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A 45,X male with a Yp/18 translocation

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Summary. A patient described as a 45,X male (Forabosco et al. 1977) was examined for the presence of Y-specific DNA by using various probes detecting restriction fragments from different regions of the Y chromosome. Positive hybridization signals were obtained for Yp fragments only. In situ hybridization with two different probes, pDP31 and the pseudoautosomal probe 113F, led to a clear assignment of the Yp sequences to the short arm of one chromosome 18. Cytogenetically, the presence of all of Yp including the Y centromere on 18p could be demonstrated replacing a segment of similar size of 18p. Thus, the Y/18 translocation chromosome is dicentric structurally, but it was shown to be monocentric functionally with the no. 18 centromere active. Gene dosage studies with the probe B74 defining a sequence at 18p11.3 demonstrated a single dose of this sequence in the patient. In agreement with these observations, the patient shows clinical signs of the 18p− syndrome. It is concluded that in XO males in general, the X is of maternal origin while the maleness is due to a de novo Y/autosome translocation derived from the father. Depending on the nature of the autosomal deficiency caused by the Y/autosome translocation, the patient may have congenital malformations.

Introduction

Recent evidence indicates that the 45,X condition associated with a male phenotype is due either to hidden mosaicism (our unpublished results) or to the presence of Y-euchromatin translocated onto an autosome. The latter condition can be detected using Y-specific DNA probes, and this in turn might facilitate the cytogenetic localization of the translocation. Such a case was reported by Schempp et al. (1985), and here we report on the series of investigations resulting in the identification of Y-chromosome material on one chromosome 18 of a 45,X male described by Forabosco et al. (1977).

Case report

G.C. was born on 2 April 1974. The clinical findings at birth were reported by Forabosco et al. (1977), who also diagnosed the 45,X karyotype with no evidence of mosaicism. Since the age of 2.3 years until 10.8 years his height was in the range between −4.5 and −6 standard deviations in comparison with normal. A slight improvement was obtained after 7 years of age with human growth hormone therapy. When 10.7 years old he was 112 cm high. In the same period his weight ranged between −5 and −6 standard deviations from normal. Bone age was repeatedly tested and was 2.7 when he was 4 years old, 4 when he was 6, 5.9 when he was 7.7, and 9.6 when he was 10.2 years old. Psychomotor development was always at the borderline between normal and retarded. He said his first words when 12 months old and began to walk at 18 months. At present he has low set hair and ears, antimongoloid slant of the eyes, hypertelorism, macrostomia, down-turned corners of the mouth, extremely high palate, dental malocclusion with multiple caries, short, broad and slightly webbed neck, shield chest with widely spaced nipples. An umbilical hernia is present. Both penis and testicles are hypoplastic: the left testicle is in the scrotum, the right testicle is at the level of the inguinal canal. There are no signs of initial pubertal development. He suffered from repeated urinary tract infections and an urography showed a bladder with trabeculation and dysmorphic neck. X-ray examination revealed no gross skeletal anomalies, but short fourth metacarpal bones were noticed bilaterally. ECG and EEG were normal and so were routine blood and urine analyses, including tests for aminoacidaemia, aminoaciduria, glycosaemia, and glycosuria. The serum levels of T_3, T_4, TSH, ACTH, FSH, LH in basal condition and after LHRH, 17-ketosteroids are all normal. The serum GH level did not increase after administration of insulin or arginine. Serum androgens after 3 days administration of hCG increased but remained in the lower normal range.

Analyses of Xg^a blood group performed by Dr. Ruth Sanger, MRC Blood Group Unit, London, on the proband and his parents gave the following results: proband: Xg^a-; father: Xg^a+; mother: Xg^a+.

H-Y antigen was determined by Antonia Mayerovà, Institute of Human Genetics Freiburg, using the cytotoxicity test after absorption of H-Y antiserum with cultivated fibroblasts of the patient. The results showed a normal male titre of H-Y antigen.

Materials and methods

Chromosome analyses were performed on lymphocyte cultures with QFQ- and GTG-banding methods on the proband

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and his parents. Chromosome preparations were also made from cultured fibroblasts of a skin biopsy of the proband using standard techniques. To demonstrate early replication patterns, the chromosome preparations of bromodeoxyuridine (BrdU)-treated cultures were differentially stained with acridine orange, resulting in RBA banding patterns. In addition distamycin A/DAPI staining was applied to the proband's preparations.

