CYTOGENETIC AND MOLECULAR INVESTIGATIONS OF AN ABNORMAL Y CHROMOSOME : EVIDENCE FOR A PSEUDO-DICENTRIC (Yq) ISOCHROMOSOME

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SAVARY J.B., VASSEUR F., FLACTIF M., WILLATT L., LEFEBVRE J., FERGUSON-SMITH M.A., DEMINATTI M.M. — Cytogenetic and molecular investigations of an abnormal Y chromosome : evidence for a pseudo-dicentric (Yq) isochromosome.

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SUMMARY : A derivative Y chromosome was found in a 55-year-old man with Lambert-Eaton paraneoplasic pseudomyastheniform disease. Small testicles, azoospermia were noticed and hormonal level values were as in the Klinefelter syndrome. A 45,X/46,XYp+ mosaïcism was described on peripheral blood lymphocytes. Cytogenetic investigations with R-G-C- and Q-banding have been performed. *In situ* hybridization with the GMGY 10 DNA probe showed two copies of proximal Yp sequences. Southern blot analyses were performed using the Y DNA probes 27a, 47z, 64a7, 50f2 disclosing specific Yp and Yq sequences from the pseudoautosomal boundary to the Yq proximal portion. The der(Y) has been defined as a dicentric isochromosome for the long arm with one active and one apparently suppressed centromere. The breakpoint leading to the der(Y), has been loca-

(Summary continued on next page)

SAVARY J.B., VASSEUR F., FLACTIF M., WILLATT L., LEFEBVRE J., FERGUSON-SMITH M.A., DEMINATTI M.M. — Investigations cytogénétiques et moléculaires d'un chromosome Y anormal (derY), arguments en faveur d'un isochromosome (Yq) pseudo-dicentrique. *(En anglais)*

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RÉSUMÉ : Un chromosome Y anormal a été mis en évidence chez un patient de 55 ans présentant un syndrome paranéoplasique, pseudomyasthéniforme de Lambert-Eaton. Le patient présentait des testicules de petite taille, et une azoospermie. Les dosages hormonaux ont donné des valeurs comparables à celles rencontrées dans le syndrome de Klinefelter une mosaïque 45,X/46,XYp+ a été mise en évidence dans les lymphocytes sanguins périphériques. Les investigations cytogénétiques ont été effectuées au moyen des marquages chromosomiques R-G-C et Q. L'hybridation *in situ* au moyen de la sonde GMGY 10 a montré deux copies des séquences Yp proximales. L'analyse par Southern-blot utilisant les sondes 27a, 47z, 64a7 et 50f2, a mis en évidence la présence de séquences spécifiques du chromosome Y, depuis la région juxta-pseudoautosomale jusque

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(Summary continued and end)

ted in the pairing segment of the Y short arm (i.e. Yp11.32). So the der(Y) was interpreted as a psu dic(Y) (qter→cen→p11.32 ::p11.32 →qter). There was thus an almost complete duplication of the Y chromosome.

KEY-WORDS : Dicentric Y chromosome. — *in situ* hybridization. — DNA probe. — Southern-blot.

(Résumé suite et fin)

la partie Yq proximale. Ce chromosome der (Y) a été interprété comme un isochromosome des bras longs, dicentrique avec un centromère actif et un centromère inactivé. Le point de cassure qui a donné naissance à ce chromosome se situerait dans la région pseudoautosomale Yp11.32. Ce chromosome der(Y) peut s'interpréter comme un psu dic(Y)(qter→cen→ p11.32::p 11.32→qter). La duplication du chromosome Y étant quasi complète.

MOTS-CLÉS : Chromosome Y dicentrique. — Hybridation *in situ*. — Sonde d'ADN. — Southern blot.

