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Clinical case report

Characterization of a (Y;4) translocation by DNA hybridization

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Summary. A phenotypically normal male with azoospermia was found to have a translocation between the short arm of the Y chromosome and the distal long arm of a chromosome 4. By cytogenetic analysis it could not be determined whether the translocation was reciprocal, nor whether it was balanced. In situ DNA hybridization with two pseudoautosomal and one Y-specific probe demonstrated that the breakpoint was on distal Yp and that there was Y chromosome material on 4q. Thus the translocation was reciprocal and could be characterized as t(Y;4)(pll;q32). There was no evidence for loss of Y-DNA sequences as judged by Southern blotting with Y-DNA probes. Thus the translocation may be balanced. We conclude that DNA hybridization can be used to refine considerably the cytogenetic analysis of such translocations.

Introduction

Cytogenetic studies have, at best, resolution limits of half a band. Usually the limits of interpretation are one or two bands. Thus deletions, translocations, and other structural abnormalities are difficult to detect when the changes are small. Even with high-resolution banding, subtle structural abnormalities may cause problems of definition if they occur in regions with uncharacteristic banding (Francke 1981). Such is the case in most telomeric regions of the human chromosomes which tend to be uniformly light on G-banding, dark on R-banding, and dull on Q-banding (ISCN 1985). When at least one breakpoint in a translocation is very close to the telomere, it is usually impossible to determine whether the translocation is reciprocal or not.

There is ample evidence that most balanced autosomal translocations are compatible with fertility, though they may lead to reduced fertility, especially in males, because of prezygotic selection of unbalanced gametes (Jacobs et al. 1975; Evans et al. 1978; Chandley et al. 1986). In contrast, most de novo Y;autosome translocations appear to lead to testicular maldevelopment and sterility (Smith et al. 1979; Fryns et al. 1985). The mechanism by which these phenotypic features arise is not well understood.

The present study of a sterile male with a (Y;4) translocation was undertaken in an attempt to answer the following questions. (1) Is the translocation reciprocal? (2) Is it balanced? (3) Do the DNA hybridization findings explain the patient's phenotype?

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Materials and methods

The patient

A healthy 32-year-old male was investigated because of childlessness after 4 years of marriage. He was the third child of healthy, nonconsanguineous parents. His three brothers were healthy and fertile. The patient was 184 cm tall. His general appearance, external genitalia, and secondary sex characteristics were all normal. After a semen analysis revealed azoospermia, a testicular biopsy was performed. Histologically, the tubules were of normal size and number but contained only Sertoli cells. No germ cells were seen. The interstitial tissue was normal. Thus the patient's features were fully compatible with the "Sertoli cell only" or del Castillo syndrome. He declined further investigation and did not wish members of his family to be studied.

Cytogenetic studies and in situ hybridization

Metaphases for cytogenetic studies and in situ hybridization experiments were obtained form Epstein Barr virus-transformed lymphoblastoid cell cultures. In situ hybridization with ^{3}H-labeled probes was done according to a method described previously (Andersson et al. 1986). The probes were labeled to specific activities of 1.5×10^7 cpm/μg (pDP230 and p708) and 9×10^6 cpm/μg (pDP105) and used at concentrations of 30–40 ng/ml. Slides were developed after 6–26 days of exposure, and metaphases were stained with 0.25% Wright's stain and photographed. To obtain better banding of chromosomes, the slides were destained by immersing them in a series consisting of 95% ethanol; then 95% ethanol, 1% HCl; and finally methanol. They were then treated with 0.03% trypsin in 0.012% EDTA-Hanks solution for 2–4 min (Popescu et al. 1985) and restained for G bands with Wright's stain.

Hybridization probes for in situ hybridization

Probe pDP230 (DXYS20) (Page et al., in press) detects homologous, pseudoautosomal sequences on Yp and Xp22.3-pter in normal human males. Probe p708 (DXYZ2) (Simmler et al. 1985) also detects homologous, pseudoautosomal sequences on both Yp and Xp22.3–pter. Probe pDP105 (DYZ4) (D. C. Page, unpublished) defines multiple Y-specific loci. In normal males, probe pDP105 hybridizes in situ to Yp and to a lesser extent to Yq (Andersson et al. 1986).

