X;Y translocation in an adolescent mentally normal phenotypic male with features of hypogonadism

RENÉE BERNSTEIN, M R PINTO, M ALMEIDA, S M SOLARSH, J MECK*, AND TREFOR JENKINS

From the Department of Human Genetics, School of Pathology, The South African Institute for Medical Research and University of the Witwatersrand, PO Box 1038, Johannesburg 2000, South Africa

SUMMARY Cytogenetic studies on a 17-year-old phenotypic male, with short stature and clinical and hormonal features of hypogonadism similar to those of an XX male, revealed an X;Y translocation, karyotype 46,Xt(X;Y)(p22;?p11?q11). He was H-Y antigen positive. X inactivation studies showed inactivation of the abnormal X in the majority of cells (60 to 70%) and inactivation of the normal X in the remaining cells. Gene marker studies, including Xg blood grouping, showed no anomalous segregation. This patient is the second reported male showing a positively identified X;Y translocation with no detectable free Y chromosome and provides further indirect evidence for an X-Y interchange in the aetiology of XX male sex reversal.

A male phenotype associated with a cytologically identified X;Y translocation and no detectable free-lying Y chromosome has been reported only once.¹ Other such cases with no detectable free Y chromosome have all been phenotypic females.²⁻⁴ Detection of heteromorphism of the X chromosomes in XX males⁵⁻⁷ is presumptive morphological evidence of an X;Y translocation or interchange in the aetiology of XX male sex reversal.

We report here the second case of morphologically definitive X;Y translocation with no detectable free Y chromosome in a phenotypic male with short stature and features of hypogonadism, and describe hormonal investigations, X inactivation studies, and H-Y antigen findings. The patient's clinical features are compared with those of an XX male.

Case report

A 17-year-old white male was referred for cytogenetic and hormonal investigation of short stature and small atrophic testes.

The proband's mother and father, aged 26 years and 28 years, respectively, at the time of his birth, are normal and not consanguineous. He has three normal sibs and there is no history of miscarriage. Delivery after an uneventful pregnancy was normal and at term. He weighed 3.2 kg at birth and all his

early developmental milestones were normal. During his first year at school, a learning disability was diagnosed which necessitated remedial teaching, but he is otherwise mentally normal, socially well adjusted, and participates fully in sporting activities. His 14-year-old brother and several paternal male relatives are 'dyslectic'.

At 7 years of age he underwent orchidopexy for bilaterally undescended testes and an associated inguinal hernia was repaired. The usual childhood ailments included an attack of mumps, without any known complications. At 17 years of age he did not shave.

The proband's father was 169 cm tall, his mother was 171.5 cm, his two older sisters, aged 21 and 19 years, were almost the same height as their mother, and his younger brother was 170 cm tall at 14 years of age. The proband's 19-year-old sister complained of menstrual irregularity for 2 years and had moderate facial hair growth.

Clinical examination at 17 years showed a physically well-developed and mentally normal male. His total height was 161.5 cm, with a crown-pubis height of 82 cm and an arm span of 161 cm. The head circumference was 54 cm. The body contours were android and body hair was male in distribution, with plentiful axillary hair but scanty pubic hair growth. There was no facial hair growth apart from a light 'downy' growth on the upper lip. No parenchymal breast enlargement was noted and his voice was that of a normal postpubertal male. The penis

*Present address: Mailman Center for Child Development, University of Miami, Miami, Florida, USA.

Received for publication 17 January 1980

was of average size, with a terminal urethral meatus but the testes, which were bilaterally descended into a normal rugose scrotum, were very small and soft. There were no somatic features of Turner's syndrome.

SPECIAL INVESTIGATIONS

Assays for endocrine function indicated normal pituitary, thyroid, parathyroid, and adrenal function. Two independent serum testosterone assays showed decreased levels of 150 ng/100 ml (5.20 nmol/l) and 128 ng/100 ml (4.44 nmol/l) (normal adult range 377 to 1090 ng/100 ml; 13.09 to 37.84 nmol/l), with a corresponding increase in serum FSH levels to 9.4 ng/ml (normal adult male range 1.0 to 6.0 ng/ml). The serum LH of 2.1 ng/ml was within the normal range for adult males (1.0 to 4.5 ng/ml). The decreased testosterone levels and the raised FSH value indicated a primary testicular dysgenesis.