INTRODUCTION

With rare exceptions the male phenotype is triggered by the presence of a Y chromosome which carries the genetic factors responsible for the determination of the male sex (Ohno, 1976 ; Ford, 1970 ; Hamerton, 1971). The study of structural anomalies of Y including male determination disturbances (Davies, 1981), advances in immunology (Watchel et al., 1975 ; Mc Laren et al., 1984 ; Simpson et al., 1987) and molecular genetics (Cooke et al., 1985 ; Buckle et al., 1985 ; Rouyer et al., 1986 ; Affara et al., 1987 ; Ferguson-Smith et al., 1987 ; Page et al., 1987) have located the testis determining factors (TDF) controlling sex determination to the short arm of the Y chromosome. These studies defined an X and Y homologous region at the tip of short arms which has been called the pairing segment or pseudoautosomal region and can be exchanged during male meiosis. The testis determining region of the Y chromosome has been located adjacent to the pseudoautosomal boundary (PABY). Two candidate genes for testicular determination were identified by deletion mapping analyses : ZFY (Page et al. 1987) and SRY (Sinclair et al. 1990 ; Berta et al. 1990), and both have been located in the sex determining region. ZFY has been excluded as the TDF candidate (Palmer et al., 1989 ; Page et al. 1990) and experiments with transgenic mice (Koopman et al. 1991) made SRY the nearly indisputable candidate for TDF.

Among the structural rearrangements altering Y chromosome morphology, dicentric Y chromosomes appear the most frequently encountered (Cohen et al. 1973). Fine analysis and compilation of such aberrations are of great interest because karyotype-phenotype correlations can help to determine the location of genes. In this paper we report a new case of dicentric (Yq) isochromosome with a duplicated pericentromeric portion of the Y short arm confirmed by *in situ* hybridization with the DNA probe GMGY 10. From cytogenetic and molecular investigations it seems likely that the breakpoints are within the pseudoautosomal region and that there is thus an almost complete duplication of the Y chromosome.

MATERIAL AND METHODS

Case report

Guy D. a 55-year-old man was affected with a Lambert-Eaton syndrome. This paraneoplasic pseudomyastheniform disease often results from a bronchial malignant growth but clinical radiological and fibroscopical investigations did not show any evidence of neoplasm. A bilateral asymetrical soft goitre with neither adenopathies nor compressive symptoms was revealed by thyroid examination, yet the contingency of malignant thyroid growth was discarded. The patient was 177 cm in height and weighed 72 kg. The phenotype was male with firm, insensitive and small testicles (2 cm × 1.5 cm × 1.5 cm). The gonadal surface was uniform. There was no gynecomastia. There was no evidence of a lack of virility apart from a sexual asthenia. Both hair distribution and mental development were normal. Twenty years ago a semen analysis showed azoospermia. Serum hormonal levels were consistent with primary testicular dysgenesis : testosterone was decreased with 2.45 ng/ml (N is 3.6-9.0) FSH was greatly in-

creased 15 ng/ml (N is 0.9-3.7) and LH (3 ng./ml) was in the normal range (N is 1.3-3.9).

Cytogenetic procedures

Sex chromatin determination was performed on buccal smears. Chromosome preparations from cultures of peripheral blood lymphocytes were made by conventional techniques. Chromosome identification was achieved by GTG, QFQ and CBG banding. Dynamic banding (RBG-GBG) with 5-BrdU incorporation during part of the last S-phase before harvesting was also used (Dutrillaux & Couturier, 1981).

In situ-hybridization

In situ hybridization was accomplished with a moderately-repetitive DNA segment probe GMGY10 assigned to the Ypter-Yp11 region (Affara et al., 1986). The probe was biotinylated and detected by peroxydase coupled to avidin using reflectance-contrast optics.

Southern blot analysis

DNA was extracted from peripheral blood leukocytes, digested with appropriated restriction endonucleases, alkali blotted onto nylon membranes and hydridized with 32P oligo-labelled probes, as previously described (Vasseur et al. 1988). Probes 47z (Geldwerth et al. 1985), 27a (Pritchard et al. 1987), 64a7 (Guellaen et al. 1984) 50f2 (Vergnaud et al. 1986) were kindly provided by J. Weissenbach (Institut Pasteur Paris).

