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Physical mapping of an Xq-proximal interstitial duplication in a male

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Summary. A viable duplication in the proximal long arm of the X chromosome in a boy with a malformative syndrome was delineated with molecular biology techniques using 14 probes from the X cen-Xq21 region. This analysis allowed us to refine the physical map of the X cen-Xq13 region.

Introduction

Several duplications involving the proximal part of the long arm of the human X chromosome have been described in the literature (Steinbach et al. 1980; Vejerslev et al. 1985; Cremers et al. 1987, 1988; Thode et al. 1988). Except for the work by Cremers et al. (1987, 1988), none were mapped using molecular biology techniques.

Clinically complex malformative syndromes may be explained by chromosomal rearrangements (mainly deletions and duplications) encompassing several genes. Thus, it is of great interest to map the boundaries of such disorders at the molecular level, in order to define several intervals that can be associated with a particular indication of these complex syndromes. A good example of such an approach is reported by Cremers et al. (1989) using a series of deletions located in Xq21 and their association (or lack of association) with clinical signs of choroideremia, mental retardation and deafness. Moreover, this kind of approach may help in the physical mapping of the proximal region of the long arm of the X chromosome.

In this work, we describe the molecular mapping of a duplication involving the Xq12-Xq13 region. The boundaries of this chromosomal rearrangement were precisely delineated. A comparison with the other duplications mapped in this region is presented.

Materials and methods

Case report

The male proband is the second child of a 32-year-old mother and a 34-year-old father, both of whom are healthy and unrelated. The child exhibited the following anomalies: growth retardation (birth weight: 2600 g, length: 45 cm), relative macrocephaly (head circumference 36 cm), and hypotonia. Mental retardation was obvious with no walking and no speech.

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Cytogenetic studies

Routine and high-resolution chromosome analysis were performed on phytohemagglutinin stimulated blood cultures. For high resolution studies, lymphocytes were synchronized by blocking with amethopterin (Sigma no C770, $10^{-7}M$). Cells were released from the block by washing and culturing in a medium containing thymidine as described by Yunis et al. (1978). The banding technique was modified by using mild heat denaturation of the preparations to obtain RHG bands. Another culture was carried out with the addition of 5-bromodeoxyuridine (BrdU) in the last 7 h of culture, followed by fluorochrome-photolysis-Giemsa technique (FPG technique), giving R-banding.

DNA markers

We used 16 probes previously mapped on the X chromosome long arm (Chance et al. 1983; Goodfellow et al. 1985; Riddell et al. 1986; Cremers et al. 1988, 1989; Schwartz et al. 1988; Yang et al. 1989). These DNA probes, kindly provided by Drs. Hors-Cayla (U12, Paris) and Yang (CIML, Marseille), are presented in Table 1.

Southern blot analysis

DNA from peripheral blood was isolated, according to Aldridge et al. (1984), from the propositus (S.P.), his father (J.P.) and his mother (C.P.). Southern blot analysis and signal density scanning were performed essentially as described by Cremers et al. (1987, 1988) except that DNA was immobilized on Hybond N (Amersham) after UV-crosslinking using a Stratalinker (Stratagene). Densitometry of the autoradiogram was performed using an image analysis system (Samba).

Results and discussion

Routine R-banding revealed a normal Y but an abnormal X chromosome, with the long arm being slightly longer than normal in the karyotype of the proband. The C-banding technique showed only one C-band corresponding to the centromeric heterochromatin.

High resolution chromosome analysis confirmed the initial observation: extra chromosomal material in the

proximal X long arm was consistent with a duplication of the Xq13 positive-R-band (Fig. 1). BrdU incorporation in the extra material was similar to that in the Xq13 band, as expected in a duplicated segment. Therefore, the karyotype of the proband was interpreted as 46,Y,dup(X)(q13); his parents had a normal karyotype.

We decided to use Southern blot analysis, using 16 molecular probes 1) to confirm the cytological observations, 2) physically to map the extent of the putative duplication, 3) to provide additional information about the fine location of the probe. Representative results, obtained with the probes described in Table 1, showed that the signal intensities were doubled in S.P. compared with his father, J.P., confirming the cytogenetical observations (Fig. 2).

