Three patients with ring (X) chromosomes and a severe phenotype

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Abstract
Three patients with mosaicism and a cell line containing a small ring (X) chromosome are described. Their phenotype is similar to several previously reported patients with a 45,X/46,X,r(X) karyotype and a phenotype far more severely affected than expected in Turner's syndrome. The clinical picture includes mental retardation, a facial appearance reminiscent of the Kabuki make up syndrome, and limb anomalies. Some of the patients also had streaky hyperpigmentation of the skin in a pattern suggesting dermal mosaicism. It has been hypothesised that the severe phenotype might be the result of the small r(X) chromosome remaining active. However, there is little critical evidence to support this suggestion, while there is considerable evidence against it, including (1) a similar phenotype in 45,X/46,X,r(Y) patients, (2) the late replication of some of the small r(X) chromosomes associated with this phenotype, and (3) the expression of XIST in some of the affected patients.

The advent of in situ hybridisation with chromosome specific DNA probes has made it possible to identify small structurally abnormal chromosomes. There have been several reports in the last few years of patients with small r(X) and r(Y) chromosomes associated with unexpectedly severe phenotypic effects including mental retardation and a dysmorphic appearance. We describe three further females with small r(X) chromosomes and discuss the range of clinical effects seen in this group of patients, their relationship to other recognised syndromes, and hypothetical pathogenetic mechanisms.

Case reports and laboratory findings

CASE 1
Case 1 (89-3927) was the first child of a healthy 17 year old mother and 20 year old father. She was born by caesarian section and weighed 1600 g after 37 weeks' gestation. Oligohydramnios, growth retardation, and dilatation of the left renal tract were noted during pregnancy. At birth she had short tapering fingers which were clasped across the palms, but could almost be straightened if the wrists were flexed. She was subsequently found to have bilateral grade IV vesicoureteric reflux requiring ureteric reimplantation. Echocardiography showed mild aortic valve stenosis with a gradient of 20 mm Hg.

On examination at the age of 20 months her length (69 cm) and head circumference (42 cm) were both well below the 3rd centile. She was severely developmentally delayed, functioning at an approximately 6 month level. Her finger contractures had improved but the fingers were short and tapering with clinodactyly of the little fingers. Her face was dysmorphic with a thin upper lip, hypertelorism, and wide palpebral fissures (fig 1).

The baby subsequently suffered from recurrent wheezing and chest infections. Her developmental and growth delay persisted. At the age of 33 months her length was 3-4 SD and her weight 2-3 SD below the mean for her age. She died at the age of 35 months.

The cyogenetic results have been reported in full by Jacobs et al. Briefly the majority of the patient's blood leucocytes had a 45,X constitution, the X being inherited from the father. However, 15 of 200 cells had a ring (X) smaller than a G group chromosome while nine cells had two ring chromosomes, one smaller than a G group and one minute. In situ hybridisation with the X centromere probe pSV2X5 confirmed that both ring chromosomes were derived from X chromosome material, presumably that of the mother. Studies of the replication status of the two ring chromosomes were attempted using a terminal pulse of BrdU, but both rings were found to be too small to give accurate results.

CASE 2
Case 2 (89-1479) was the second child of a healthy 32 year old mother and 33 year old father. The pregnancy was complicated by polyhydramnios and she was born after spontaneous labour at 37 weeks' gestation, weighing 2950 g. Limb abnormalities were noted at birth. She had a short right arm and the right hand had a thumb and two fingers only, all digits being broad and short. The left arm and hand were normal in size but all the digits were broad and short. The right leg and foot were normal but the left femur was slightly short and the left fibula was absent. There was syndactyly of the lateral three toes. The unequal leg length necessitated a below knee amputation at 8 years.

