X;15 translocation in a retarded girl: X inactivation pattern and attempt to localise the hexosaminidase A and other loci

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SUMMARY Cytogenetic studies on a retarded girl showed a complex X;15 translocation, karyotype 45,X,-15,+t(X;15). The translocation X chromosome was non-randomly partially inactivated, the inactivation being mainly confined to the X segment and in some cells only to the X long arm. Gene marker studies failed to show anomalous segregation of the hexosaminidase A gene or any other gene markers tested.

Nearly 50 patients with an X-autosome translocation have been reported and attempts made to correlate the phenotypic expression with the type and site of translocation, as well as with the X inactivation pattern (Cohen *et al.*, 1972; Summitt *et al.*, 1974; Therman and Patau, 1974; Gilgenkrantz *et al.*, 1975; Bartsch-Sandhoff *et al.*, 1976; Hagemeijer *et al.*, 1977). A further 18 X-autosome translocations are listed by Borgaonkar and Dolling (1977). Every autosome except chromosomes 10 and 20 has been implicated and in at least 5 cases the autosome has been identified as chromosome 15 (Engel *et al.*, 1971; Lucas and Smithies, 1973; Sujansky *et al.*, 1973; Laurent, 1971, quoted by Gilgenkrantz *et al.*, 1975; Bartsch-Sandhoff *et al.*, 1976).

The X inactivation pattern of sufficient numbers of such cases has now been studied to establish that balanced X-autosome translocations usually show non-random inactivation of the normal X chromosome, while in unbalanced translocations, the derivative abnormal X is usually completely or partially inactivated, with resultant diversity of phenotypic expression. For a valid correlation to be made between the phenotype and the karyotype in these patients, it is important to determine whether X inactivation has spread from the X segment to the attached autosome. Such an exercise was often difficult with autoradiographic techniques, but incorporation of 5-BrdU during DNA synthesis (Dutrillaux *et al.*, 1973) has provided a simple and

accurate means of studying the replication patterns of chromosomal DNA, including the inactivation patterns of the X chromosome.

We report the investigation of a markedly retarded girl, who was found to have a complex translocation of chromosomes X and 15 (45,X,-X,-15,+t(X;15)) in the majority of her peripheral blood metaphases, and a minor mosaic 45,X (Turner) cell-line. The translocation X chromosome was found to be non-randomly partially inactivated. Serogenetic studies were performed on the child and her parents in an attempt to localise genes on chromosomes X and 15.

Case report

A 4-year-old girl was brought to hospital in Windhoek, Namibia/South West Africa from a remote country area for investigation of marked mental and physical retardation. She was the fourth born of 5 children, and was delivered normally after an uneventful pregnancy. Both parents and the 3 older sibs, all boys, are phenotypically normal, as was the youngest sib, a girl, who died recently of 'congested lungs'. There is no known history of abnormality in either parent's family. The mother is of (black) Tswana origin, while the father has mixed Tswana/Caucasoid ancestry.

The birthweight is not known, but the child was said to be weak and had difficulty in sucking. The mother thought the child was normal until 3 months of age, but development was then noted to be slow

and her condition has progressively deteriorated. At 5 years of age she has no intelligible speech, but recognises her parents; she has never sat or crawled and locomotion is by means of 'wriggling' movements only. Clinical examination at 5½ years of age showed a grossly mentally and physically retarded child. Her height of 78 cm, weight of under 10 kg, and head circumference of 46 cm are all well below the 3rd centile. She adopts a peculiar posture in her cot, lying on her back with flexed hips and extended knees (Fig. 1a,b). Hand and leg movements are uncoordinated and uncontrolled but a 'grasp' reflex is present; she is slightly hypertonic. She has a cleft palate, large

simple low set ears, a beaked nose, down-curved upper lips, and a narrow forehead with a very low hairline. The fingers are long and tapering with proximally inserted thumbs, there is bilateral fifth finger clinodactyly, the palmar creases are normal, but detailed dermatoglyphic studies were not performed. The skin shows large confluent depigmented areas, confined mainly to the trunk and proximal parts of the limbs, somewhat resembling incontinentia pigmenti (Fig. 1c). There are no obvious dental anomalies and there is no clinical evidence of internal abnormalities.

