Original articles

An investigation of ring and dicentric chromosomes found in three Turner's syndrome patients using DNA analysis and in situ hybridisation with X and Y chromosome specific probes

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Abstract

We have studied three patients with features of Turner's syndrome, two with a 45,X/46,X,r(?) and the third with a 45,X/46,X,dic?(Y) karyotype. Because Turner's syndrome patients with a mosaic karyotype containing a Y chromosome are known to have a high risk of developing gonadal tumours, we used DNA analysis and in situ hybridisation with X and Y specific probes to identify the chromosomal origin of the rings and dicentric chromosomes in the three index patients. Both ring chromosomes were shown to be of X origin, while the dicentric was composed of Y chromosome material. We discuss the importance of using a combination of molecular and cytogenetic analyses in such cases.

One of the consequences of Turner's syndrome is abnormal gonadal development in females. Patients

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with Turner's syndrome can present with a variety of chromosome complements and only 50% have a 45,X karyotype alone. A cell line with a Y chromosome is present in 5.5%, and a further 3% of cases have an unidentifiable marker chromosome presumably derived from an X or Y chromosome.¹

Patients with mosaicism involving a Y chromosome are a clinically important subgroup because they have a 15 to 20% risk of developing gonadoblastoma or dysgerminoma²; these tumours rarely occur in Turner's syndrome patients without a Y chromosome.³ Therefore, it is important to establish unequivocally the origin (X or Y) of marker chromosomes found in Turner's syndrome patients, something which is often difficult to achieve consistently using conventional cytogenetic (G or G11 banding) methods.^{4,5}

The availability of X and Y specific DNA probes has made it possible by DNA and in situ hybridisation analyses to detect the presence of Y chromosome material in patients with sex chromosome abnormalities, including small rings.⁵⁻⁷ By using a combination of conventional cytogenetic methods, in situ hybridisation, and DNA analyses, we have studied two patients with a 45,X/46,X,r(?) karyotype and one patient with a 45,X/46,X,dic?(Y) karyotype, and determined the chromosomal origin of the ring or dicentric chromosome in each case.

Materials and methods

CASE 1

Case 1 presented at the age of 15 years 1 month because of short stature and delayed puberty. Her height was 142.4 cm (-3.3 SD). She had other features suggestive of Turner's syndrome including widely spaced nipples, an increased carrying angle,

and multiple cutaneous naevi. Raised basal gonadotrophin levels were present with luteinising hormone (LH) of 47 IU/l and follicular stimulating hormone (FSH) greater than 80 IU/l. Pelvic ultrasound defined a uterus but no gonadal tissue.

CASE 2

Case 2 presented at the age of 9 years 9 months because of short stature; her height was 117.3 cm (-3.0 SD). Her parents were short and she had been of low birth weight at 2100 g. No dysmorphic features indicative of Turner's syndrome were present. Other investigations found LH to be 5.9 IU/l and FSH 77 IU/l. Pelvic ultrasound showed a uterus 2 cm long but no identifiable gonadal tissue.

CASE 3

Case 3 presented at the age of 12 years 5 months because of short stature and behavioural problems. Her height was 133.4 cm (-3.4 SD). She had features suggestive of Turner's syndrome including characteristic facies, widely spaced nipples, increased carrying angle, and multiple cutaneous naevi. Basal gonadotrophins were not raised (LH <1 IU/l and FSH 6 IU/l). Pelvic ultrasound defined a uterus but no gonadal tissue.

CYTOGENETICS

Cytogenetic analysis was carried out on metaphases obtained from phytohaemagglutinin (PHA) stimulated peripheral lymphocytes using standard techniques. Initial chromosome analysis was performed using GTG banded metaphases⁸ and subsequently metaphases were CBG banded.⁹

PROBES

Cosmid Y84 was originally isolated from a human Y chromosome library and contains three copies of a 5.5 kb *Eco*RI repeat.¹⁰ In conditions of high washing stringency, it strongly hybridises to the 5.5 kb *Eco*RI

fragment characteristic of the aliphoid centromeric repeat of the human Y chromosome.¹⁰

Probe p75/79 was derived from a subclone of cosmid 75 isolated from a Y chromosome library and hybridises to a Y specific 2 kb fragment located between Ypter and Yq11.¹¹ Y190 hybridises in situ to the Yp proximal heterochromatin¹² and pSV2X5 is derived from an aliphoid X centromere specific sequence cloned into pSV2Neo.¹⁰

DNA PREPARATION AND IN SITU HYBRIDISATION

Genomic DNA was prepared from peripheral blood of the three patients and normal male and female controls. DNA extraction, enzyme digestion, and Southern blotting were carried out by modifications of standard procedures described elsewhere.^{13 14} For in situ hybridisation, biotinylated probes were hybridised overnight to standard metaphase spreads, washed at high stringency, and the sites of hybridisation detected using a double antibody amplification

Figure 1 GTG banded X chromosome and GTG (left) and CBG (right) banded ring and dicentric chromosomes from each case. The dicentric chromosome present in case 2 is shown after in situ hybridisation with probes Y84 and Y190.

Results of chromosome analysis, DNA analysis, and in situ hybridisation (numbers of metaphases studied given in brackets).