As a baby she fed badly owing to palatal incoordination and had slow weight gain. Daily injections of growth hormone started at the age of 7 produced a good growth response. She wore glasses from 10 months old for hypermetropia and had recurrent ear infections. Echocardiography showed a bicuspid aortic valve and dilated aortic root. Renal ultrasound was normal. When examined aged
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10 years her head circumference was 52 cm (50th centile). She had strikingly long palpebral fissures (outer canthal distance 120 mm (above the 97th centile), inner canthal distance 34 mm (75th to 97th centile)), prominent ears, narrow palate, smooth philtrum, thick hair, short neck, and low posterior hairline (fig 2). She was moderately mentally retarded (IQ around 65) and her height was just below the 3rd centile after 2½ years of growth hormone and oestrogen therapy.

The initial cytogenetic results were reported by Jacobs et al. Briefly she was found to be a 45,X/46,X,r(X) mosaic with approximately equal proportions of the two cell lines in blood lymphocytes. The normal X was maternal in origin. The ring was smaller than a G group chromosome and was shown by in situ hybridisation with the X centromere probe pSV2X5 to be derived from an X, presumably that of the father. At that time replication studies of the ring chromosome were considered inconclusive because of its small size. Subsequently a cyst was removed from the right side of her neck and fibroblasts from this tissue cultured for cytogenetic analysis. Thirty-six cells had a 45,X complement and 42 a 46,X,r(X) complement. Replication studies on the fibroblasts containing the ring gave unequivocal results on 18 cells, of which 17 appeared late replicating (fig 3) and one early replicating. mRNA was extracted from the cultured fibroblasts and tested for XIST expression and on two occasions there was clear evidence of XIST expression suggesting inactivation of the r(X) in at least a proportion of cells.2

CASE 3
Case 3 (91-1113) was the second child of a healthy 25 year old mother and 36 year old father. She was born after a 36 week pregnancy in which fetal movements were thought to be reduced, weighing 2300 g. She breathed spontaneously but was hypotonic and had early feeding difficulties. Slow development was evident from birth. Streaky skin markings were noticed for the first time at 9 months. From the age of 1 year she had occasional grand mal seizures.

At the age of 7 years her height was 107 cm (below the 3rd centile), her head circumference was 54 cm (90th to 97th centile), inner canthal distance 3.7 cm (above the 97th centile), interpupillary distance 6.0 cm (97th centile), and outer canthal distance 8.5 cm (75th centile). She was severely mentally handicapped, unable to stand alone, with no speech and few social responses. Her palpebral fissures appeared wide, with a slight upward slant, her nasal bridge was broad and prominent, the alae nasi were hypoplastic, the nostrils anteverted, and the columella short. Her
eyebrows were highly arched and her eyelashes long. She had low set ears, a thin upper lip, and a long chin (fig 4). Her fingers were thick and tapering and there was clinodactyly of the little fingers and soft tissue syndactyly of the second and third toes. She was moderately hypotonic and had generalised joint laxity. The skin felt loose and dry, and was scaly over the lower limbs. There was hyperpigmentation in streaks and whorls affecting the skin of the neck, shoulders, axillae, flanks, and groins.

The patient was referred for chromosome studies as a neonate in 1984 and on standard G band analysis was found to have a 46,XX/46,X,+mar karyotype. The marker was present in nine of 62 cells (14%) and appeared to be a small ring of unknown origin. The mother had a normal chromosome constitution while the father had a 47,XXY constitution in all 30 cells examined. In 1992 we repeated the cytogenetic studies in order to determine the origin of the ring chromosome. We obtained a blood sample and two skin biopsies, one from a normal area and one from a hyperpigmented area. Among the 73 cells scored from the lymphocyte culture, six (8%) were found to have 46 chromosomes including the ring which was about the size of a G group chromosome, the remainder having a normal female complement. All 50 fibroblasts from the normal skin biopsy had a 46,XX constitution while of the 46 cells scored from the hyperpigmented skin biopsy 44 were 46,XX and the remaining two had a 45,X constitution. In situ hybridisation of the leucocyte cultures using the X centromere probe pSV2X5 showed the ring chromosome to be of X chromosome origin.