RESULTS

Sex-chromatin patterns were compatible with the phenotypic sex. The patient showed an X-chromatin negative and a Y-chromatin positive buccal smear with a large fluorescent corpuscle. Some cells exhibited a fluorescent Y duplex (fig. 1). Cytogenetic results were obtained from 165 banded metaphase plates. An abnormal Yp+ chromosome der(Y) was noticed. As shown in table I the patient displayed a 45,X/46,X der (Y) mosaïcism with an excess of the der(Y) cell line (89.1 %) as compared with the 45,X cell line (9.7 %). One cell 47,X der(Y) der(Y) and one cell with a ring der(Y) chromosome were also observed. The banding appearances of the der(Y) are presented in figure 2. The abnormal Y chromosome exhibited a symetrical Yq pattern with GTG, RBG and QFQ bandings (fig. 2a-b-c). CBG and GBG bandings displayed two parts of apparently unequal length (fig. 2d-e). With Q-banding procedures both ends of the der(Y) were strongly fluorescent. Likewise tips of CBG banded der(Y) displayed intensively stained heterochromatic segments. Dynamic bandings showed that ends of the chromosome marker were late replicating : faintly stained with RBG banding and darkly stained with GBG banding (fig. 2b-e). Moreover the tips of the der(Y) exhibited the typical Yq lateral asymmetry (fig. 2b) with RBG banding (Verma, 1988). With CBG and GBG procedures, the centromeric area exhibited banding patterns of a dicentric chromosome.

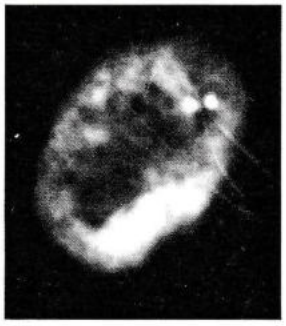

Fig. 1. — Chromatin buccal smear with fluorescent Y duplex.

TABLE I. — **Karyotype distribution in lymphocytes.**

Karyotypes	no. of cells	percentage
45X	6	9.7
46X der(Y)	147	89.1
46X r der(Y)	1	0.6
47X der(Y) der(Y)	1	0.6
total	**165**	**100**

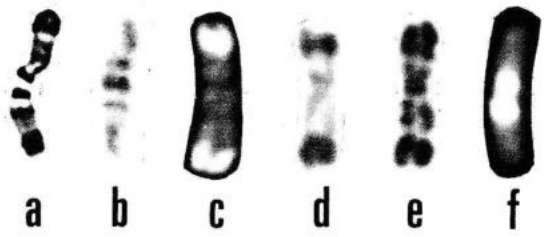

Fig. 2. — Cytogenetic appearances of the der(Y) chromosome with GTG (a) RBG (b) QFQ (c) CBG (d) and GBG (e) banding. *In situ* hybridization of the der(Y) with the GMGY10 DNA probe (f).