A single complement fixation test for parotitis virus (V) and (S) at 17 years of age showed a titre of >1:8, indicating an antibody response to a previous infection.

The proband's 19-year-old sister was investigated for menstrual irregularity of 2 years' duration. Clinical examination, hormonal assays, and laparoscopic examination were all normal and histology of both gonads showed normal ovarian tissue.

DERMATOGLYPHIC INVESTIGATIONS

The palmar creases of both hands of the proband were normal. The finger ridges of both hands consisted of a whorl on four fingers and an ulnar loop on the fifth finger. The total finger ridge count was 222 and the total *a-b* ridge count was 75. The palmar axial triradii were in the normal 't' position with normal *atd* angles. The thenar and hypothenar spaces of both hands showed an open pattern. Distal loops were present in interdigital spaces III and IV of both hands.

Methods

CYTOGENETIC STUDIES

Peripheral blood metaphases were derived from synchronised cell cultures.⁸ A fibroblast culture was established from a forearm skin biopsy by a standard closed culture technique. Banding procedures and X chromosome inactivation studies were performed by previously described methods.¹

GENE MARKER STUDIES

A wide range of gene marker studies was carried out on the proband, his 19-year-old sister, and both his parents to exclude non-paternity as well as to search for anomalous segregation. In addition to the gene

marker studies described for our first case,¹ haptoglobin and transferrin polymorphisms were analysed by techniques described by Giblett,⁹ as well as glyoxalase I¹⁰ and carbonic anhydrase I and II.¹¹

H-Y ANTIGEN ASSAY

A fibroblast culture of the patient's cells was established from a skin biopsy. The cells were tested for H-Y antigen by means of the *Staphylococcus aureus* binding technique using the method of Goldberg *et al.*¹² Three groups were assayed for the H-Y antigen: a positive control from normal male fibroblasts, a negative control from normal female fibroblasts, and the patient's fibroblasts. In each group, 100 cells were examined by light microscopy and the number of *Staphylococcus aureus* bound to each cell was recorded. Any cell bearing more than 25 Staphylococci was given a score of 26, since it was impossible to give an accurate count with more Staphylococci attached. The data were analysed by a *t* test for comparison of means.

Results

CYTOGENETIC STUDIES

Chromosome analysis of Giemsa banded metaphases showed two X chromosomes, one of which was abnormal. The abnormal X had extra chromatin material attached to the distal end of Xp. There was no morphologically identifiable Y chromosome (figs 1, 2a). Quinacrine banding showed the typical brilliant fluorescence characterising the distal long arm of a Y chromosome, located at the terminal portion of the X short arm (fig 2c). Centromeric banding confirmed the presence of darkly staining heterochromatin of Yq attached to the terminal portion of Xp. The abnormal X showed only one distinct X chromosome centromere, but a fairly darkly stained area proximal to the dark distal heterochromatin was noted in the region of the presumed X;Y fusion site, which could represent a suppressed Y centromere (fig 2b). On reverse banding the telomeric p22 positively staining band of the abnormal X was similar in size to that of the normal X homologue. None of the above banding techniques detected any obvious morphological loss of the telomeric p22 band of the translocation X, nor was it possible to see a definite Y centromere.

An attempt was made by means of measurements to establish whether the translocated Y had lost the whole or part of its short arm in the translocation process. The results are shown in the table. The centromeric index of the father's X does not differ significantly from the proband's normal X chromosome. The centromeric index of the proband's

FIG 1 G banded karyotype showing extra chromatin material attached to the distal portion of Xp (arrowed).

FIG 2 Appearance of the normal X chromosome (left column) and abnormal t(X;Y) chromosome (right column). (a) G banding, (b) C banding, (c) Q banding.

