Cytogenetic and clinical studies in gonadal dysgenesis with 46,X,Xt(qter→p221::p223→qter) karyotype: review and phenotype/karyotype correlations

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SUMMARY Chromosome analysis by Q, R, and C banding was performed in a case diagnosed clinically as gonadal dysgenesis and the karyotype was shown to be 46,X,Xt(qter→p221::p223→qter). Localisation of the breakpoints in the fused X chromosomes and replication studies have led to a hypothesis on the origin of the translocation. A comparison of clinical and cytogenetical findings in this and other published cases has also been made in an attempt to detect some phenotype/karyotype correlations.

Cases of known X:X translocations in man are quite rare. Some of these, published before the introduction of banding techniques, have been described as known or presumptive X:X or X; autosome translocations, mostly on the basis of the clinical picture.¹

Since 1972, 12 more cases have been described and exhaustively studied by means of combined banding techniques, including one of the older cases² which has been studied again with modern banding techniques (table 1).³ The chromosomal characterisation of these new cases is often very accurate and includes the localisation of the breakpoints⁴ as well as hypotheses on the possible mechanisms of the chromosomal abnormality.⁵⁻⁷ Many cases, however, either lack sex chromatin studies and histological studies of gonadal tissues, or give rather scanty clinical and endocrinological pictures of the patients.

We present the case of an adult female, diagnosed clinically as a case of gonadal dysgenesis, in whom exhaustive clinical, endocrinological, histological, and cytogenetical studies have been performed.

Case report

CLINICAL FINDINGS

The patient, aged 22, was first referred to the Department of Obstetrics and Gynaecology of the University of Chieti because of amenorrhoea of 3 years' duration. She was the second born of seven children

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of normal parents. All the sibs are phenotypically normal and in good health. There is no family history of amenorrhoea, spontaneous abortions, or congenital malformations.

The age of both parents at the time of birth was 23. Pregnancy and delivery were uneventful and birthweight was 3200 g. No lymphoedema was noted during early infancy. No feeding difficulties or major complications were noted during childhood. Menarche occurred at the age of 14; subsequent menstruation occurred only after steroid withdrawal until the age of 19. Secondary sex characteristics developed at the usual age. At the age of 19 isolated spontaneous bleeding was recorded. A laparoscopy, performed in another hospital, showed: "a small uterus and two ovaries of the size of small almonds". When first seen by one of us (QDN), she was a 22-year-old girl of normal intelligence, 149 cm tall, weighing 45.5 kg. No signs of Turner's syndrome could be observed. Gynaecological examination showed normal genitalia and a small uterus but no gonads could be felt on external examination. Axillary hair was normal, pubic hair was scanty, and the breasts were small. The patient did not complain of hot flushes or other signs of premature menopause. Endocrine findings, showing high gonadotrophin and low oestrogen values, are reported in table 2. Several courses of therapy with HCG (human chorionic gonadotrophin), HMG (human menopausal gonadotrophin), and clomiphene failed to induce

TABLE 1 Cytogenetic data in cases of females with X;X translocation identified by banding techniques

Authors	Clinical diagnosis	Sex chromatin	Karyotype	Inactive X
Distèche <i>et al</i> ³	Signs of Turner's syndrome	Large, bipartite (OS and VS)	46,X,Xt (end to end fusion p22::p22)	Abnormal X
Van den Berghe <i>et al</i> ¹⁵	Turner's syndrome	Rather large	45,X/X,Xt (Xp Xq)	Abnormal X
Therman <i>et al</i> ⁵	Ovarian dysgenesis	Large, bipartite (OS and fibroblasts)	46,X,Xt(pter→q27::q27→pter)	Abnormal X
Kim <i>et al</i> ¹ Mother	Few Turner's stigmata	Large	45,X/46,X,Xt(qter→p22::p22→qter)	Abnormal X
Daughter	Many Turner's stigmata	Large	46,X,Xt(qter→p22::p22→qter)	Abnormal X
Ruthner and Golob ¹⁶	Gonadal dysgenesis	Very large	45,X/46,X,Xt(p::p)	Abnormal X
Laurent <i>et al</i> ¹⁷			46,X,Xt(qter→p22::q12→pter)	Abnormal X
Fraisse <i>et al</i> ¹⁸	Signs of Turner's syndrome	Single and double masses	46,X,Xt(telomeric fusion p::p)	Abnormal X
Sillesen <i>et al</i> ¹⁹	Turner's syndrome	Normal, large, bipartite, tripartite	45,X/46,X, idicX(qter→p22::p22→qter)	Abnormal X
Sinha <i>et al</i> ²⁰		Very large	45,X/46,X,Xt (end to end fusion q::q)	Abnormal X
Mirzayants and Baranovskaya ²¹	Gonadal dysgenesis	Very large	45,X/46,X,Xt(q27::q27)	Abnormal X
de la Chapelle <i>et al</i> ⁷	Pure gonadal dysgenesis		45,X/46,X,Xt(pter→q21::q11 or q13→pter)	Abnormal X
Laća <i>et al</i> ²²	Pure gonadal dysgenesis	Large	45,X/46,X,Xt (q24::q24)	Abnormal X
Our case	Ovarian dysgenesis	Normal, large, bipartite, double (OS and fibroblasts)	46,X,Xt(qter→p221::p223→qter)	Abnormal X