Methods

CYTOGENETIC STUDIES

Synchronised peripheral blood cultures were established by a modification of the technique of Yunis (1976). Banding studies were carried out by modifications of well described banding procedures: (i) trypsin-Giemsa banding (Priest *et al.*, 1975); (ii) quinacrine mustard fluorescence (Rowley, 1976, personal communication); (iii) centromeric banding (Sumner, 1972); and (iv) acridine-orange reverse banding after incorporation of 5-BrdU during the last 6 hours of culture (Dutrillaux *et al.*, 1973) and photographed with black and white film and colour film using the photographic techniques described by Verma and Lubs (1975). Buccal epithelial cells were stained with Klinger-thionine stain to show X chromatin.

GENE MARKER STUDIES

Blood groups were determined by the methods recommended by Race and Sanger (1968) and comprised the following systems: ABO, Rhesus, MNSs,

(a)

(b)

(c)

Fig. 1 Clinical appearance of patient at 5 years. (a) Facial features; (b) characteristic posture; (c) appearance of skin lesion.

P, Kell, Duffy, and Xg. Haptoglobins (Hp), transferrins (Tf), erythrocyte enzymes 6 phosphogluconate dehydrogenase (6PGD), glucose-6-phosphate dehydrogenase (G6PD), acid phosphatase (AP), adenosine deaminase (ADA), first and second loci phosphoglucomutase (PGM₁ and PGM₂), adenylate kinase (AK), and the peptidase (Pep A, B, C, and D) systems were investigated by the methods recommended by Giblett (1969). Red cell carbonic anhydrases (CA) I and II were studied by the method of Hopkinson *et al.* (1974), esterase D (EsD) by the technique of Hopkinson *et al.* (1973), glutamic pyruvate transaminase (GPT) by the technique of Chen and Giblett (1971), isocitrate dehydrogenase (ICD) by the technique of Chen *et al.* (1972), and glyoxalase (GLO I) by the technique of Kompf *et al.* (1975). Serum hexosaminidase was assayed by the method of Kaback (1973).

Results

Preliminary chromosome analysis of unbanded metaphases disclosed a modal number of 45, with a mosaic pattern 45, - C/45, - C, - D, + mar C.

Giemsa banding showed a translocation between an X chromosome and chromosome 15 in 139 of 160 cells (87.0%) screened (Fig. 2). A minority of 21 cells had a karyotype of 45,X (Fig. 3). In these latter cells, all six D group chromosomes were present and the

translocation chromosome was absent. The translocation was complex in nature. The rearrangement was initially thought to involve a simple tandem translocation of 15q onto the X chromosome, but G, Q, and R bands of the normal X and 15 chromosomes and the translocation X did not correspond at all when the translocation was interpreted in this manner. The short arm of the translocation chromosome did, however, pair well with the normal 15, and the distal portion of the long arm of the translocation X paired well with the short arm of the normal X (Fig. 4a, b). The translocation was therefore interpreted as follows (Fig. 5). (a) Two breaks on the X chromosome, one centromeric and the other on Xq, with deletion of Xq2?5→Xqter. (b) Centromeric inversion of Xq2?5→X?p11?q11. (c) Centromeric or proximal long arm breakage of chromosome 15 and translocation of 15q onto the centromeric breakpoint of the X, that is, t(X;15) (p11;q11) or (q11;p11).

Centromeric banding showed only one definite centromere, but was non-contributory in elucidating the origin of this centromere, as differences in size between the translocation X, normal X, and normal 15 were too subjective for a definitive identification of the centromere involved. C-banding did not show a typical second centromere at the presumed fusion point between Xq and Xp, but in many cells a darkly staining band was visible at this site (Fig. 4c) and a secondary constriction in this area was also observed.

Fig. 2 Giemsa-banded karyotype showing only one chromosome 15 and translocation of the other chromosome 15 onto an X chromosome (t).

in some G-banded cells (Fig. 4b, I and II), indicating that there could be a second non-functional centromere.