TABLE Mean ratios from measurements of proband's and father's sex chromosomes

	Proband <i>n</i> = 8 cells	Father <i>n</i> = 7 cells	Difference between means (D)	Standard error of (D) (SE)	D/SE†
$\frac{Xp}{X} \times 100^*$	39 ± 1.96	40 ± 1.58	1	0.9148	1.0931
$\frac{t(Xp;Y)}{t(X;Y)} \times 100^*$	55 ± 4.36		3	1.6168	1.8555
$\frac{(Xp+Y)}{(X+Y)} \times 100^*$		58 ± 1.29			
$\frac{t(X;Y)}{X}$	1.41 ± 0.1328		0.02	0.0486	0.4115
$\frac{X+Y}{X}$		1.43 ± 0.0333			

*Centromeric index; †value >2.5 would indicate significant difference.

t(X;Y) does not differ significantly from the calculated centromeric index derived from the combined length of the father's (X+Y), nor do the ratios of the proband's t(X;Y) and the father's (X+Y) to the normal X differ significantly. In none of these calculations was the difference between the means more than 1.86 times greater than the standard error of the difference between the means. It is therefore concluded that most of the Y chromosome, including at least the proximal portion of Yp, is present in the translocation chromosome. The karyotype is

therefore interpreted as 46,Xt(X;Y) (p22;p11) rather than (p22;q11).

A total of 147 peripheral blood metaphases from two independent cultures and 151 skin fibroblast metaphases were screened (298 cells); in all but two peripheral blood cells the t(X;Y) chromosome was present. In the latter two cells, the karyotype was 45,X (0.67% of the total cells). No free-lying Y chromosome was detected in any of the cells from the two tissues studied.

The X inactivation pattern of the normal and t(X;Y) chromosomes was studied in 34 informative reverse banded metaphases. In 24 cells (70.5%) the

translocation X showed the dull orange fluorescence associated with a late replicating X chromosome and the normal X was brightly fluorescent (fig 3a). Ten of 34 cells (29.5%) showed inactivation of the normal X (fig 3b). Buccal epithelial cells showed an X chromatin body of average size in 24% of 200 nuclei. No obvious variation in the size of the Barr bodies was detected. A fluorescent Y body was present in 78% of 200 nuclei screened. (Chromosome analysis did not show any fluorescent chromosome variants capable of being misinterpreted as a Y body in interphase nuclei.) Sequential staining for Y and X chromatin in 41 informative nuclei showed unassociated X and Y bodies indicative of inactivation of the normal X in 16 cells (39%) (fig 4a) and associated X and Y chromatin bodies suggestive of inactivation of the translocation X in 25 nuclei (61%) (fig 4b).

Both parents had normal karyotypes. The father's Y chromosome was of average size, and fluorescence did not show any morphological evidence of a pericentric inversion or any other Y chromosome abnormality. Chromosome analysis of peripheral blood and gonadal metaphases of the proband's sister showed a normal female karyotype.

GENE MARKER STUDIES

These gave no indication of parental exclusion or anomalous segregation for any of the systems studied. In particular the index case and his sister were Xg(a+); his mother was also Xg(a+) while his father was Xg(a-).

H-Y ANTIGEN STUDIES

The patient's cells were not significantly different from the male control in the mean number of *Staphylococcus aureus* attached per cell ($p = 0.775$).

FIG 3 Reverse A-O banding after 5-BrdU incorporation. (a) The pale inactivated t(X;Y) chromosome is to the left of each of three pairs of X homologues. (b) The pale inactivated normal X chromosome is to the right of each of three pairs of X homologues.

FIG 4 Sequential staining for X and Y chromatin in epithelial interphase nuclei. (a) Two nuclei showing associated X and Y chromatin indicative of t(X;Y) inactivation. (b) Two nuclei showing unassociated X and Y chromatin indicating normal X inactivation.

However, both the patient and the male control were significantly different from the female control ($p = 0.0001$). Thus, the patient is H-Y antigen positive.

Discussion

The phenotypic expression of a Y to X translocation will be determined by the sites of breakage on the X and Y chromosomes, the mechanism producing the translocation, and the X inactivation pattern.

