb bands in Fig. 3c, 7.8-kb band in Fig. 3d), whereas her father (lane 2) had a normal male pattern and her mother (lane 1) had a normal female pattern. The data summarized in Table 1 confirmed the cytogenetic data indicating that patient 1 had a deletion of a portion of the short arm of the Y chromosome. Probes 118, 52d, and 50f2 showed some male-specific bands that were not deleted in patient 1 (lane 3, 7.8- and 6-kb bands in Fig. 3a, 10- and 0.8-kb bands in Fig. 3b, and 6-, 4.5-, and 1.7-kb bands in Fig. 3c) as compared to her father (lane 2), indicating that these three probes recognized sequences located in regions of the Y chromosome other than that deleted in patient 1. These bands have been mapped to the long arm or the pericentromeric region of the Y chromosome (4).

Patient 2 did not appear to be missing Y chromosome-specific bands with probes 118 and 52d. However, with probe 50f2, the father of patient 2 (Fig. 3c, lane 5) and patient 2 (Fig. 3c, lane 6) were missing the 6-kb, Y chromosome-specific band, which represents a variant band. With probe 47b, patient 2 (Fig. 3d, lane 6) showed a missing Y chromosome-specific band at 7.8 kb identical to the one missing in patient 1 (Fig. 3d, lane 3) but present in normal males, including the father of patient 2 (Fig. 3d, lane 5). Studies with two additional probes, p12f2 and p12f3, which are located on the long arm of the Y chromosome (13), showed normal male hybridization patterns on patients 1 and 2 (data not shown).

Table 1 summarizes the hybridization data, which indicate that patients 1 and 2 have different deletions. The only DNA fragment these patients are both missing in common is homologous to probe 47b.

## DISCUSSION

We have described the molecular analysis of two female individuals with a Y chromosome. A common region of the Y chromosome was found to be missing in both individuals. This region was detected by probe 47b, which is adjacent to probe 47c (subclone of the same cosmid). Interestingly, the latter probe has been found to be most often present in patients who are 46,XX males (2-4). One or both of the deletions described here are likely to be interstitial, extending on either side of the location of 47b. The overlapping portion of the deletions is likely to be essential for male determination. The Y chromosome rearrangements in our 46,XY female patients could be more complex than simple deletions. They could also involve inversions or translocations.