	Karyotype	DNA analysis		In situ hybridisation analysis of ring/dicentric chromosomes		
		Y84 (Ycen)	p75/79 (Yp)	pSV2X5 (Xcen)* 46,X,r(X) (7)	Y84 (Ycen)†	Y190 (Yp)
Case 1	45,X/46,Xr(?) (16) (8)	—	—			
Case 2	45,X/46,X,dic?(Y) (5) (15)	+	+		46,X,dic(Y) (7)	46,X,dic(Y) (12)
Case 3	45,X/46,Xr(?) (14) (6)	—	—	46,X,r(X) (15)		

*The normal X acted as an internal control for X centromere specific signal.

†Normal 46,XY control slides were processed under the same experimental conditions in order to check the efficiency and specificity of the Y specific probes.

step followed by precipitation of diaminobenzidine onto an avidin-horseradish peroxidase complex in the presence of hydrogen peroxide. Full details of the in situ technique have been published elsewhere.¹⁵

Figure 2 Genomic DNA extracted from peripheral blood, restricted with *EcoRI*, and hybridised with probe Y84. Sizes of restriction fragments (in kb pairs) are given on the right. Each lane contains 5 µg DNA. Lane 1=case 1, lane 2=case 2, lane 3=case 3, lane 4=normal female, lane 5=normal male, lane 6=1:1 normal male:normal female DNA.

Figure 3 Genomic DNA extracted from peripheral blood, restricted with *EcoRI*, and hybridised with p75/79. Sizes of restriction fragments (in kb pairs) are given on the right. Each lane contains 5 µg DNA. Lane 1=case 1, lane 2=case 2, lane 3=case 3, lane 4=normal female, lane 5=normal male, lane 6=1:1 normal male:normal female DNA.

Results

Detailed results of the cytogenetic, DNA, and in situ hybridisation analyses are shown in the table and figs 1 to 3. Cytogenetic analysis of the father of case 2 (dic?Y) showed an apparently normal GTG banded and CBG banded Y chromosome. Fig 1 shows the GTG and CBG banded appearance of the ring and dicentric chromosomes of each patient.

Southern blotting and hybridisation with Y84 and p75/79 showed that the Y specific 5.5 kb *EcoRI* fragment detected by Y84 and the 2.0 kb Y specific fragment detected by p75/79 are both absent in cases 1 and 3 but present in case 2 (figs 2 and 3).

Probes p75/79 and Y84 were hybridised to artificial mixtures of normal male and female DNA (results not shown). The Y specific 2.0 kb (p75/79) and 5.5 kb (Y84) fragments were visible, though faint, when male DNA comprised as little as 5% of the total mixture.

In situ hybridisation with probe pSV2X5 on cases 1 and 3 gave two discrete signals, one from the structurally normal X and the other from the ring chromosome. In situ hybridisation on case 2 with probes Y190 and Y84 showed two strong signals from the dic?Y, both distally located (fig 1).

Discussion

At high washing stringency, probe Y84 hybridises to a 5.5 kb *EcoRI* fragment specific to the alphoid centromeric repeat of the Y chromosome.¹⁰ A centromeric probe is useful in cases where a cytogenetically unidentifiable ring or dicentric chromosome is suspected to be derived from a Y chromosome, as sequences which map to distal Yp or Yq may be lost during the formation of these chromosomes, although their centromeric sequences are preferentially retained.

Our hybridisation results using Y84 and p75/79 to a panel of artificial mixtures of normal male and female DNA suggest that each probe can be used to detect Y chromosome sequences at a frequency of >5% male DNA. As the dicentric chromosome in case 2 is present at a frequency of approximately 75% (SD 10%), and the ring chromosomes in cases 1 and 3 are present at a frequency of approximately 30% (SD 10%), we conclude that, under the conditions of stringency outlined above, probes Y84 and p75/79 hybridise to Y chromosome sequences with sufficient sensitivity to detect or exclude these sequences in each of our three patients.

The results presented in the table and figs 2 and 3 unequivocally show that the Y specific sequences recognised by probes Y84 and p75/79 are present in case 2 but absent in cases 1 and 3. These results are confirmed by the in situ hybridisation studies where probes Y84 and Y190 hybridised to the dic?Y in case 2, giving two strong distally located signals. In cases 1

and 3 the X centromere specific probe pSV2X5 showed specific hybridisation signals to both the small ring chromosomes and the normal X chromosomes, thus confirming that the small ring chromosome present in both of these patients is derived from the X chromosome.

The increased risk (15 to 20%) of gonadal malignancy in patients with 45,X/46,XY or 45,X/46,X,dic(Y) karyotypes is well documented,^{3 16 17} although the number of patients from which these risk estimates are derived is small. As it is known that the prevalence of tumours in patients with a 45,X cell line and a cell line with a structurally abnormal X is low,¹⁸ the results presented above indicate that case 2 has an increased risk of developing gonadoblastoma or dysgerminoma, while cases 1 and 3 have a low risk of developing these tumours.

In conclusion, using a combination of conventional cytogenetic methods, in situ hybridisation, and DNA analysis, we have unequivocally established the origin (X or Y) of the ring and dicentric chromosomes in each of our three cases and have been able to assign a low risk (cases 1 and 3) or an increased risk (case 2) of malignancy to each patient. We advocate that such molecular studies are undertaken in similar cases as an aid to the clinical management of these patients.

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