A male phenotype in mammals is primarily determined by testicular differentiating genes on the Y chromosome¹³ and these genes can be serologically detected as H-Y antigen.¹⁴ There is good evidence for the existence of multiple copies of the H-Y gene, fetal testicular differentiation depending on the presence of a critical number of H-Y gene copies.¹⁵ A male phenotype in X;Y translocations is therefore presumptive evidence that the translocation did not involve a critical number of H-Y genes, provided that there is no undetected mosaicism for a cell line with a free lying Y chromosome. Examination of 298 peripheral blood and fibroblast metaphases showed no trace of such mosaicism yet the phenotype was male and the subject was H-Y antigen positive.

From studies of the phenotype of subjects with structural abnormalities of the Y chromosome, the locus for male determining genes was assigned to the short arm of the Y.^{16,17} Localisation of H-Y antigen genes to the Y short arm by Koo *et al*¹⁸ and Moreira-Filho *et al*¹⁹ serologically confirms these observations, but in one H-Y antigen positive female, cytological studies indicated a loss of the Y short arm in an X;Y translocation and the H-Y locus was therefore assigned to Yq¹⁸ (case 8). It must therefore be assumed that H-Y antigen positivity associated with a female phenotype in their patient is the result of either loss of a critical number of H-Y gene copies on Yp or of the inactivation pattern. Another interpretation of the case of Koo *et al*¹⁸ is, however, possible. If the fusion of Xp and Y followed a break in the region of Yq11, then this is indirect evidence that copies of H-Y genes are situated on Yq as well as Yp, but if a pericentric inversion of the Y preceded the translocation, the cytologically visible proximal portion of Yq would in fact consist of Yp genetic material. A pericentric inversion was postulated as one of the mechanisms by which an X;Y translocation could occur at meiosis.^{1,2} In our patient, who is an H-Y antigen positive male, comparison of the size of the proband's t(X;Y) and the combined size of the father's (X+Y) suggests that most of the Y chromosome, including at least a portion of Yp, is translocated onto the X, although a definite Y centromere could not be seen.

If a critical number of male determining genes on the Y are intact, the second factor determining the phenotypic sex in an X;Y translocation is the X inactivation pattern. The type of X inactivation observed in previously described cases has not conformed to any definite pattern (reviewed by Bernstein *et al*¹). In the present case inactivation of both the normal X and t(X;Y) chromosome was found and the resulting phenotype was that of a male with testicular dysgenesis. We could not, however, establish whether the translocated Y chromosome in those cells where the t(X;Y) was inactivated was in fact fully inactivated because the Y is normally a late replicating chromosome. Inactivation studies on X; autosome translocations have shown that inactivation of only the X portion can occur, while the translocated autosome remains active.^{20,21} Therman and Patau²² postulated that the X inactivation process can spread to an autosome attached to Xp but not to autosomal material attached to Xq. If this type of inactivation spreading holds true for Y translocations onto Xp it would be expected that the Y and its testicular differentiating genes would be inactivated in those cells where the abnormal X was inactivated.

Inactivation of the abnormal X and its attached Y chromosome in more than half the cells studied would have an effect corresponding to a 45,X/47,XXY mosaicism, but enough testicular differentiating genes must have been active to produce the male phenotype, and although the inactivation pattern in blood lymphocytes and epithelial cells was the same (>60% inactivation of the t(X;Y) chromosome) this need not necessarily be the pattern present in germinal or other tissue.

Another factor which could have modified the phenotype in this case is the possible loss of the translocation X chromosome in some cells, resulting in a minor 45,X mosaic cell line. Only two such cells were detected out of a total of 298 peripheral blood and skin fibroblast metaphases analysed, and these cells could have resulted from random technical loss rather than an *in vivo* mosaicism. There were no somatic features of Turner's syndrome apart from short stature, and there was no evidence of asymmetrical gonadal dysgenesis. Both gonads were clinically testes, albeit small ones (a gonadal biopsy was, however, not done).