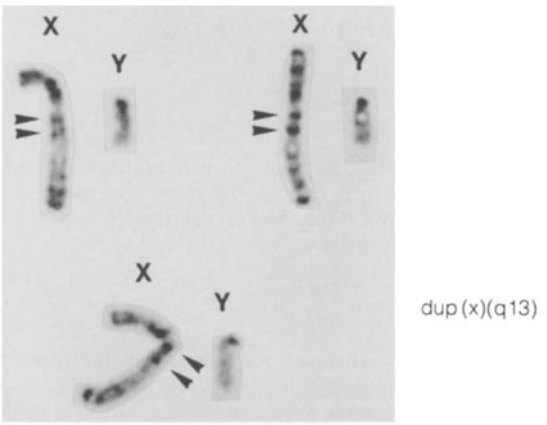

Fig. 1. RHG-banded X and Y chromosomes of the patient S.P. *Arrowheads* indicate the duplicated bands

Table 1. Representation of the extent of S.P.'s duplication, delineated using Southern blots. Probes were provided by M.C. Hors-Cayla and H.M. Yang. The intervals (represented by lines) have been determined by Cremers et al. (1988); the broken line represents a new interval defined by the duplication described in this paper. Quantitation of the signal intensity was as described in materials and methods

Locus	Name	HGM 10 (1989) location on the X chromosome	No. of copies
DXS136	pX12g	q11–q12	1
DXS1	p8	q11.2–q12	2
DXS61	St72	q12–q13-1	2
PGK1p1	pHPGK-7e	q12	2
PGK1p1	825	q12	2
DXS162	cpX73	q13	2
DXS55	St10	q13	2
DXS56	pL2.98	q13	2
PGK1	pHPGK-7e	q13	2
DXS169	pX104	q21.1	2
DXS72	pX65H7	q21.1	2
DXS95	pXG-7	q21.2–q21.3	1
DXS3	p19.2	q21.3	1
DXYS1	pDp34	q21.3	1
DXYS8	p115i	q13–q22	1
DXS17	S21	q22	1
F9	p1	q26.3–q27.1	1

The intensity of the signal given by probe DXS1 was the same in the mother and the affected boy. Conversely, with DXS 136, the signal intensity was the same in the boy and his father, but doubled in the mother. Thus, as these two probes were located in the same interval of the proximal long arm of the X chromosome (Cremers et al. 1988), we concluded that the proximal boundary of the duplication mapped between these two probes. These results were confirmed by densitometric screening of the autoradiographs (data not shown). Similar results obtained with several probes, recognizing altogether 16 loci, are presented in Table 1. Comparing our findings with the physical localization of the probes presented by Cremers et al. (1988), Schwartz et al. (1988) and Yang et al. (1989), we concluded that the duplication in our proband started between DXS136 and DXS1. The distal breakpoint mapped between DXS72 and DXS95, probably at the interface of band Xq13.3 and Xq21.1.

As regards the physical mapping of probes previously located in this region, S.P.'s duplication allowed us to map the locus DXS136 more proximally that DXS1. In addition, these results gave us interesting data on the location of probe DXS55, confirming its previous localization (Xq13). Finally, these data confirmed the location of DXS72 (more centromeric with respect to the XY homology region located in the Xq21 band).

Thus, the duplicated segment in our proband extended more proximally than in the previously reported cases of duplication. However, except for the duplicated DXS1 locus, our patient's rearrangement was similar to that of patient K.M. studied by Cremers et al. (1988). The distal border of S.P.'s duplication lay in a region (between DXS169 and DXS72) where other breakpoints have been located. Out of 8 chromosomal rearrangements reported by Cremers et al. (1988, 1989), all had one breakpoint between these two probes. Moreover, 3 of these breakpoints (one duplication and two deletions) mapped between DXS72 and DXS95, as did the distal boundary of S.P.'s duplication.

This non-random distribution of chromosomal rearrangement breakpoints may be explained as follows. 1) The frequency of rearrangements may be similar elsewhere in the X chromosome but these were not observed either because they have no phenotypically visible effect or because they are lethal. Among the chromosomal abnormalities occurring in the proximal long arm, deletions were observed only in Xq21 (a region thought to be poor in genes). 2) There is an under-representation of DNA sequences from the chromosome segment that harbors these breakpoints in the panel of probes used (Cremers et al. 1988). 3) There is an enhanced proneness to chromosome breakage.