In situ hybridization was performed in two of the laboratories involved using two different probes.

a. In Pavia, in situ hybridization was performed essentially according to the method of Bartram et al. (1983). The pDP31 probe DNA (Page et al. 1984) was labelled by nick-translation using ^{3}H-TTP and ^{3}H-dCTP to a specific activity of 4.5×10^6 cpm/μg. Autoradiography was performed with Kodak NTB2 emulsion and the preparations were exposed for 14 days, developed, and stained for Q-bands. On the preparations obtained the Q-banding patterns and the distribution of the grains were analysed at the same time.

b. In Freiburg, the pseudoautosomal probe 113F (Simmler et al. 1985) was radiolabelled by ^{3}H-TTP, ^{3}H-dCTP, and ^{3}H-dATP to a specific activity of 6×10^7 cpm/μg using the oligolabelling technique originally described by Feinberg and Vogelstein (1983, 1984). In situ hybridization and autoradiography were performed according to published procedures (Lau et al. 1985). After autoradiography the chromosomes were identified with quinacrine mustard using the method of Caspersson et al. (1970). To locate the silver grains slides were then stained in 8% buffered Giemsa solution.

Hybridization probes

The following DNA probes were used in this study:
(1) pDP31 and pDP34, defining locus DXYS1. Both probes hybridize to Xq13-q21.2 and to Yp (Page et al. 1984). (2) p782, defining locus DXS85 at Xp22.3-p22.2 (Hofker et al. 1985). (3) p47z, defining locus DXYS5. The probe hybridizes

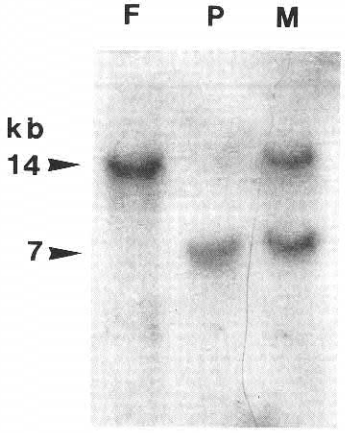

Fig. 1. Maternal origin of proband's X chromosome. EcoRI-digested DNA from the proband's father (*F*), the proband (*P*), and his mother (*M*) was fractionated on a 0.7% agarose gel, transferred to nitrocellulose filter, and hybridized with the X chromosome-specific probe p782 (DXS85). The size of the resulting fragments in kb is indicated on the left