TABLE 2 Clinical, anatomical, and functional data in female patients with X;X translocation identified by banding techniques

Authors	Age	Clinical features			Gonads	Genitalia			Secondary sex characteristics			Hormonal studies				
		Height (cm)	Turner's stigmata	Intelligence	Menses	Ovaries	Oestrogens	Gonadotrophins	External	Uterus	Breasts	Hair	17KS	11 OHCS	Cortisone	Thyroid
Distèche <i>et al</i> ³	15	128	+	Low	PA	↑	↓		Inf	Small	—	—	N	N		N
Van den Berghe <i>et al</i> ¹⁵	14	130	+		PA	N	↓	Streaks, no germ cells	Normal	Small	—					N
Therman <i>et al</i> ⁵	31	182			PA	↑		Streaks	Inf	Small	—	±	N			
Kim <i>et al</i> ¹ Mother	34	152	±		SA				Normal	Normal	+	+				
Daughter	16	147	+	Normal	SA	↑				Small	+	±	N	N		
Ruthner and Golob ¹⁶	25	153	±		PA	↑		Streaks, no germ cells		Normal	+	+				
Laurent <i>et al</i> ¹⁷	24	153		Normal	SA				Normal	Normal						
Fraisse <i>et al</i> ¹⁸	53	137	+	Low	PA	↑			Inf	Normal	+	+	N	N	N	N
Sillesen <i>et al</i> ¹⁹	11	124	±	Normal		↑	↓		Normal		—	—				
Sinha <i>et al</i> ²⁰	15				PA											
Mirzayants and Baranovskaya ²¹	23	173			PA	↑			Normal	Small	+	±	N	N	N	N
de la Chapelle <i>et al</i> ⁷	18	158	—		PA		↓	Streak at right, left absent	Inf	Small	+	±				
Laća <i>et al</i> ²²	29	153		Low	PA	↑				Small	+		N	N	N	
Our case	22	149	—	Normal	SA	↑*	↓	Streaks bilaterally, few primordial follicles	Normal	Small	+	±	N	N	N	N

*Radioimmunoassay of serum gonadotrophin levels: HPRL=15 ng/ml, FSH=70 mIU/ml, LH=147 mIU/ml. N=normal values. ↑=high values. ↓=low values. PA=primary amenorrhoea. SA=secondary amenorrhoea. Inf=infantile.

menstruation. A mini laparotomy performed in April 1979 showed a small uterus and very small streak gonads. Histological examination of the gonadal biopsy showed ovarian stroma, few primordial follicles, and hypertrophy of interstitial cells. No corpora lutea were observed.

A clinical diagnosis of ovarian dysgenesis⁸ was therefore made. On the basis of functional studies this case can also be classified as 'premature ovarian failure'.⁹

CYTOGENETIC STUDIES

Sex chromatin studies were performed on buccal mucosa smears and on fibroblast cultures after thionine staining.¹⁰ Chromosome analysis was performed by standard methods on leucocyte

cultures from the proband and her parents and on tissue cultures from ovarian biopsy of the proband. Tissue cultures were set up in a CO₂ incubator and cultured in Ham's F10 medium (Difco) supplemented with 20% fetal calf serum (Gibco).

