X;Y translocation in a girl with short stature and some features of Turner's syndrome: cytogenetic and molecular studies

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Abstract

A 13 year old girl referred for chromosome analysis because of disproportionate short stature (short neck, curved legs, pectus excavatum) with an initial clinical diagnosis of Turner's syndrome was found to have the karyotype 46,X,+der(X) in 100% of her blood lymphocytes. By means of conventional differential staining (QFH/AcD, FPG, and RBA banding) supplemented with distamycin A treatment, the karyotype of the proband was interpreted as 46,X,t(X;Y)(p22.3;q11). The rearranged marker X chromosome was found to be active in 91% of lymphocytes studied. PCR analysis with Y chromosome specific oligoprimers showed the presence of some Y chromosome long arm DNA in both lymphocyte and gonadal tissue biopsy cells. At laparoscopy the patient was found to have small gonads with a rudimentary uterus and fallopian tubes. Histological examination of gonadal tissue showed primary follicles with dystrophic changes of the germ cells and numerous follicular cysts (polycystic ovaries). The proband's phenotype and its correlation with the genetic imbalance of the rearranged X chromosomes, as well as with non-random t(X;Y) chromosome inactivation, are briefly discussed.

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About 50 to 60% of Turner's syndrome patients possess structurally rearranged X chromosomes or have a mosaic karyotype.¹⁻³ Of special interest are patients with Y chromosome material.⁴ The latter can be present as a low level Y chromosome mosaicism or as cytogenetically identifiable markers. The identification of Y chromosome material may indicate an increased risk of developing gonadoblastoma.⁵

In this report we present the results of our studies of a 13 year old girl with some features of Turner's syndrome and a 46,X,t(p22.3;q11) karyotype. Her rearranged X chromosome carrying a major part of Yq DNA material was active in over 90% of peripheral lymphocytes.

Case report

The patient was 12 years old when referred for endocrinological analysis because of short stature. On external examination she showed

obvious body disproportion including short limbs with curved joints, a relatively long trunk, a short neck, and mild pectus excavatum. There was no neck webbing and no ear abnormalities. Hormonal analysis showed reduction of progesterone level, all other hormones (prolactin, FSH, LTH, and oestradiol) being normal. Normal pubertal development (breast growth, pubic hair, regular menses) was evident at the age of 13. Ultrasound examination showed a markedly underdeveloped uterus (33 × 20 × 35 mm), abnormally small gonads, and short fallopian tubes with reduced ampullae. After cytogenetic analysis at 14 years of age, a laparoscopy was carried out, which showed an extremely hypoplastic uterus and bilateral (20 × 17 × 10 mm) polycystic ovaries. Histological examination of gonadal tissue showed primary follicles with obvious dystrophic changes in their germ cells and numerous follicular cysts. No lymphoid infiltration, focal calcification, or Sertoli cells were detected.

Material and methods

CYTOGENETICS

Metaphase cells were obtained from PHA stimulated blood lymphocytes using standard and specialised techniques (chromatin undercondensation with distamycin A (DA) treatment and BrdU labelling for replication pattern and R banding analysis). Chromosomes were examined using various staining methods including FPG and RBA banding and Hoechst 33258/Actinomycin D banding.

DNA STUDIES

DNA samples extracted from peripheral blood lymphocytes or from ovarian tissue by means of conventional proteinase K methods were used in PCR with specific oligoprimers corresponding to Yp11.2 (SRY) for the short arm, to Yp11.1 (DYZ3) for the centromere, and to Yq12 (DYZ1) corresponding roughly to the middle part of the Yq heterochromatin, and to the extreme tip of Yq12 (DYZ2). Physical mapping of Y chromosome and relevant primer sequences were as reported elsewhere.⁶

Results

CYTOGENETIC STUDIES

All metaphase spreads of PHA stimulated lymphocytes showed 46 chromosomes with only one normal X chromosome and one metacentric marker (46,X,+mar). With QFH banding

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(Hoechst 33258/AcD staining) the marker chromosome appeared to be a metacentric X chromosome with the brightly fluorescing heterochromatin block attached to its short arm (fig 1A). DA treatment of the lymphocyte cultures for the last 24 hours prevented condensation of the translocated fragment (fig 1B), thus favouring its heterochromatin nature.