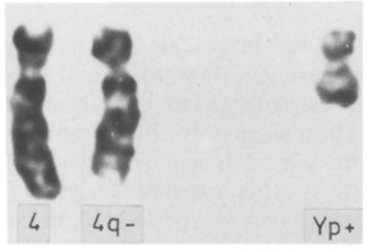
Southern hybridization

DNA extraction from lymphoblastoid cells, restriction enzyme digestion, electrophoresis, transfer, and hybridization of DNA were performed as previously described (Page and de la Chapelle 1984). DNA probes detecting Y-specific restriction fragments used in Southern blotting experiments are listed in Table 2.

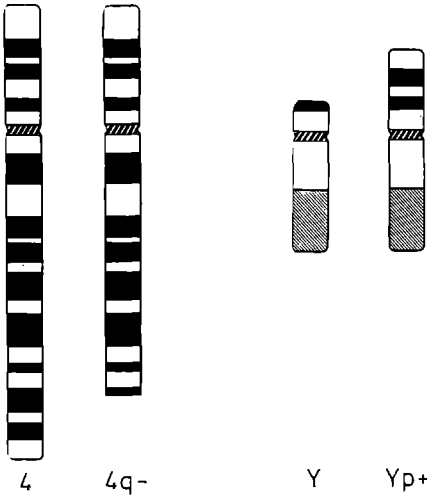
Results

Cytogenetic findings

There was an apparent translocation between the short arm of the Y chromosome and the long arm of chromosome 4. As judged by G- and Q-banding, the long arm of the Y was normal while the short arm of the Y was approximately 2.5 times longer than normal. Bands q32–35 appeared to be missing from one chromosome 4, and the extra material on Yp was compatible with being 4q32–35 (Fig. 1a and b). The breakpoint in band 4q32 might be tentatively assigned to q32.2. Thus the translocation could be described as a t(Y;4)(p11;q32.2). Detailed cytogenetic inspection did not yield any clues as to the exact breakpoint on Yp. Similarly, close inspection of the abnormal 4q did not reveal whether Yp material was present or absent. Thus it could not be determined whether the translocation was truly balanced and whether it was reciprocal. All other chromosomes appeared normal.



a



b

Fig 1. **a** G-banded translocation chromosomes and the normal chromosome 4 of the t(Y;4) patient; **b** diagram of the translocation showing normal and abnormal chromosomes 4 and the abnormal Y chromosome. A normal Y is also shown

In situ hybridization

To assess the breakpoint on Yp and whether Yp material was present on the 4q− chromosome, we hybridized two pseudoautosomal probes (pDP230 and p708) and one Y-specific probe (pDP105) to metaphase chromosomes in situ (Table 1). With both pseudoautosomal probes, the terminal part of the long arm of the 4q− chromosome was heavily labeled (Fig. 2a and b). The Yp+ chromosome showed no labeling with pDP230 and very few grains with p708. With Y-specific probe pDP105 the short arm of the Yp+ chromosome was heavily labeled while no other chromosome was significantly labeled (Fig. 2c). From these results we conclude that (1) the translocation breakpoint in Yp is located between the pseudoautosomal loci (detected by pDP230 and p708) and the short-arm locus defined by pDP105 (interval 3, D.C.P, unpublished) and (2) the translocation is reciprocal, with the most distal end of Yp being translocated to the end of the long arm of the 4q− chromosome.

Southern hybridization

Hybridization to Southern blots of genomic DNA did not reveal the absence of any Y-DNA sequences. We tested for all eight deletion intervals on the Y chromosome (Table 2). All Y-DNA sequences for which we tested were found to be present. This is compatible with the translocation being balanced. However, we cannot exclude the possibility of deletion of Y-DNA sequences for which we did not test.