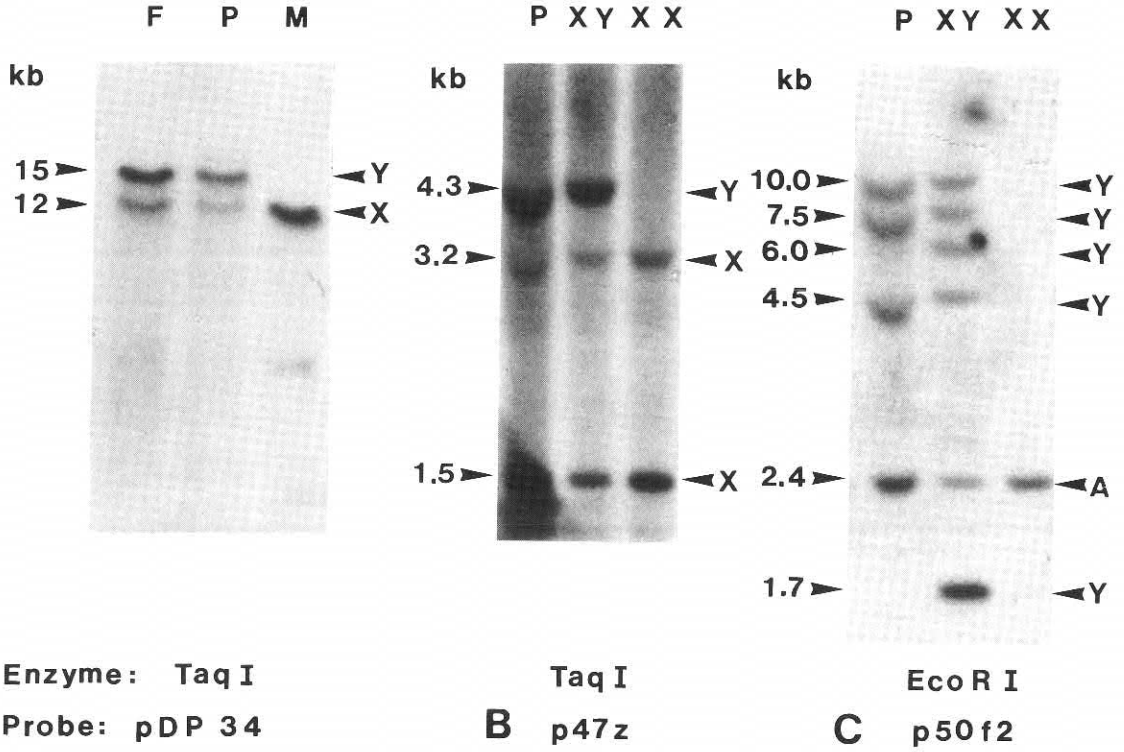

Fig. 2A–C. Detection of Y-specific fragments in proband's DNA. DNA samples were digested with the enzymes indicated, fractionated on 0.7% agarose gels, and the genomic blots were hybridized with the respective DNA probes. The size of the resulting fragments in kb is indicated on the left, while their chromosomal origin is given on the right: Y, Y-specific band; X, X-specific band; A, autosomal band. **A** The following DNA samples were used: F, proband's father; P, proband; M, proband's mother. **B, C** The following DNA samples were used: P, proband; XY, normal male control; XX, normal female control