With FPG and RBA banding there was a faint differentiation along the marker with relevant breakpoints located within Xp22.3 and Yq11 (fig 1C,D). Chromosome replication analysis after 5-BrdU treatment showed early replication of the rearranged X chromosome in 91 (fig C,D) and late replication in nine of the total 100 cells scored.

DNA ANALYSIS

PCR with DNA samples from lymphocytes and ovarian cells gave positive signals for both Yq heterochromatin segment (DYZ1 and DYZ2) probes but were negative for Yp (SRY) and centromeric heterochromatin (DYZ3) probes (fig 2). All Y chromosome probes were negative when tested on control samples from the proband's mother.

Discussion

The results of the present study show a 46(X;Y)(p22.3;q11) translocation. Differential

staining (RBA, FPG, QFH) and identification of DYZ1 and DYZ2 by PCR suggest recombination between Xp22 and Yq11 in our proband. Microscopically detectable X;Y translocations are quite rare.⁴ The majority of reported translocations of this kind have their breakpoints in Xp22 and Yq11.^{4,7-9} Extensive DNA sequence homology of these chromosomal segments predisposing to illegitimate recombinations in meiosis has been suggested⁸ and in some cases proved.⁴

Some of the reported X;Y translocations are known to be sporadic events, while others are inherited.^{4,9} We were unable to trace the origin of the translocation in our proband as her parents were not available for karyotyping. However, the absence of DNA sequences corresponding to Y specific DNA by PCR in the maternal DNA samples indicates a paternal origin of the rearranged chromosome, perhaps as a result of an abnormal recombination event in male meiosis. With some minor exceptions most of the females with Xp22;Yq11 translocations are reported as phenotypically normal and even fertile.^{4,9} Although short stature is often a feature of such females^{4,8,9} our patient had hypogonadism, a short neck, a wide chest, disproportionate short stature (curved joints, short limbs), and reduced progesterone levels. These findings suggested the initial diagnosis of Turner's syndrome. Thus the clinical manifestations of the X;Y translocation in our proband seemed to be more severe than those in other

Figure 1 The proband's normal X and marker Y chromosomes (arrowed) after QFH/AcD banding (A), distamycin A/Giemsa staining (B), RBA banding (C), and FPG banding (D).

Figure 2 Electrophoregram of PCR amplified products of (A) locus SRY, (B) DYZ3 (Yp11.1, centromere region), (C) DYZ1 (Yq12 middle), and (D) DYZ2 (Yq12, extreme tips) from the control male XY (3), from the proband's blood samples (2), and from the proband's gonadal tissue (1). Molecular weights are shown.

cases,^{4,7-9} for unknown reasons. However, unlike in other data on structurally rearranged X chromosomes,¹⁰ including some cases of similar X;Y translocations,⁹ the abnormal X chromosome in our proband was early replicating and thus genetically active in 91% of blood cells studied. If this proportion is the same in cells of other tissues, the obvious Turner's syndrome stigmata in our case might be the result of genetic imbalance of the rearranged X chromosome. Recently, it has been suggested that Turner's syndrome is a result of monosomy (haploinsufficiency) of homologous genes RPS4X and RPS4Y, common to both Xq and Yp chromosomes, encoding for ribosomal S4 proteins and usually escaping inactivation.¹¹ It has also been postulated, though not proved so far, that RPS4 genes are prone to inactivation on structurally abnormal gonosomes.¹¹ There are two homologous RPS4X genes in our proband, one carried by the normal and the other one by the rearranged X chromosomes. Whether Yq heterochromatin translocated to Xp could result in impairment of RPS4X function and how it correlates with early replication of the mar (X) chromosome in our proband remains unknown.

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