Although both our patients with a t(X;Y) chromosome were males, their clinical presentation was very different. The present patient was mentally normal, whereas the prepubertal boy previously described¹ was markedly mentally retarded and had other physical abnormalities. In the latter patient a loss of the distal portion of Xp was postulated on the basis of anomalous Xg blood group segregation, but

in the present case there is no clinical or cytological evidence to suggest that there is any loss of X chromosomal genes. Xg blood group segregation studies in the present family also failed to throw any light on this aspect.

The clinical features in the present patient were very similar to those of XX males, namely short stature associated with male hypogonadism, and he differed in some respects from Klinefelter males although he had two X chromosomes and a Y chromosome. The mean height of XX males is slightly smaller than the mean height of normal males,²³ whereas Klinefelter males are usually taller than normal males and they have relatively long legs.²⁴ Our patient was short and his crown-pubis height was proportional to the total height. He also had undescended testes which were surgically brought into the scrotum, a feature rarely found in Klinefelter's syndrome, whereas genital anomalies are more frequent in XX males.¹⁷ The patient's dermatoglyphic patterns were normal, although the high total ridge count of 222 is more typical of the situation found in 45,X females, who have a mean ridge count of 166, rather than of Klinefelter males who have a decreased mean ridge count of 118.²⁵

The possible mechanisms responsible for sex reversal in XX males are reviewed by Evans *et al.*⁷ In those cases with an autosomal mode of transmission this may be dominant as in Sxr XX mice, or recessive as in polled homozygous goats and possibly man. In all these instances of sex reversal, affected subjects are H-Y antigen positive.^{6, 26} A dominant or recessive mode of inheritance in these circumstances could depend on whether a critical or subcritical number of H-Y gene copies are translocated onto an autosome.^{15, 27}

The H-Y antigen positivity, and the clinical and endocrinological resemblance of this patient to an XX male, lends further credence to the X-Y interchange hypothesis of Ferguson-Smith²⁸ as an explanation for apparent sex reversal in XX males. Morphological heteromorphism of the X chromosomes in eight of 12 XX males, and identification of Y specific DNA in one of three of these males,⁷ is very convincing evidence that the latter mechanism is the most frequent one in XX male sex reversal. The demonstration of morphologically unequivocal X;Y translocations presupposes that minute submicroscopic translocations resulting in genetic interchange of critical or subcritical numbers of H-Y gene copies during end-to-end short arm X-Y pairing at meiosis can occur as well.

We wish to thank Dr B Bloomberg for the results of hormonal assays, Dr S Hirschowitz for clinical

details of the proband's sister, Miss P Moores for confirmatory Xg blood group studies, Mrs N de Meyer, Mr A B Lane, Mrs R Turnbull, and Mr D Dunn for technical assistance, Dr Ellen Goldberg who supplied the H-Y antibody and the *Staphylococcus aureus* used for the H-Y antigen assay, and Dr Robert Meck who performed the statistical analysis of the H-Y antigen data on the PROPHET computer system. The operation and continuing development of the PROPHET system is sponsored by the Chemical/Biological Information-Handling Program, Division of Research Resources, National Institutes of Health.