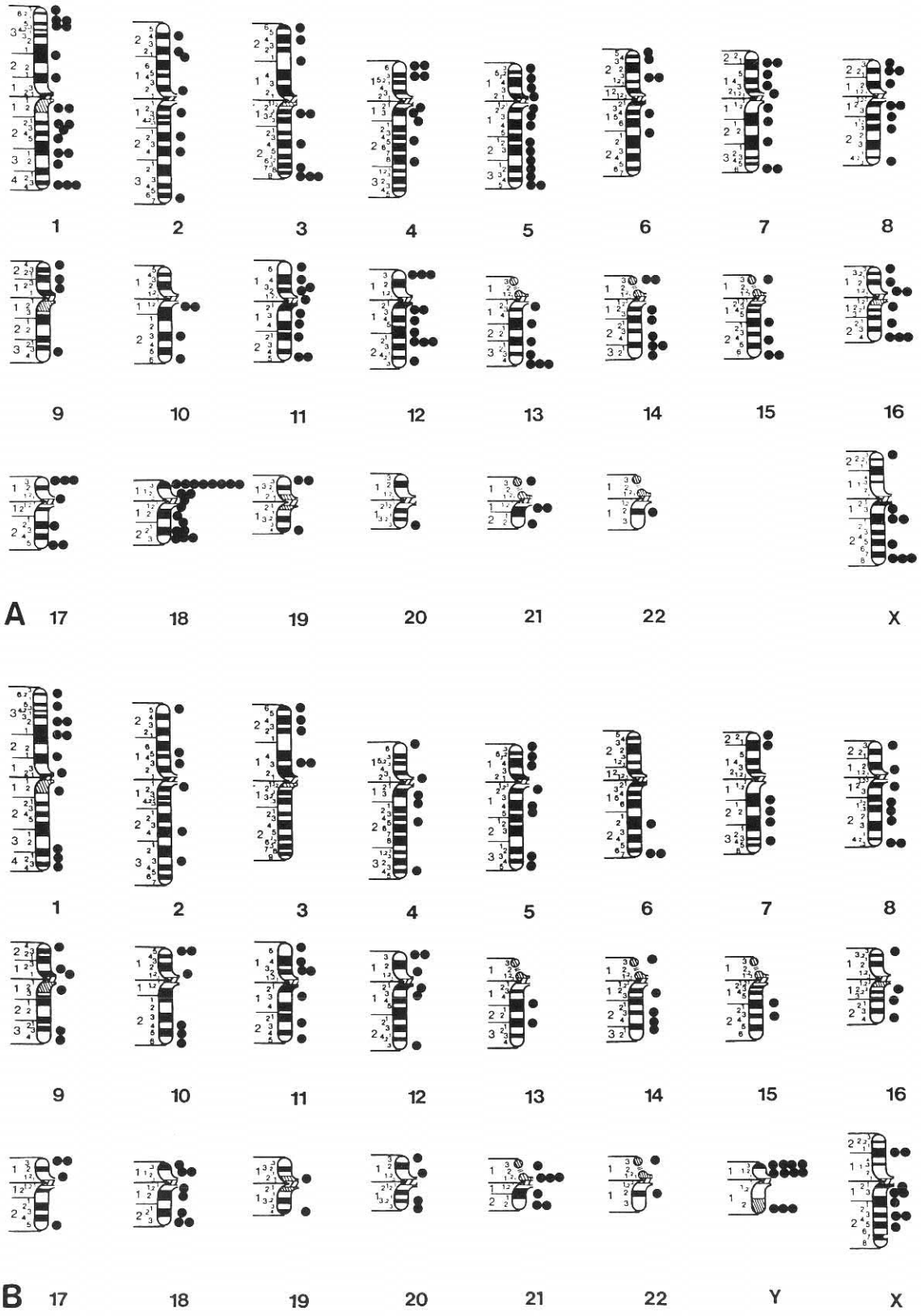

Fig. 3A, B. In situ hybridization with probe pDP31. **A** The distribution of silver grains (186) on the 45 chromosomes of the XO male. Fifty three metaphases were examined for silver grains with an average of 3.5 grains on chromosomes per cell. **B** The distribution of silver grains (139) on the 46 chromosomes of the father. Thirty one metaphases were examined for silver grains, with an average of 4.5 grains on chromosomes per cell

to Xq12-q22 and to Yp (Guellaen et al. 1984). (4) p50f2, defining multiple loci. The probe hybridizes to Yp, Yq, and to an autosome (Vergnaud et al. 1986). (5) B74, defining locus D18S3 at 18p11.3 (Mattei et al. 1985). (6) pHINS500, equivalent to pHINS6.0, defining the insulin locus at 11q15 (Elbein et al. 1985). (7) 113F, which detects a repeated pseudoautosomal sequence referred to as DXYZ2 (Simmler et al. 1985).

Southern blot analysis

Total human DNA was isolated from white blood cells essentially as described by Baas et al. (1984). Ten micrograms of DNA was digested to completion with TaqI or EcoRI (Boehringer Mannheim) according to the manufacturer's conditions and fractionated on 0.7% horizontal agarose gels. The DNA was transferred to nitrocellulose filters and subsequently hybridized to nick-translated probes as described by Gal et al. (1985). Alternatively, the DNA was blotted onto Gene Screen nylon membranes (NEN), UV cross-linked by irradiation with a germicidal bulb for 5 min, and hybridized to "oligolabelled" probes (Feinberg and Vogelstein 1983, 1984) in 0.5 *M* sodium phosphate buffer, pH 7.2/7% sodium dodecyl sulphate (SDS)/ 1 m*M* EDTA (Church and Gilbert 1984). For experiments with probe B74, a pre-hybridization step of the labelled probe with an excess of sonicated, denatured human DNA was included (Litt and White 1985) to reduce background due to repetitive sequences present in this probe. After hybridization for 16 h at 65°C, filters were washed twice at 65°C for 1 h each in 0.4 *M* sodium phosphate buffer, pH 7.2/1% SDS or in 40 m*M* sodium phosphate buffer, pH 7.2/1% SDS, depending on the probe used. Autoradiography was for 48 h at −70°C using Kodak XAR-5 film and Dupont Cronex II or Siemens Titan 2 HS intensifying screens. Autoradiograms were scanned on an LKB Ultrascan XL laser densitometer.

