An XO/X Ring X Chromosome Mosaicism in an Individual with Normal Secondary Sexual Development

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Ring chromosomes in man, in association with congenital abnormalities, have been reported involving a chromosome in the 17-18 group (Genest, Leclerc, and Auger, 1963; Gropp, Jussen, and Ofteringer, 1964; Lucas, Kemp, Ellis, and Marshall, 1963; Wang, Melnyk, McDonald, Uchida, Carr, and Goldberg, 1962), a chromosome in the X-6-12 group which is probably not an X (Turner, 1963), a member of the 13-15 group (Smith-White, Peacock, Turner, and Den Dulk, 1963), a No. 1 chromosome (Gordon and Cooke, 1964), and on five occasions an X chromosome (Bain, Gauld, and Farquhar, 1965; Hustinx and Stoelinga, 1964; Lindsten and Tillenger, 1962; Lüers, Struck, and Nevinny-Stickel, 1963; Pfeiffer and Büchner, 1964). We report the findings in a further patient with a presumptive ring X chromosome.

Case Report

The patient was referred for paediatric opinion because at the age of 13 years she was still only 125 cm. tall. However, her secondary sexual development was normal and she first menstruated shortly after her 13th birthday. After a few months without periods she began to menstruate regularly. Two buccal smears taken at this time failed to show sex chromatin, and it seemed possible, that despite her normal secondary sexual development and regular menstruation, she might have a sex chromosome abnormality. She was referred for detailed chromosomal study.

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Sex Chromatin and Chromosome Analysis. Of the 120 nuclei examined in buccal smear preparations, 6 (5%) had a single, normal-sized, sex chromatin body. Sex chromatin bodies are found by this observer (A.M.B.) with a frequency of 25-60% in normal females and are not observed in normal males. Further sex chromatin preparations were obtained from skin culture. These latter preparations were made in parallel with a control culture from a normal female. The preparations derived from the normal female were sex chromatin positive, but of 300 cells examined in the skin culture derived from the propositus, not one showed a sex chromatin body. A blood smear preparation was analysed and 3 drumsticks (0.6%) were found in 500 polymorphonuclear leucocytes. Mittwoch (1964) in an investigation of 12 normal females found a mean drumstick count of 3.66%.

Chromosome counts showed two cell types with 45 and 46 chromosomes, in three independent blood cultures and a skin culture.

Analysis demonstrated XO and XO plus a ring chromosome karyotypes (Table I). The ring chromosome was of variable size (Fig. 1).

The pattern of DNA synthesis was studied in the third blood culture, labelled for 4 hours with tritiated thymidine (Bishop and Bishop, 1963). In all, 300 cells were examined: 41 had a count of 46 chromosomes. 14 of these 41 were labelled, and 8 showed a concentration of labelling over the ring chromosome with slight labelling over the other members of the complement (Fig. 2). These 8 cells were presumably at the end of DNA synthesis when labelling occurred. It was, therefore, assumed that the ring chromosome was a structurally abnormal, late-labelling, X chromosome. A

FIG. 1. Various forms of the ring as observed in different cells. The ring on the left being the size observed in most of the cells.

FIG. 2. A cell, labelled at the end of DNA synthesis, with its corresponding autoradiograph, showing late labelling of the ring.

TABLE I

Tissue	Chromosome Counts					
	45		46		47	
	Counted	Analysed*	Counted	Analysed†	Counted	Analysed‡
Blood culture	1	27	4	3	0	0
	2	69	0	11	0	0
	3	255	4	41	4	4
Skin culture		23	4	3	0	0

* 44A + XO.

† 56 cells were 44A + X ring X. One cell contained 44A + X + acentric fragment and another 44A + X dicentric ring X.

‡ 44A + X ring X ring X.

late-labelling member of the X-6-12 group was not seen in the cells with a count of 45 chromosomes.

It was concluded that the patient was a 44 + XO/44 + X ring X mosaic.

Discussion

A comparison may be made between this patient and the 5 others in whom it was believed a ring X chromosome had been observed (Table II). Although each patient had one or more of the clinical abnormalities characteristic of gonadal dysgenesis (Turner's syndrome), the only abnormal clinical feature common to them all was short stature. Our patient bears some resemblance to Case 3 in that each had normal secondary sexual development and regular periods in association with dwarfism.

A cytological comparison (Table II) shows that all were mosaics of XO and X ring X (XX_R) cells. The frequency of XO cell lines and the size of the ring chromosome in cultures established from these patients were variable and plainly cannot be related to the clinical findings.