Discussion

Cytogenetic and DNA hybridization studies showed that the t(Y;4) patient had a terminal and reciprocal translocation between Yp and 4q. In situ hybridization studies confirmed the breakpoint to be in Yp, so that all sequences homologous to pDP230 are translocated to the 4q− chromosome while some p708 sequences may remain on the Yp+ chromosome. The Yp breakpoint thus seems to be located within or immediately proximal to the pseudoautosomal region. The translocated Y

Table 1. Grain counts on the metaphases from the t(Y;4) patient after in situ hybridization of ^{3}H-labeled probes

Probe	No. of mitoses	No. of cells with grains on chromosomes		No. of grains on chromosomes		
		Yp+ (%)	4q− (%)	Total	Yp+ (%)	4q− (%)
pDP230	50	3 (6)	38 (76)	349	4 (1)	65 (19)
p708	62	10 (16)	29 (47)	383	12 (3)	39 (10)
pDP105	63	49 (78)	7 (11)	191	34 (35)	7 (4)

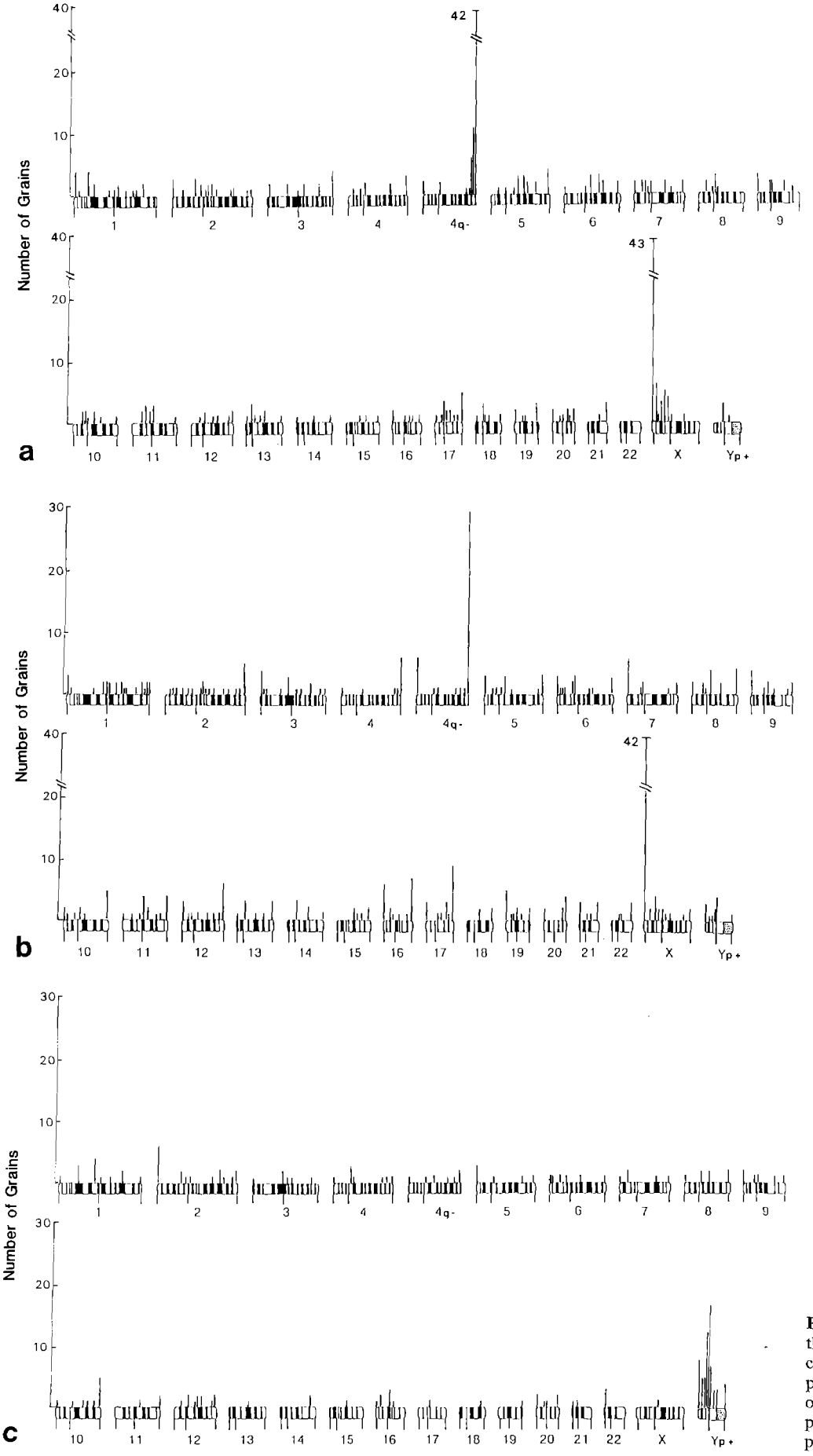


Fig. 2a–c. Histograms showing the localization of grains on the chromosomes of the t(Y;4) patient after in situ hybridization of ^{3}H-labeled probes: **a** probe pDP230, **b** probe p708, **c** probe pDP105