Acridine-orange reverse banding, after 5-BrdU incorporation during the last 6 hours of culture,

showed non-random inactivation of the translocation X chromosome in all 66 of 75 reverse banded metaphases containing the translocation chromosome (Fig. 6). The inactivation was, however, partial, involving mainly the X segment, which was extended, fuzzy, and emitted a dull orange

Fig. 3 Giemsa-banded karyotype of minor mosaic 45,X cell line.

Fig. 4 Morphological appearance of normal X chromosome (left), inverted $t(X;15)$ chromosome (centre), and normal chromosome 15 (right). (a) Q-banding; (b) G-banding; (c) C-banding. ((aI) and (bI) and (aII) and (bII) are sequentially G→Q banded).

fluorescence characteristic of the X inactivation demonstrated by this technique (Hagemeijer *et al.*, 1977). Inactivation extended beyond the centromere into the proximal portion of the translocated autosomal segment in the majority of cells (Fig. 6, I, V, VI, VII, and XII). It was not possible, however, to determine exactly how far the inactivation process

had spread beyond the centromere into the autosome, as the proximal portion of 15q is normally less intensely fluorescent on reverse banding. In only 2 of 66 cells did the inactivation appear to involve the entire translocation chromosome (Fig. 6, IV and VIII), but this could have been an artefact. Further variation of the inactivation pattern of the inverted

Fig. 5 Diagram explaining proposed mechanism of origin of X;15 translocation (see text).

Fig. 6 Partial A-O reverse banded karyotypes of normal X chromosome (left), inverted $t(X;15)$ chromosome (centre), and normal chromosome 15 (right) after incorporation of 5-BrdU during late synthesis. (I-VIII, printed in black and white from colour transparencies; IX-XII, photographed with black and white film.)

Xq and Xp segments was noted in 32 of 66 cells. In these metaphases, the inactivation of the inverted Xq did not appear to extend into the distal region which is presumed to be the X short arm (Fig. 6, VI, VII, VIII, and XII).

Both parents had normal karyotypes.

Buccal smear examination showed 36% X chromatin positive nuclei in 250 cells screened. The Barr bodies were normal in size in 95% of the cells (Fig. 7a), but in 5% of the nuclei, a large X chromatin body was observed (Fig. 7b).

GENE MARKER STUDIES

Variation was not found in the child or her parents (all 3 were apparently homozygous for the common allele) in the following systems: Kell, P, G6PD, ADA, PGM₂, Peptidase A, B, C, and D, ICD, AK, EsD, CA₁, Tf, and Hex A. The findings in the other systems are shown in the Table.

Discussion

In patients with X;autosome translocations, the resultant phenotype depends not only on the loss or gain of genetic material dependent on the type of translocation, but also on the X inactivation pattern present. This inactivation generally conforms to a pattern which is consistent with the least amount of

genotypic and phenotypic imbalance, and presumably results from selection of the most viable genotype (Leisti *et al.*, 1975). Another factor which may influence the type of inactivation is the postulated existence of an inactivation centre in the proximal portion of Xq (Therman and Patau, 1974) or both Xq and Xp (Cohen *et al.*, 1972). This implies that the site of breakage on the X chromosome also determines the X inactivation pattern: if the inactivation centre is intact it is probable that the normal and abnormal X are randomly inactivated initially, and subsequent selection of the most viable phenotype and genotype occurs (Therman and Patau, 1974). Gilgenkrantz *et al.* (1975) believe that the X inactivation pattern depends on whether an X segment is translocated onto an autosome, in which case the normal X is inactivated, the converse applying where an autosome is translocated onto the X.

Whatever the mechanism, balanced X;autosome translocations are associated with non-random inactivation of the normal X chromosome (Summitt *et al.*, 1974; Therman and Patau, 1974; Leisti *et al.*, 1975; Hagemeijer *et al.*, 1977). In one case (Cohen *et al.*, 1972), both the normal X and the translocation X were inactivated in 32% of cells. The only exception to this pattern so far documented is reported by Thelen *et al.* (1971), where a balanced X;autosome translocation was associated with inactivation of the entire translocation X in nearly 80% of cells.