Evidence on the origin of these ring chromosomes was obtained from autoradiography and sex chromatin analysis. Late replication, characteristic of heteropycnotic X chromosomes in excess of one, whether structurally normal or abnormal (Gilbert, Muldal, Lajtha, and Rowley, 1962; Muldal, Gilbert, Lajtha, Lindsten, Rowley, and Fraccaro, 1963), was observed for the ring chromosomes studied in Case 4 (Rowley, Muldal, Lindsten, and Gilbert, 1964) and in our patient. It was, therefore, apparent that in these two the ring was an X chromosome. Cases 2, 3, and 5 were sex chromatin positive, though far fewer cells than normal had a sex chromatin body. It is reasonable to assume that the chromosome abnormalities observed in these latter cases also involved the sex chromosome complement and that the ring chromosomes observed were X chromosomes. However, the patient described by Pfeiffer and

Büchner (1964) was sex chromatin negative. Furthermore, the ring chromosome did not appear to be differentially labelled. Pfeiffer and Büchner suggested that the interstitial, late-replicating (Atkins and Gustavson, 1964; Gianelli, 1963) parts of the X chromosome were absent from the ring chromosome observed in their patient. It does, however, seem possible that their ring chromosome was not an X chromosome but a Y chromosome. The Xg^a blood group findings did not clearly distinguish between these alternatives (Nijenhuis and Gemser-Runia, 1964).

Chromosome analysis on a skin culture preparation derived from our patient showed the presence of XX_R cells with a frequency of approximately 11%, yet not one of the 300 interphase nuclei examined for sex chromatin showed a well-defined heteropycnotic body adjacent to the nuclear membrane. Sex chromatin bodies were observed in skin culture preparations by Hustinx and Stoelinga (Case 3) when the frequency of XX_R cells was 1% and by Lindsten and Tillinger (Case 4) when the frequency of XX_R cells was 10% (Table II). However, the ring X chromosome in our patient was smaller than the ring chromosomes observed by these authors. It might be that this ring X chromosome was too small, at least in skin culture preparations, to be visible as a distinguishable heteropycnotic body. Alternatively, it may have been that the frequency of XX_R cells in preparations studied for chromosome analysis was different from that studied for sex chromatin analysis. Furthermore, sex chromatin preparations were made several weeks later than the chromosome preparations, and the XX_R cell line may have been lost due to well-known instability of the ring chromosomes. The observations that we have made on the variable size of the ring and the presence of two rings in some cells are evidence of this instability.

It was concluded that breakage of an X chromosome, during meiosis or early cleavage, led to loss

TABLE II

Reference	Clinical Description	Cytology					
		Chromosome Analysis		Size of Ring Circumference of X _R /Length of Normal X	Sex Chromatin Analysis		Labelling of Ring with Tritiated Thymidine
		Blood culture	Skin culture		Buccal Mucosa	Skin Culture	
Case 1 Pfeiffer and Büchner (1964)	12-year-old girl; 'typical features of Turner's syndrome'; no laparotomy	40% XO cells 60% XX _R cells		Approx. three-quarters	Negative		Ring X not late-labelling; corresponds with observations of unlabelled pericentric and terminal regions of normal X
Case 2 Lüers <i>et al.</i> (1963)	20-year-old woman, 140 cm. height; no menses; infantile secondary sex characters; no laparotomy	73% XO cells 17% XX cells 6% XX _R cells			9%		
Case 3 Hustinx and Stoelinga (1964)	15-year-old girl, 137 cm. height; menstruation normal; secondary sex characters normal; webbed neck; small mandible and syndactyly; no laparotomy	88% XO cells 12% XX _R cells	99% XO cells 1% XX _R cells	Approx. 1.0†	5%	9.6%	
Case 4 Lindsten and Tillenger (1962)	22-year-old woman, 143 cm. height; menstruation regular at first but later oligomenorrhoea; well-developed secondary sex characters; slight cubitus valgus; scarcity of primordial follicles and only 2 graafian follicles	63% XO cells 35% XX _R cells	90% XO cells 10% XX _R cells*	0.6-1.0	Negative	Positive	Ring X the only late-labelling X
Case 5 Bain <i>et al.</i> (1965)	5-year-old girl, 92.5 cm. height	33% XO cells 63% XX _R cells		Variable	10%		
Our case (1965)	13-year-old girl, 125 cm. height; menstruation normal; secondary sex characters normal; no laparotomy	86% XO cells 14% XX _R cells	89% XO cells 11% XX _R cells	Variable up to three-quarters	5%	Negative	Ring X the only late-labelling X

* Culture of biopsy specimen of ovary yielded 100% XO cells. Culture of lymph node yielded 90% XO and 10% XX_R.

† Estimated from authors' data.

of chromosomal material and ring formation giving rise to the XX_R cell line. It is probable that subsequent loss of the unstable ring X chromosome gave rise to the XO cell line.

Summary

A 13-year-old girl with XO/X ring X mosaicism is reported. The only clinical abnormality observed was dwarfism. The investigations on the sex chromatin and chromosomes in buccal smear, skin,

and blood and autoradiographic studies are discussed. The cytological and clinical data are compared with those in previously described patients.

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