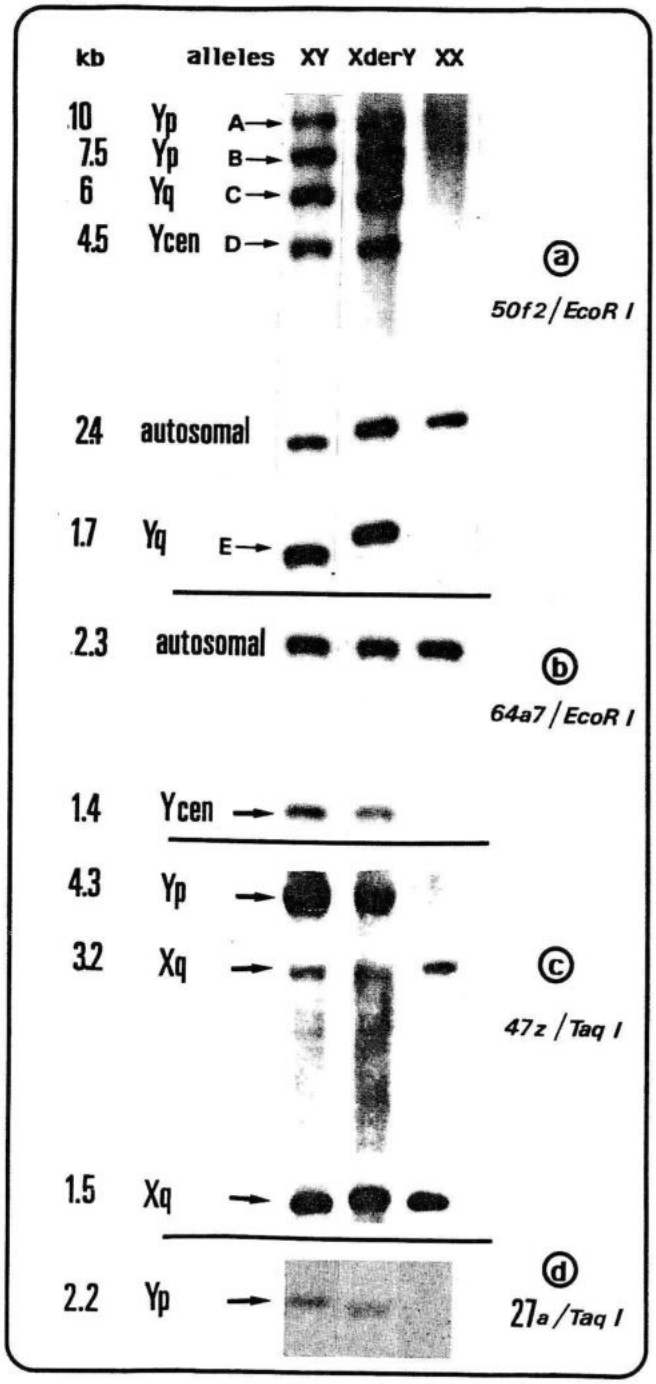

Fig. 3. — Southern-blot analyses of DNA from a normal XY male (lane 1), the der (Y) patient (lane 2) and a normal XX female (lane 3). DNA were restricted with either EcoRI or TaqI and probed with 50f2 (a), 64a7 (b), 47z (c) and 27a (d).

In situ hybridization results with the GMGY10 DNA probe showed two copies of proximal Yp sequences separated by a non-hybridizing region (fig. 2f).

DNA from the patient was investigated with probes disclosing Y sequences in the proximal Yq, in the pericentromeric area, and from the Yp11 segment to the pseudoautosomal boundary. EcoRI digested DNA probed with 50f2 (fig. 3-a) revealed the specific proximal Yq 6kb (50f2-C), 1,7kb (50f2-E) alleles, the Y pericentromeric 4,5kb (50f2-D) allele and the Yp 10kb (50f2-A), 7,5kb (50f2-B) alleles. These specific Y sequences were present in normal male (lane 1), in the patient (lane 2) and absent in normal female (lane 3). A non Y specific autosomal 2,4kb fragment is noticed in every subject. Likewise, EcoRI digested DNA probed with 64a7 (fig3-b) revealed the specific pericentromeric 1,4kb fragment in normal male (lane 1), in the patient (lane 2). This 1,4kb fragment was absent in normal female (lane 3). TaqI digested DNA probed with 47z (fig3-c), revealed the Yp11 specific 4,3kb allele in normal male (lane 1),in the patient (lane 2). This fragment is absent in normal female (lane 3). The specific Xq 3,2kb and 1,5kb alleles were present in every subject. TaqI digested DNA probed with 27a (fig3-d) disclosed the specific distal 2,2kb allele located in the Yp11.3 pseudoautosomal boundary, in normal male (lane 1), in the patient (lane 2). This fragment is absent in normal female (lane 3).

DISCUSSION

Cytogenetic analysis of our patient showed a complex karyotype with a structural aberration in addition to a 45,X mosaïcism. This observation raises two questions : the nature of this abnormal Y chromosome and the phenotype-karyotype correlation.

Results with QFQ, CBG and RBG bandings gave good evidence for the duplication of the Yq12 portion. This was supported by the tips of the der(Y) : brightly fluorescent with QFQ, and intensively stained with CBG banding. Moreover, the tips were late replicating with dynamic RBG and GBG bandings, and presented the classical lateral asymetry with RBG procedure. These data strongly support at least a complete duplication of the Yq heterochromatic segment. A symetrical Yq banding pattern obtained with QFQ, RBG and GTG suggested an Yq isochromosome. Nevertheless CBG and GBG bandings revealed two parts of apparently unequal length on the der(Y), with an asymmetrical banding around the putative centromeric area. This result should point out a dicentric chromosome with one active centromere and one apparently suppressed centromere.

In situ hybridization with the GMGY10 DNA probe exhibiting two copies of proximal