Results

The renewed chromosome analysis of the proband confirmed the 45,X karyotype, while both parents showed a normal karyotype. The possibility of mosaicism was ruled out after scoring 200 metaphases from lymphocytes and 200 from fibroblasts. The study of Xg blood group suggested that the proband's X chromosome was of maternal origin. This was confirmed by Southern blot analysis using the X chromosome-specific probe p782 (DSX85) which reveals two polymorphic EcoRI fragments of 14 kb and 7 kb in size (Hofker et al. 1985). As can be seen from Fig. 1, the proband's mother is heterozygous for this polymorphism, while his father's X chromosome carries the 14 kb allele. Therefore, the proband's X chromosome, which is characterized by the 7 kb allele, must be of maternal origin.

To test for the presence of Y chromosome material in the 45,X male, Southern blot analyses were carried out using the DNA probes pDP34, p47z, and p50f2, all of which detect Y-specific DNA fragments, in addition to X chromosomal or autosomal fragments (Page et al. 1984; Vergnaud et al. 1986). As shown in Fig. 2, the proband's DNA reveals Y-specific fragments with all three probes used. The Y-specific fragments revealed by probes pDP34 and p47z are both located on Yp. Particularly revealing is the result obtained with probe p50f2 (Fig. 2C). In addition to the autosomal fragment, only three of the five Y-specific fragments are present in the 45,X male DNA. The two largest fragments have been assigned to the short arm of the Y chromosome, and the 4.5 kb fragment to the centromeric region. The two fragments of 6.0 kb and 1.7 kb, which are absent in the proband's DNA, are located on Yq (Vergnaud et al. 1986). The conclusion from this analysis is that the 45,X male carries Y chromosome material from most of Yp and from the centromeric region, while at least part of Yq is missing. Note also that the hybridization signals of all bands revealed by these probes are the same in the proband's DNA and in the controls, indicating that the Y chromosome material is present in the normal stoichiometric amount relative to the other chromosomes in this 45,X male.

In situ hybridization was performed first with probe pDP31 (Page et al. 1984), which is homologous to X and Y sequences, in the proband and his father to look for a possible translocation of Y material that could be cytogenetically undetectable. The results of this analysis are summarized in Fig. 3; the distribution of the grains observed in the father (Fig. 3B) indicates the localization on the Y chromosome of the sequence homologous to the pDP31 probe, while in the proband many grains were observed on chromosome 18 (Fig. 3A). The pronounced labelling expected as well at Xq13-q21 in both cases could not be demonstrated because the chromosome preparations were not of sufficient quality; thus, there will have been some confusion among C group chromosomes of similar size.

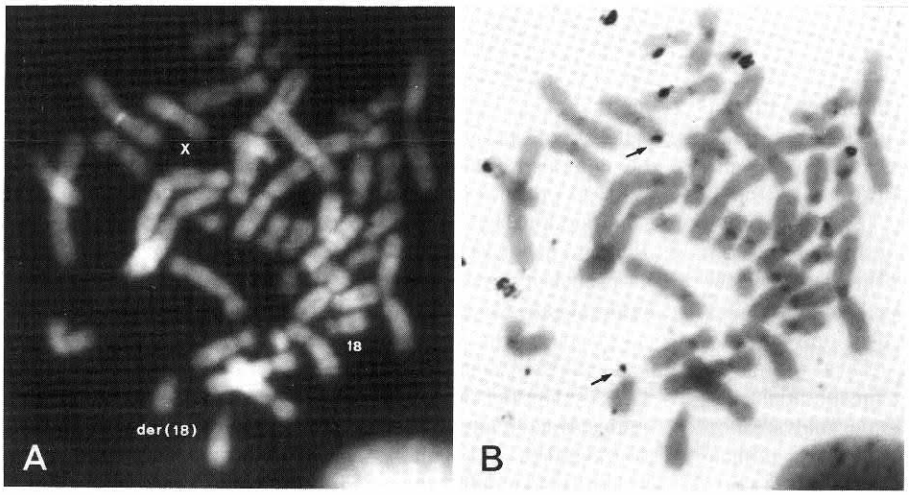

Fig. 4A, B. In situ hybridization with probe 113F in a metaphase spread of the proband. The chromosomes were identified by Q-banding (**A**). Note the silver grain location (**B**) on the distal short arm of chromosome der(18) and X, respectively *(arrows)*