Standard chromosome preparations were stained with Giemsa or aceto-orcein. The following banding techniques were used: Q banding with quinacrine mustard (QM, Sigma), modified C banding,¹¹ and R banding with acridine orange¹² or modified Giemsa staining¹³ on cultures treated for 7 hours with BUdR (final concentration 200 µg/ml).

Replication studies were performed on cells from both tissues labelled with ³HTdR (final concentration 0.5 µCi/ml) or treated with BUdR (final concentration 200 µg/ml) for the last 7 hours of culture.

Autoradiography was performed with Kodak stripping film AR 10. The emulsion coated slides were exposed in the dark at 4°C for 5 days.

The analysis of breakpoints was performed on leucocyte and fibroblast cultures treated with BUdR in a final concentration of either 200 µg/ml, to obtain highly despiralised late replicating X chromosomes, or 100 µg/ml to obtain banded but less despiralised late X chromosomes.

Results

Sex chromatin bodies were observed in 38% of buccal mucosa cells and in 53% of the fibroblasts.

FIG 1 Sex chromatin in cells from oral smears (upper row) and fibroblasts (lower row): (a) normal sized single mass, (b) large single mass, (c) bipartite mass, (d) double masses.

FIG 2 Giemsa stained cell from the proband. The arrow indicates the abnormal chromosome.

FIG 3 Q banded karyotype from leucocyte cultures of the proband.

FIG 4 Identification of the abnormal chromosome. Composite partial karyotype showing Q banded (left) and R banded (BUdR 100 μ g/ml, right) normal and rearranged X chromosomes. The rearranged chromosome is compared to the normal X (printed in duplicate) from the same cell. The line indicates the centromeres.

About one third of chromatin positive cells from both tissues showed a single, normal sized mass, often darkly stained (fig 1a). The other two thirds showed a very large, more faintly stained, single

mass (fig 1b), or a large bipartite mass (fig 1c), or two normal sized masses with medium intensity of staining, always located close to each other (fig 1d).

Chromosome analysis performed on 316 cells from blood cultures and 47 cells from fibroblast cultures showed 46 chromosomes, with a C group chromosome replaced by a large submetacentric chromosome resembling a chromosome 2 (fig 2). Q and R banding techniques showed the presence of a single normal X chromosome. The rearranged chromosome similar to No 2 was made up of two more X chromosomes fused by their short arms (fig 3, 4). This chromosome had two C bands (fig 5a, b). The first one was located in its primary constriction and corresponded to the centromere of one of the two fused X chromosomes. The second was bipartite and located on the long arm of the rearranged chromosome, at the same site as the centromere of the second X (fig 5c).

A detailed analysis of breakpoints on the short arms of the two fused Xs was made in 27 cells from both tissues, treated with different BUdR concentrations, by comparing the two arms of the rearranged X chromosome with each other (fig 6). For better identification of breakpoints the rearranged

FIG 5 C bands in cells from fibroblast (a) and leucocyte (b) cultures. The arrows indicate the rearranged chromosome showing two C bands. The bipartite C band is located at the same site as the centromere of the second X (c).

FIG 6 Identification of the breakpoints in the short arms of the fused X chromosomes: (a) comparison between the two arms of the rearranged chromosome (printed twice) from two different cells (BUdR, 200 µg/ml); (b) comparison of the two arms of the rearranged chromosome with each other and with the diagram of late-replicating X (BUdR, 100 µg/ml). The solid line indicates the centromeres, the dotted line the fusion sites. The arrows indicate the breakpoints.

chromosome was also compared with a standard R banding scheme of a late replicating X.¹⁴ The results show that the chromosome with a single C band is broken in sub-band 221, and the other, with a bipartite C band, in band 223 (fig 6b).

The rearranged chromosome has therefore been characterised as 46,X,dic(X)(qter→p221::p223→qter).⁴ The patient is therefore trisomic for Xqter→p221, disomic for p222→p223, and monosomic for p223→pter.

Replication studies on 270 cells from the two tissues showed that the rearranged chromosome was always preferentially inactivated with respect to the normal X chromosome, both in ³HTdR and in BUdR treated cells (fig 7). Sex chromatin and chromosome findings in our case and other published cases are reported in table 1. The karyotypes of both parents were normal.