Finally, we noticed the following, relative to the loci present in the Xq proximal region and associated with various phenotypes. The putative X inactivation center,

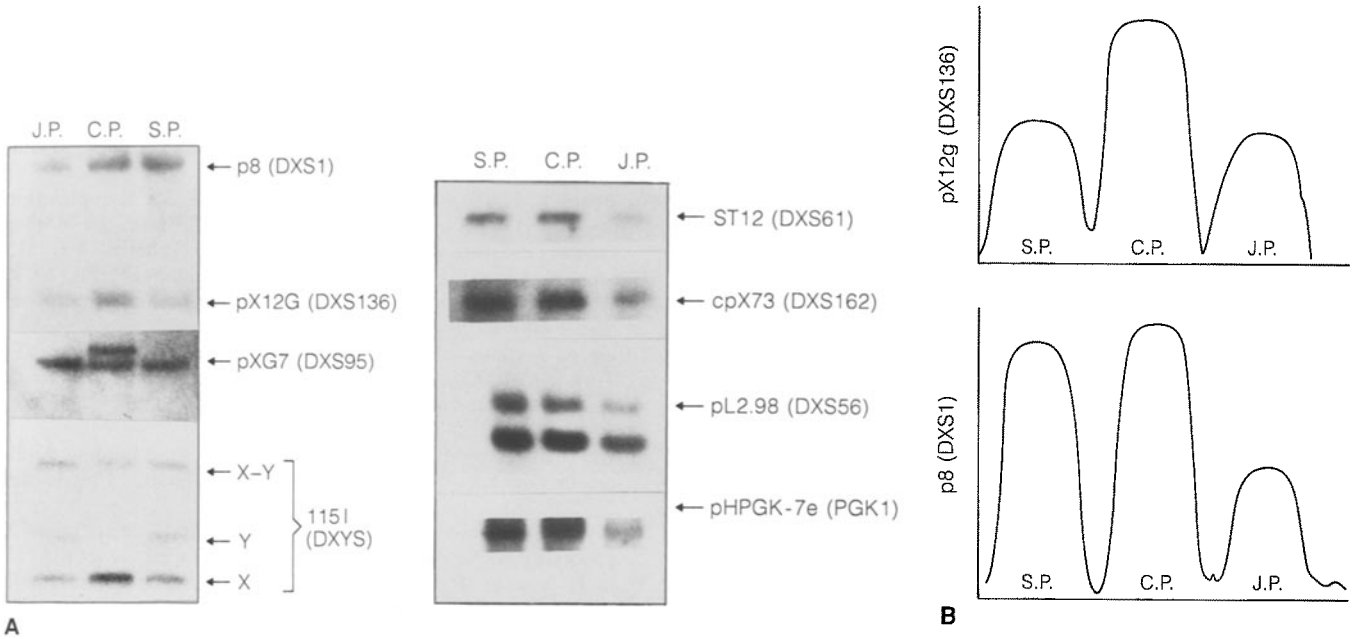

Fig. 2. A Southern blot analysis of genomic DNA of a patient (S.P.) and his unaffected parents (mother C.P. and father J.P.). Blots were hybridized with the following probes: *Hin*dIII-digests: p8 (DXS1), pX12g (DXS136), pHPGK7e (PGK1, only the 2.7-kb PGK1 specific band is shown). *Msp*I-digests: St 12 (DXS61), cpX13 (DXS162), pL2.98 (DXS56). *Taq*I-digests/ pXG-7 (DXS95, this probe reveals a two allele polymorphism in the mother), p115i (DXYS8,X and Y-specific bands are indicated). **B** Densitometric analysis of a blot hybridized with p8 and PX12g. The density of the bands were transversally determined on the autoradiogram, using an image analysis system

previously associated with the proximal long arm (Therman et al. 1979), and more precisely between the Xq12.2–Xq21.1 bands (Mattei et al. 1981), was probably duplicated in S.P.'s chromosome (2 doses as in the female). It will thus be particularly interesting to determine the methylation status of the duplicated segment. Recently, a gene (Xist) located in this region and only expressed on the inactive X chromosome (in all cells containing more than one X chromosome) has been described (Brown et al. 1991). Is Xist expressed when the inactivation center is duplicated (in 2 doses)? We are currently exploring these hypotheses.

Concerning the correlation of phenotype and karyotype, we compared S.P.'s duplication phenotype with that of other duplications in the proximal region of Xq. The observation of growth retardation in our case, as in the cases of Vejerslev et al. (1985), Steinbach et al. (1980), Schwartz et al. 1986), and Thode et al. (1988), suggests that stature may be influenced by genes in the region Xq13–Xq21. These results are in agreement with those of Goldman et al. (1982), who have proposed the existence of "statural determinants" close to the centromere on the long arm of the X chromosome. Moreover, several loci of X-linked disorders have been assigned to the proximal region of the X chromosome long arm (Harper et al. 1989), e.g., that of Menkes syndrome, X-linked Charcot-Marie-Tooth disease, anhidrotic ectodermal dysplasia and immunodeficiency 4. Failure to detect such diseases in our patient strongly suggests that these disorders do not result from an excess gene. An alternative explanation could be that one copy of the duplicated segment is inactivated. In this case, the phenotype of the patient results from the breakpoints associated with the duplication.

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