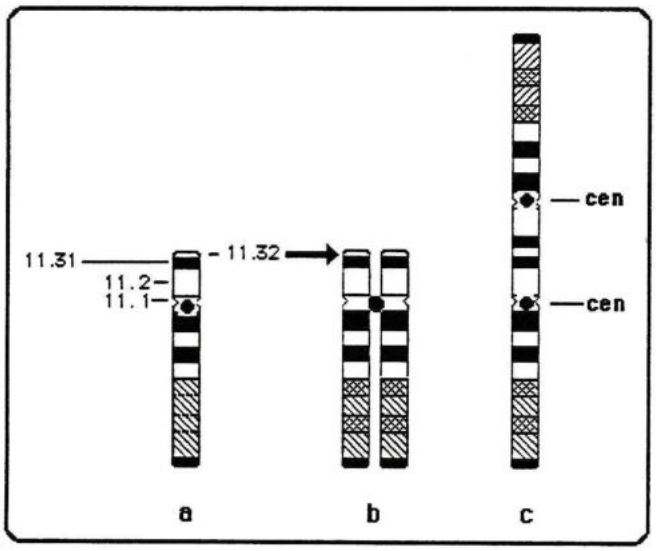

Fig. 4. — G-banded idiogram of the Y chromosome according to Magenis et al. (1985). Explanation of theoretical model for psu dic(Y)(qter→cen→p11.32::p11.32→qter). Normal Y chromosome (a) replicated normal Y chromosome in meiosis I (b) with breakpoint in Yp11.32 (arrow) and fusion of chromatid ends leading to a dicentric chromosome with entirely Yq and partial Yp duplication (c) cen are centromeres.

Yq sequences separated by a non-hybridizing region, made evidence for the duplication of pericentromeric sequences of Yp and corroborated a dicentric chromosome.

Molecular analyses showed the presence of specific Y DNA markers on the der (Y), specially the proximal Yq (50f2-C, 50f2-E), the pericentrometric (64a7, 50f2-D), the Yp11 (50f2-A, 50f2-B, 47z), and the pseudoautosomal boundary Yp11.3 (27a) sequences. The quantitative analysis of Southern blots was uninformative probably because of the presence of the 45,X cell line. Thus the der(Y) contained in at least one dose, Y DNA sequences, from the pseudoautosomal boundary Yp11.32 to the proximal Yq, and specially TDF located in the Yp11.3 region (Vergnaud et al. 1986 ; Disteche et al., 1986 ; Page et al., 1987). This ruled out the der(Y) as a monocentric Yq isochromosome because cases of 46,X i(Yq) have a duplicated Yq segment without Yp. They are phenotypically female without masculinization (Jacobs & Ross, 1966 ; Davis, 1981). The male phenotype of the patient, cytogenetic data (duplication of Yq12, dicentric appearance with CBG and GBG banding), duplication of pericentromeric sequences revealed by *in situ* hybridization, and molecular analyses showing the presence of Y sequences from the proximal Yq to the pseudoautosomal boundary, are in agreement with this der(Y) as being a dicentric Yq isochromosome (fig. 4). Moreover, the asymmetrical patterns with GBG banding together with *in situ* hybridization allow to point out the presence of one active centromere and one apparently suppressed one.

Compilation of Y structural abnormalities are of great interest because karyotype-phenotype correlations can help to determine the location of genes. Yq isochromosomes are usually associated with female sexual differentiation, masculinization in patients with a dicentric Yq isochromosome have also been described (Ferguson-Smith et al., 1969 ; Magenis et al., 1985 ; Ferguson-Smith et al., 1987). It seems likely that in masculinized patients the breakpoint in Yp lies distal to TDF, while in those whose sexual differentiation is female the breakpoint lies proximal to TDF. The male phenotype of our patient is a good evidence for the presence of TDF. The phenotypic changes related with this der(Y) remained difficult to state. Out patient was a mosaic with a 45,X cell line and it was impossible to determine the extend of the effect of the mosaïcism on the phenotype. The 45,X cell line may be related with the abnormal Y chromosome instability previously reported by Cohen et al. (1973). A similar model could explain the 47,X der(Y) der(Y) cell and the 46,X der(Y)r one.

The origin of the aberrant Y chromosome in the paternal germ cells remains unknown. However the simplest explanation would be a Yp breakage followed by fusion of chromatid ends in meiosis I leading to a dicentric chromosome with entire Yq and partial Yp duplication. This model suggests a variable Ypter deletion according to the location of the breakpoint. In our patient, the presence of DNA sequences from the pseudoautosomal boundary locates the breakpoint, distal to the DYS104 locus defined by probe 27a. As shown in figure 4 there is thus an almost complete duplication of the Y chromosome without any significant loss of Y material. Thus the der(Y) chromosome has been interpreted as a psu dic(Y)(qter→cen→p11.32::p11.32→qter) according to the international Cytogenetic Nomenclature (ISCN,1985).

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