Fig. 7 X chromatin in interphase nuclei. (a) Upper row, normal size Barr bodies. (b) Lower row, large Barr bodies.

Table Genetic polymorphisms showing variation in index case (child) and/or her parents

	ABO	MNSs	Rhesus	Duffy	Xg	6PGD	AP	PGM ₁	CA ₂	GPT	GLO1	Hp
Father	B	Ms/Ms	R ₀ /R ₀	(a+b-)	a-	AC	B	2-1	2-1	2-1	2-2	1
Mother	O	Ms/Ns	R ₂ /R ₀	(a-b+)	a+	A	AB	2-1	2-1	2-1	2-1	2-1
Child	B	Ms/Ns	R ₂ /R ₀	(a+b+)	a+	AC	B	1	1	1	2-2	2-1 mod

In unbalanced X; autosome translocations, as is the case in our patient, the translocation X is usually wholly or partially inactivated, but exceptions to this pattern have been observed (Mikkelsen and Dahl, 1973). The diversity of phenotypes associated with unbalanced translocations can be ascribed to several factors. Firstly, as in autosomal translocations, the nature of the translocation and the genetic content of the autosome involved will determine the severity of phenotypic expression. Secondly, the extent of spreading of the inactivation process from the X portion into the translocated autosomal segment will influence the degree of effective autosomal monosomy or trisomy. Thirdly, if there is incomplete inactivation of the X segment of the translocation chromosome, this could result in some degree of effective X disomy, which Therman and Patau (1974) believe could lead to a lethal genetic imbalance if fully expressed. This may be of some relevance in regard to our findings and will be discussed below. Lastly, non-random inactivation of one of the X chromosomes could result in effective hemizyosity of a deleterious recessive gene (Therman and Patau, 1974).

In our patient, some or all of these factors could have contributed to the abnormal phenotype. The nature of the translocation itself was complex and resulted in both deletion of distal Xq and a presumed inversion of the remaining Xq. The presence of a minor 45,X (Turner) cell line further modifies the phenotype and is consistent with the selective elimination of the structurally abnormal X (Cohen *et al.*, 1967) before the autosomal translocation occurred. It is difficult to assess whether the X autosome translocation has resulted in a structural loss of the proximal part of 15q. There was no morphologically detectable loss of 15q and centromeric banding did not assist in precisely defining the origin of the centromere at the X autosome fusion site.

The abnormal X translocation chromosome was non-randomly inactivated and, as expected, the inactivation was partial, affecting mainly the X segment in the majority of cells. Complete inactivation would have resulted in an effective monosomy 15 in the cells containing the X autosome translocation (87% of peripheral blood cells), which would almost certainly have resulted in an inviable genetic imbalance. In 2 of 66 cells, the inactivation appeared to involve the entire translocation chromosome and, though this could have been technical, the presence of large X chromatin Barr bodies in 5% of buccal epithelial nuclei suggests that in a minority of cells the translocation X chromosome may, in fact, have been completely inactivated. In the metaphases containing a partially inactivated translocation X, spreading of the inactivation did extend beyond the

centromere into the long arm of chromosome 15, but the extent of this spreading is not easy to detect morphologically.

If, in the translocation process, there were the loss of a genetic locus, anomalous segregation might result, giving an apparent paternal or maternal exclusion. No such anomaly was found with respect to a number of genetic polymorphisms most of which have had their loci assigned to chromosomes other than X or 15. The only autosomal ones not yet assigned and which might have been informative in this family are CA₂, GPT, and GLOI.

The hexosaminidase A locus has been assigned to chromosome 15 (McKusick and Ruddle, 1977), but the fact that the proband showed a proportion of Hex A well within the non-carrier range indicates that this locus has not been lost in the translocation process or inactivated under the influence of the X chromosome.