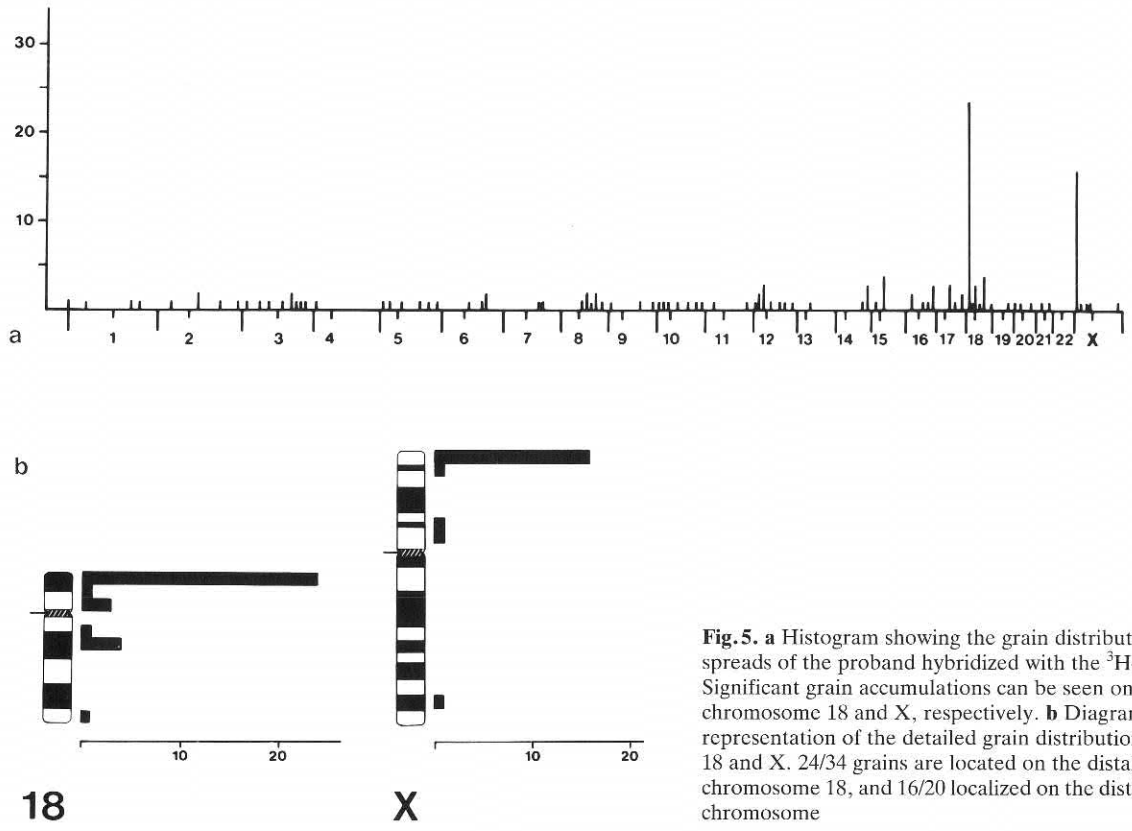

Fig. 5. a Histogram showing the grain distribution in 22 metaphase spreads of the proband hybridized with the ^{3}H-labeled probe 113F. Significant grain accumulations can be seen on the short arms of chromosome 18 and X, respectively. **b** Diagrammatic representation of the detailed grain distribution over chromosomes 18 and X. 24/34 grains are located on the distal short arm of chromosome 18, and 16/20 localized on the distal short arm of the X chromosome

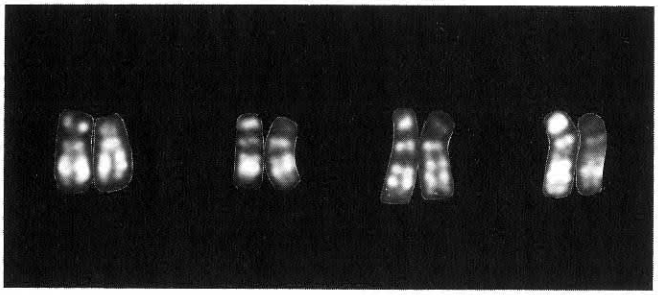

Fig. 6. Four selected chromosome pairs No. 18 of the proband showing early replication patterns (RBA-bands). The der(18) chromosome is placed on the right each. In contrast to the normal chromosome 18, only two weak early replicating bands are seen in the short arm of chromosome der(18)