Discussion

All available clinical and chromosomal findings on cytologically well documented X;X translocations are shown in tables 1 and 2.

In all cases with complex X chromosome rearrangements the difficulty in establishing cause-effect relationships between chromosome constitution and the final phenotype also results from the frequently observed mosaicism with coexistence of an XO cell line, and from the complex mechanisms of selective cell growth and X chromosome inactivation. Some general evidence may be tentatively drawn from published reports.

It can be seen from table 1 that one constant feature is ovarian dysgenesis with or without Turner's stigmata, resulting in primary or secondary amenorrhoea, high values of gonadotrophins, and

FIG 7 Preferential inactivation of the rearranged chromosome (arrowed): (a) labelling with $^3\text{HTdR}$, (b) Giesma staining after 200 $\mu\text{g/ml}$ BUdR, (c) acridine orange staining after 200 $\mu\text{g/ml}$ BUdR.

the presence of streak ovaries. Another fairly constant feature in cases of Xp deletion is small stature, which is lower than the average population height. In fact, it emerges from table 1 that only those subjects carrying deletions of the long arm of the X chromosome are of average, or higher than average, stature. Short stature has been explained by some

authors on the assumption that genes controlling body height are located on the short arm of the X,⁷ while severe impairment of both gonadal development and function, observed in all deletion cases, cannot be explained solely on the basis of Xp deletion.

In our case, gonadal dysgenesis without Turner's stigmata and with normal intelligence are in agreement with published data. Normal general appearance and secondary sex characteristics could be explained on the basis of the long term oestrogen-progesterone replacement therapy. The height (149 cm), however, is within the lower limits of the normal distribution for a southern Mediterranean population. This might be related to the cytological demonstration of monosomy for only a minute segment of Xp (p223→pter).

There are striking phenotypic differences between non-mosaic females with almost total X trisomy, as in our case, and triple X females. The mechanism of X chromosome inactivation does not seem to offer a satisfactory explanation for their phenotypic differences. Since it is known that in mammalian female embryos both Xs are functioning in the earliest stages of development,²³ and since the phenotype of gonadal dysgenesis has been explained by the lack of one X chromosome during this critical period, it is possible that monosomy for even very small segments of the X chromosome and trisomy for other segments may negatively influence the final phenotype. The rapid and progressive regression of the ovary in our case can also be interpreted in this way.

The finding of normal karyotypes in the parents makes this a de novo case of translocation, as has been observed in most published cases. The analysis of banding patterns in BUdR treated cells showed that the breakpoints are located in two different sub-bands (p221 and p223, respectively) in the two X chromosomes (fig 6). It follows that the translocation must have arisen from an asymmetrical break occurring either during premeiotic G_1 in the two X chromosomes in the maternal germ line, or in G_1 in the sister chromatids of the X in either the maternal or the paternal germ line. This seems to be the most reasonable explanation on cytological grounds. Furthermore a symmetrical location of breakpoints would also imply a second, interstitial, deletion in band p221 of the acentric X chromosome (fig 6). Both these mechanisms, as well as other more complex ones which could be proposed, call for an early inactivation of one of the centromeres of the rearranged chromosome. In fact, an essential condition for cell survival is that one of the centromeres becomes inactive. This does not necessarily imply the existence of a cause-effect relationship

between the two events of translocation and centromeric inactivation.

Replication studies have shown that the rearranged chromosome is always preferentially inactivated. This finding is in agreement with data obtained by other authors. Sex chromatin results are also in agreement with published data (table 1). The finding of a single, normal sized, deeply stained sex chromatin mass in about one-third of cells can be explained by different degrees of heteropyknosis in different cells. The same large rearranged X chromosome may therefore appear, according to the degree of condensation and possibly to the phase of the cell cycle, as a single heavily stained mass, a single large faintly-stained mass, a bipartite mass, or two single masses.

Replication studies have shown preferential inactivation of the rearranged chromosome in two tissues, leucocytes and fibroblasts, both having the same embryological (mesodermal) origin. It can be inferred, on the basis of the results obtained by sex chromatin studies on buccal smears, that another tissue of different (ectodermal) origin, that is, oral mucosa, must also have the same chromosomal complement and the same preferential inactivation of the rearranged X chromosome.

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