The incomplete inactivation of the Xp portion of the translocation in nearly 50% of the cells is of great interest because of its possible contribution to the abnormal phenotype by producing an effective disomy of Xp in some of the cells (Therman and Patau, 1974). The lack of complete inactivation of Xp in these cells could be explained by discontinuity and dissociation of Xp from the postulated inactivation centre on Xq as a result of the inversion of Xq. Therman and Patau (1974) observed that inactivation seems to be able to spread beyond the short arm, but not beyond the long arm. If this is in fact true it would explain the lack of apparent inactivation of both the autosomal and Xp segments. If these speculations are correct, however, it is difficult to explain why the same pattern of inactivation of the X segment did not apply to all the observed cells.

The possibility that the child had incontinentia pigmenti is an intriguing one. This rare condition is thought to be X linked; most cases have been female and it is assumed to be lethal in males (Morgan, 1971). The loss of the incontinentia pigmenti locus in the translocation process, or its non-random inactivation, would result in the manifestation of the disease if the mutant gene for the condition were present on the normal X chromosome; why it was not lethal in this particular hemizygote is not clear. It might be that the locus is on that part of Xp which is incompletely inactivated in nearly 50% of the cells, that is, effectively disomic.

Comparison of the phenotypic features in the X inactivation pattern of our patient with other reported t(X;15) patients was not possible in 4 of 5 such cases previously described, because these patients had a balanced translocation of an X segment onto chromosome 15, with inactivation of the normal X chromosome. One subject was phenotypically normal (Bartsch-Sandhoff *et al.*, 1976), two

patients suffered from primary amenorrhoea (Lucas and Smithies, 1973; Sujansky *et al.*, 1973), while Laurent's patient (Gilgenkrantz *et al.*, 1975) was phenotypically abnormal, but there is no clinical description of the abnormalities, and the karyotype anomaly is not clear.

The only case report with which comparisons may be made is that of Engel *et al.* (1971), who described a child with multiple anomalies who had a very similar karyotype to our patient, 45,X,-15,tan(15qXq+)+. No associated mosaic cell line was found. This infant was far more severely affected than our patient and died at 3 months of age. The multiple anomalies included hydrocephalus and congenital heart disease, neither of which was present in our case. The only clinical features in common appear to be cleft palate and low set ears. The severe multiple anomalies in the patient of Engel *et al.* (1971) can be explained by the differing X inactivation pattern. In 75% of cells, the translocation X was late replicating with entire or partial inactivation of the autosomal segment in most of these cells. In 11% of cells, the normal X was inactivated and 10% of metaphases showed both normal and translocation X inactivation. The abnormal phenotype is, therefore, presumably the result of effective monosomy 15 in a large number of cells.

The patient of Engel *et al.* (1971) and another severely abnormal child reported by Neuhäuser and Back (1967) are the only two previously reported cases of unbalanced X autosomal translocation with a modal number of 45. One other patient with a karyotype of 45,X/45,X,-22,+t(X;22) is listed in Borgaonkar and Dolling (1977), and the description of this case and the X inactivation pattern is awaited with interest.

As further patients with unbalanced X;autosome translocations are described, and techniques for the precise localisation of X inactivation improve, a clearer pattern of the mechanism of X inactivation will emerge. This will confirm or refute the present theories relating to an inactivation centre, its precise location, and how it functions.

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Addendum

Since submitting this report, a relevant paper has been published describing 3 further cases of X; autosome translocation (Zabel, B. U., Baumann, W. A., Pirntke, W., and Gerhard-Ratschow, K. (1978). X inactivation pattern in three cases of X autosome translocation. *American Journal of Medical Genetics*, **1**, 309–317). Two of these, a phenotypically normal mother and her abnormal child had an X;15 translocation. In the chromosomally balanced mother, the normal X was preferentially inactivated, but in the child the abnormal X_t was late replicating in 75% of the cells, whereas in the remainder of the cells the normal X was inactivated. The abnormalities were therefore ascribed to partial trisomy 15q. The third case showed an X;21 translocation in which the normal X was inactivated in 70% and the Xp chromosome in 30% of the cells, but the chromosome 21 bearing the translocated Xp was never inactivated. The abnormal phenotype was ascribed to possible disomy of Xp, as in our patient, and lends further support for the existence of only one inactivation centre located in the proximal part of Xq (Therman and Patau, 1974).