Analyses of 22 metaphase spreads hybridized with the pseudoautosomal probe 113F (Simmler et al. 1985) revealed that 24% of all silver grains (34/142) were located on chromosome 18, and 14% (20/142) on the X chromosome. Of the 34 silver grains distributed over chromosome 18, 71% (24/34) were localized on the distal short arm, and of the 20 grains observed over the X chromosome, 80% (16/20) occurred in the distal region of the short arm (Figs. 4, 5). We conclude that Y material is translocated onto the short arm of one chromosome 18 in the patient.

An attempt was then made to obtain morphologic evidence of this translocation using chromosome analyses performed on fibroblasts. A detailed study of the early replication pattern (RBA) revealed an altered pattern in one chromosome 18 short arm (Fig. 6). Normally the short arm of chromosome 18 shows two extended very early replicating bands and appears therefore as brightly fluorescent in total. In contrast, the short arm of one of the chromosomes 18 shows only two weak early replicating bands, resembling the early replication pattern of the Y chromosome short arm. Distamycin A/DAPI staining revealed an additional distinct fluorescence in the proximal short arm of the der(18) chromosome, being directly comparable to the Y centromeric distamycin A/DAPI fluorescence intensity (Fig. 7). Thus, the cytogenetic evidence indicates that the patient is monosomic for a portion of 18p and lacks a larger part of the Y-chromosome long arm.

As a corollary to the cytogenetic evidence for 18p monosomy in the 45,X male, the DNAs of the proband and his parents were characterized in Southern blot experiments with a DNA probe from the short arm of chromosome 18. The probe used, B74 (D18S3), has been localized to band 18p11.3 (Mattei et al. 1985) and detects TaqI and MspI RFLPs (Willard et al. 1986). As the family was non-informative for both RFLPs, deletion of one D18S3 allele in the patient's DNA could only be demonstrated by gene dosage. DNA was digested with TaqI to produce clearly separated, nonpolymorphic bands on simultaneous hybridization of the resulting blot with probe B74 and probe pHINS500, an insulin probe from chromosome 11 used as internal standard (Fig. 8). All family members were homozygous for the common 2.8 kb/2.0 kb TaqI allele detected by the insulin probe (Willard et al. 1985). The 1.2 kb band is a constant insulin band not described by Willard et al. (1985). The hybridization signals were quantified by densitometric tracing and measurement of the peak areas. The ratios of the 3.0 kb B74-signal to the 2.8 kb insulin-signal and of the 1.4 kb B74-signal to the 1.2 kb insulin-signal, respectively, for

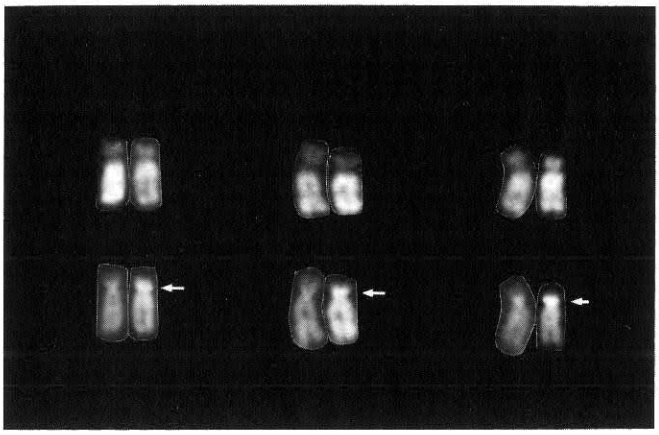

Fig. 7. Distamycin A/DAPI-banded chromosome pairs No. 18 of the proband *(lower row)* after previous identification by Q-banding *(upper row)*. The der(18) chromosome is placed at the right of each pair. *Arrows* indicate the distinct distamycin A/DAPI fluorescence in the proximal short arm of the der(18) chromosome. Note the separate location of the distamycin A/DAPI fluorescence on the sister chromatids, suggesting inactivation of the Y centromere