Table 2. Southern hybridization of Y-DNA probes to genomic DNAs from the t(Y;4) patient and normal males and females

Interval[a]	Probe/locus	Enzyme	Stringency[b]	Reference	Presence (+) or absence (−) of Y-specific restriction fragment		
					Patient	Normal males	Normal females
1	pDP132	TaqI	H	D. C. Page, unpublished	+	+	−
2	pDP61	TaqI	H	D. C. Page, unpublished[c]	+	+	−
3	50f2/A,B	EcoRI	M	Guellaen et al. (1984)	+	+	−
3	pDP105/A	TaqI	M	D. C. Page, unpublished	+	+	−
4A	pDP34	TaqI	H	Page and de la Chapelle. (1984)	+	+	−
4B	pDP97	EcoRI	H	D. C. Page, unpublished[d]	+	+	−
4B	50f2/D	EcoRI	M	Guellaen et al. (1984)	+	+	−
5	12f	TaqI	H	Bishop et al. (1984)	+	+	−
6	50f2/C,E	EcoRI	M	Guellaen et al. (1984)	+	+	−
6	pDP105/B	TaqI	M	D. C. Page, unpublished	+	+	−
7	pY431-HinfA	TaqI	M	K. Smith, unpublished	+	+	−

[a] Deletion intervals on the Y chromosome are as described by Vergnaud et al. (1986) and Page (1986)
[b] H, High stringency (hybridization at 47°C, wash at 65°C); M, medium stringency (hybridization at 42°C, wash at 55°C)
[c] Derived from plasmid 115 (Geldwerth et al. 1985)
[d] Derived from cosmid Y97 (Wolfe et al. 1985)

material on the 4q− chromosome then consists of the terminal part of Yp including pseudoautosomal sequences, but we cannot determine if other Y-specific material is translocated too. Since interval 3 on Yp is still left on the Yp+ chromosome, we can conclude that the breakpoint is on distal Yp and the portion translocated is physically small, which is compatible with the absence of any cytogenetially visible part of Y on the 4q− chromosome.

Sterility is frequently observed in males with de novo Y;autosome translocations (Fryns et al. 1985), but its causes are not well understood. In considering the etiology of our patient's sterility, rearrangements or abnormalities of two Y chromosomal genes should be taken into account. The testis-determining gene, *TDF,* is localized proximal to the pseudoautosomal region (Vergnaud et al. 1986) and thus could be affected by the translocation. However, the role of *TDF* is believed to be that of primary testis determination, since loss of *TDF* leads to the absence of testes (e.g., XY gonadal dysgenesis females; Page 1986; Disteche et al. 1986). Though it presently cannot be determined whether *TDF* is translocated to chromosome 4, involvement of *TDF* in the patient's sterility seems unlikely. Another gene, *AZF* (azoospermia factor) has been localized to Yqll based on azoospermia in males with deletions of Yq (Tiepolo and Zuffardi 1976). The testicular histology of our patient does not resemble that described by Tiepolo and Zuffardi. Furthermore, as the translocation clearly affects Yp, and not Yq, and as our Southern analysis suggests that Yq is intact, direct involvement of *AZF* seems unlikely.

In the present patient, the translocation could lead to the formation of a tetravalent at meiosis I; a breakdown of the meiotic process could result. However, the histology showed total absence of germ cells, suggesting a defect in the germ line prior to meiosis.

We conclude that DNA hybridization analysis can be used to inprove considerably the accuracy with which chromosome abnormalities are described. With the rapidly increasing number of mapped and characterized DNA probes available (Human Gene Mapping 8 1985), there will eventually be probes that can be used to help characterize abnormalities in almost all parts of the chromosomes. These developments should lead to the establishment of much more precise clinical-cytogenetic correlations than before.

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