References

- Bernstein R, Wagner J, Isdale J, Nurse GT, Lane AB, Jenkins T. X-Y translocation in a retarded male. Clinical, cytogenetic, biochemical, and serogenetic studies. *J Med Genet* 1978;**15**:466-74.
- Khudr G, Benirschke K, Judd HL, Strauss J. Y to X translocation in a woman with reproductive failure—a new rearrangement. *JAMA* 1973;**226**:544-9.
- Borgaonkar DS, Sroka BM, Flores M. Y-to-X translocation in a girl. *Lancet* 1974;**i**:68-9.
- Van den Berghe H, Petit P, Frys JP. Y to X translocation in man. *Hum Genet* 1977;**36**:129-41.
- Madan K, Walker S. Possible evidence for Xp⁺ in an XX male. *Lancet* 1974;**i**:1223.
- Wachtel SS, Koo GC, Breg WR, *et al.* Serologic detection of a Y-linked gene in XX males and XX true hermaphrodites. *N Engl J Med* 1976;**295**:750-4.
- Evans HJ, Buckton KE, Spowart G, Carothers AF. Heteromorphic X chromosome in 46,XX males: evidence for the involvement of X-Y interchange. *Hum Genet* 1979;**49**:11-31.
- Yunis JJ. High resolution of human chromosomes. *Science* 1976;**191**:1268-70.
- Giblett ER. *Genetic markers in human blood*. Oxford: Blackwell, 1969.
- Kompf J, Bissbort S, Gussmann S, Ritter H. Polymorphism of red cell glyoxalase I (E.C.: 4.4.1.5). A new genetic marker in man. *Humangenetik* 1975;**27**:141-3.
- Hopkinson DA, Coppock JS, Mühlemann MF, Edwards YH. The detection and differentiation of the products of the human carbonic anhydrase loci CA_I and CA_{II} using fluorogenic substrates. *Ann Hum Genet* 1974;**38**:155-62.
- Goldberg EH, Arrington T, Tokuda S. Detection of H-Y antigen on human male leukocytes. *J Immunol Methods* 1978;**23**:203-6.
- Ford CE. Cytogenetics and sex determination in man and mammals. *J Biosoc Sci* 1970;**suppl 2**:7.
- Wachtel SS, Ohno S, Koo GC, Boyse EA. Possible role for H-Y antigen in the primary determination of sex. *Nature* 1975;**257**:235-6.
- Wachtel SS, Ohno S. The immunogenetics of sexual development. In: Steinberg AG, Bearn AG, Motulsky AG, Childs B, eds. *Progress in medical genetics*. vol 3. Philadelphia, London, Toronto: Saunders, 1979: 109-42.
- Jacobs PA. Structural abnormalities of the sex chromosomes. *Br Med Bull* 1969;**25**:94-8.
- Simpson JL. *Disorders of sexual differentiation*. New York: Academic Press, 1976.
- Koo GC, Wachtel SS, Krupen-Brown K, *et al.* Mapping the locus of the H-Y gene on the human Y chromosome. *Science* 1977;**198**:940-2.

- ¹⁹ Moreira-Filho CA, Otto PG, Frota-Pessoa O. H-Y gene expression in apparent absence of the long arm of the Y chromosome. *Am J Med Genet* 1979;**4**:135-9.
- ²⁰ Hagemeijer A, Hoovers J, Smit EME, Bootsma D. Replication pattern of the X chromosomes in three X/autosomal translocations. *Cytogenet Cell Genet* 1977;**18**:333-48.
- ²¹ Bernstein R, Dawson B, Kohl R, Jenkins T. An X;15 translocation in a retarded girl: X inactivation pattern and attempt to localise the hexosaminidase A and other loci. *J Med Genet* 1979;**16**:254-62.
- ²² Therman E, Patau K. Abnormal X chromosomes in man: origin, behaviour and effects. *Humangenetik* 1974;**25**:1-16.
- ²³ de la Chapelle A. Analytical review. Nature and origin of males with XX sex chromosomes. *Am J Hum Genet* 1972;**24**:71-105.
- ²⁴ Milne JS, Lauder IJ, Price WH. Anthropometry in sex chromosome abnormality. *Clin Genet* 1974;**5**:96-106.
- ²⁵ Cushman CJ, Solton HC. Dermatoglyphics in Klinefelter's syndrome. *Hum Hered* 1969;**19**:641-53.
- ²⁶ Bennett D, Mathieson BJ, Scheid M, *et al.* Serological evidence for H-Y antigen in Sxr, XX sex-reversed phenotypic males. *Nature* 1977;**265**:255-7.
- ²⁷ Wachtel SS, Basrur P, Koo GC. Recessive male-determining genes. *Cell* 1978;**15**:279-81.
- ²⁸ Ferguson-Smith MA. X-Y chromosomal interchange in the aetiology of true hermaphroditism and XX Klinefelter's syndrome. *Lancet* 1966;**ii**:475-6.

Requests for reprints to Dr R Bernstein, Cytogenetics Unit, Department of Human Genetics, South African Institute for Medical Research, University of the Witwatersrand, Johannesburg 2000, South Africa.