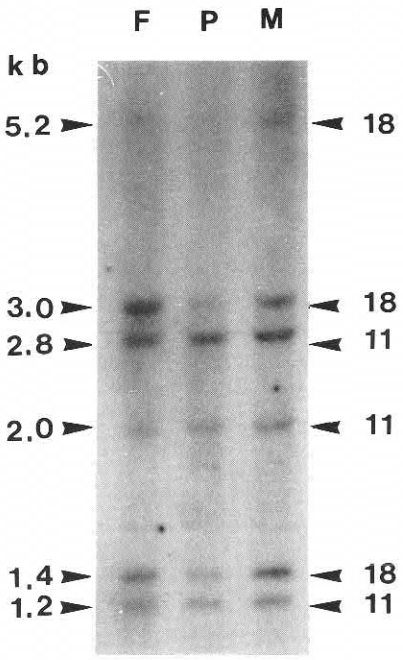

Fig. 8. Gene dosage blot shows 18p monosomy in proband's DNA. TaqI-digested DNA from proband's father (*F*), the proband (*P*), and his mother (*M*) was fractionated on an 0.8% agarose gel, transferred to Gene Screen nylon filter, and hybridized simultaneously with an 18p specific probe, B74, and an 11p-specific probe, pHINS500. The sizes of the resulting fragments are shown on the left and their chromosomal origin on the right

father (F), proband (P), and mother (M) were as follows: F: 1.17:1 and 1.33:1, P: 0.5:1 and 0.82:1, M: 1.14:1 and 1.35:1.

Discussion

The presence of Y-specific DNA in this "XO male" patient was clearly demonstrated by Southern blot analysis. Y-specific signals were obtained with the DNA probes pDP34, p47z, and p50f2. The localization on the Y chromosome of the fragments detected by these probes indicates that at least a larger part of the short arm of the Y chromosome must be hidden somewhere in the genome.

This Y-material was shown to be located on the short arm of one chromosome 18 by in situ hybridization with both pDP31 and the pseudoautosomal probe 113F. Thus, a directed chromosome analysis became possible confirming by replication pattern that the short arm of one chromosome 18 was compatible with the short arm of the Y chromosome, but not with a normal 18p. The cytogenetic evidence of an 18p deletion was confirmed by demonstrating an uniplex dosage for the sequence D18S3 located at 18p11.3. As a consequence of this deletion, the patient should show clinical signs of the 18p− syndrome, and this is indeed the case. As seen by distamycin A/DAPI staining, in addition to the functional centromere of chromosome 18, the Y centromere is present as well. The fact that the Y centromere appears as two separate fluorescent dots on the two chromatids of the short arm of the Y/18 translocation chromosome (Fig. 7) suggests that it is nonfunctional.

In the XO male with a Y/15 translocation described by Schempp et al. (1985), the centromere of the Y was shown to be translocated together with Yp, but has become nonfunctional since the translocation chromosome was functionally monocentric for the No. 15 centromere. Similarly, in the present case the Y/18 translocation chromosome appears to be dicentric structurally, but monocentric functionally with the No. 18 centromere active. With respect to the origin of this translocation, breaks must have occurred proximal in Yq and in 18p, resulting in the dicentric Y/18 chromosome and in an acentric fragment composed of Yq and 18p which was lost.

Interestingly, in the two XO males studied by us, the X chromosome was of maternal origin, and consequently the paternal contribution was a Y/autosome translocation. Principally, however, the paternal transmission of an X/Y translocation must not be excluded in XO males, since nullisomy for the distal Xp is compatible with life (Tiepolo et al. 1977). However, in addition to the paternal translocation, a maternal nondisjunction for the X chromosome would be required in such a case, and since such a coincidence is highly improbable, we can predict that XO males in general will have a maternal X chromosome.

If it can be generalised that XO males originate from Y/autosome translocations, they always should be deficient for a segment of the autosome involved. Depending on the nature of the segment concerned, the deficiency may lead to congenital malformations as in the present case of 18p−, or not as in the case of a Y/15 translocation resulting in 15p− described by Schempp et al. (1985). It may be concluded that for the development of a male phenotype, specific Y-DNA sequences are required. Since the patient is H-Y positive, it follows that the presence of H-Y antigen is independent of most Yq.

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