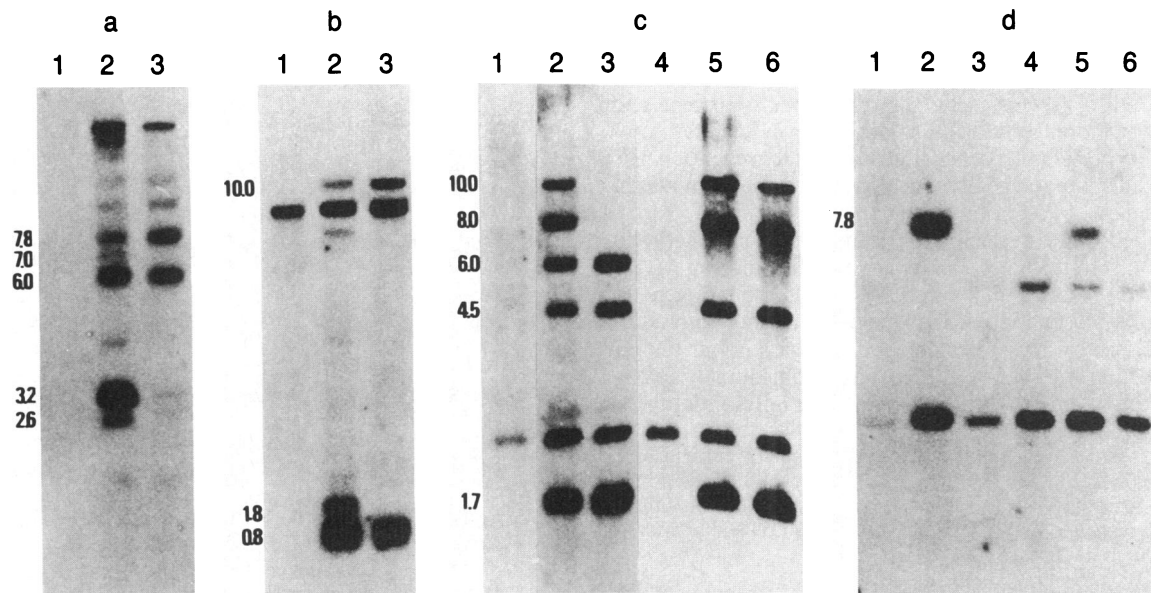


FIG. 3. Autoradiographs of Southern blots containing *EcoRI*-digested DNAs from the mother of patient 1 (lane 1), the father of patient 1 (lane 2), patient 1 (lane 3), the mother of patient 2 (lane 4), the father of patient 2 (lane 5), and patient 2 (lane 6). Ten micrograms of DNA was loaded on each lane of 0.7% agarose gels. After transfer to a Nytran membrane,  $^{32}\text{P}$ -labeled probes 118 (a), 52d (b), 50f2 (c), and 47b (d) were hybridized to the blots. The male-specific bands are indicated by their size in kb.

The hypothesis of exchange between the X and Y chromosomes, put forward by Ferguson-Smith (5), predicted that some individuals would be 46,XX males carrying Y chromosome material on one X chromosome, and others would be 46,XY females missing Y chromosome material. The description of 46,XX males with a small translocation of Y chromosome-specific material (1-4) and the present report agree with this prediction. The recent finding of pseudoautosomal sequences near the telomere of the X and Y chromosomes provides a way to explain exchanges between the two nonhomologous sex chromosomes (15-17). Cytologically, it appears that the region of pairing at meiosis extends into the nonhomologous region of the sex chromosomes and it was suggested that exchanges might occur due to abnormal delay in the segregation of the chromosomes (18).

The two patients reported here have different but overlapping deletions, which could explain that although both patients have features of Turner syndrome, they differ in some respects. Patient 2, but not patient 1, developed gonadoblastoma; however, the gonads were removed at an early age in patient 1.

Two previous cases of Yp deletions have been reported. Rosenfeld *et al.* (6) described a patient who had typical Turner phenotype with lymphedema at birth and short stature. Her gonads were fibrous with no primordial follicles or seminiferous tubules. Magenis *et al.* (7) recently reported a Yp deletion in another patient with features of Turner syndrome but of normal height, like our patients 1 and 2. That patient developed gonadoblastoma, like our patient 2. By *in situ* hybridization, deletion of a Y chromosome-specific

Table 1. Hybridization data indicating that patients 1 and 2 have different deletions

Probe	Locus	Band, kb	Location	Patient 1*	Patient 2†
pDP34	<i>DXYS1</i>	15.0	Yp	+	-
118	<i>DYS8</i>	7.8	Yq	+	+
		7.0	Yp	-	+
		6.0	Yc	+	+
		3.2	Yp	-	+
		2.6	Yp	-	+
52d	<i>DXYS11</i>	10.0	Yq	+	+
		1.8	Yp	-	+
		0.8	Yc	+	+
50f2	<i>DYS7</i>	10.0	Yp	-	+
		8.0	Yp	-	+
		6.0	Yq	+	-‡
		4.5	Yc	+	+
		1.7	Yq	+	+
47b	<i>DXYS5</i>	7.8	Yp	-	-

For each probe, a + indicates the presence of the Y chromosome-specific band and a - indicates the absence of that band. The bands were generated by *Taq* I digestion of DNA for pDP34 and *EcoRI* digestions of DNA for the other probes. The bands scored correspond to sequences detected on the Y chromosome only and their location is indicated as Yp (short arm), Yq (long arm), or Yc (pericentromeric region) (see Figs. 2 and 3). Some of the probes also detect sequences located on autosomes or the X chromosome.

\*Phenotype, Turner stigmata; sex, female; gonads, streak.

†Phenotype, Turner stigmata; sex, female; gonads, streak and gonadoblastoma.

‡This fragment was also absent from the father of patient 2.

sequence was demonstrated in this patient (19). Patients with isochromosomes of the long arm of the Y chromosome or with isodicentric Y chromosomes missing portions of the short arm may also have stigmata of Turner syndrome and develop gonadoblastoma (8, 9). However, interpretation of the phenotype-genotype relationships in these patients is more difficult due to the presence of 45,X mosaicism and of two Yq regions in addition to the absence of Yp.

It is likely that some 46,XY female patients with no apparent chromosomal deletion have Y chromosome deletions that are smaller than can be detected on a cytogenetic level. The study of these 46,XY female patients with additional Y chromosome-specific probes should aid our understanding of the role of the different Y chromosomal regions and Y chromosome-specific genes in male differentiation.

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