A further blood sample was obtained in order to study the replication status of the r(X) using a terminal pulse of BrdU. Two hundred cells were scored; 184 had a 46,XX constitution with a single late replicating X chromosome, seven had a 45,X chromosome constitution the single X being early replicating, and nine had a 46,X,r(X) constitution. In seven of the nine cells with a r(X) the ring was clearly late replicating, while no decision was possible on the remaining two. Thus the ring appears to be late replicating in all, or the great majority, of cells in this patient. While nine cells were found to have a 45,X constitution, they represented only 2% of the 431 cells scored and we do not regard this as clear evidence of a 45,X cell line.

DNA from this patient and her mother was tested with two probes, DMD43 and 5'DYSII4, both recognising sequences from within the dystrophin gene on Xp21. In both instances the patient was clearly seen to have inherited one allele from the mother and one from the father excluding uniparental disomy for the normal X chromosome.

**Discussion**

Berkovitz et al9 reviewed 36 reported patients with ring (X) chromosome without mentioning mental retardation. Before the use of molecular cytogenetics, however, the origin of small rings was unclear and those associated with mental retardation would presumably have been assumed to be autosomal.

Kushnick et al10 drew attention to the occurrence of mental retardation and dysmorphic features in two patients with a 45,X/46,X,r(X) karyotype. Grompe et al11 reported a further case. In all three cases the rings were small. Van Dyke et al12 noted that, of 15 patients with a ring (X) chromosome among a series of cases of Turner's syndrome, six were mentally retarded. Their ring chromosomes were smaller than those of the mentally normal cases. A later paper9 gives the clinical details of these patients plus two further patients with mental retardation and small ring (X) chromosomes. In the authors' experience, all patients with a 45,X/46,X,r(X) karyotype where the ring (X) was smaller than a chromosome 20 had mental retardation; however, in a note added in proof they refer to a further case of their own plus two published patients911 with a small ring (X) chromosome and normal intelligence. Reviewing their eight patients plus 13 previously reported patients with small ring (X) chromosomes, van Dyke et al11 conclude that, compared with Turner's syndrome, these patients showed an increased frequency of mental retardation (86%), seizures (54%), strabismus (at least 38%), ear infections (at least 33%), and pigmentary dysplasia (at least 19%). They were shorter and had smaller heads than the average Turner patient.

It is clear that retarded ring (X) patients as a group have a greater degree of facial dysmorphism than is expected in Turner's syndrome. Comparing the published photographs, the common features, not always emphasised by the authors, include hypertelorism, a broad nose, anteverted nostrils, a wide mouth with thin upper lip, and long palpebral fissures which appear to slant upwards in some cases and downwards in others. Our cases 2 and 3 resemble facially cases 2, 5, 6, and 7 of van Dyke et al and our case 1 resembles their case 4 as well as the patient of Grompe et al11 and patient 2 of Kushnick et al. Several patients had syndactyly.

![Figure 4](http://jmg.bmj.com/) Case 3 aged 7 years.
Kajii et al. recently presented a series of 11 Turner patients and one male with hypoplasias, all of whom were thought to show features of the Kabuki make up syndrome. Seven of the 12 had a 45,X/46,X,r(X) karyotype, three had other structural abnormalities of the X, with a 45,X cell line detected in two, one had only 45,X detected, and the male was 45,X/46,X,r(Y). These patients, although photographs are not yet published, presumably had the syndrome discussed above. We had independently noticed the resemblance of our ring (X) patients to Kabuki make up syndrome. Among 62 patients with the latter syndrome reviewed by Niikawa et al., 14 patients showed that (IPI) showed that X;autosome translocations had been the pigmenti (IP1) syndrome. By these patients.

We feel that the cases with small ring chromosomes are reminiscent of but different from Kabuki make up syndrome. They are less likely to show lower eyelid eversion, flattening of the nasal tip, arched eyebrows, and prominent ears seen in most of the Kabuki patients.

The relationship between chromosomal mosaicism and abnormalities of skin pigmentation was reviewed by Thomas et al., 15 on the basis of eight personal patients and 36 other published cases. Four of the patients of Thomas et al. and one from the review of previous publications had abnormalities confined to the sex chromosomes (45,X/46,XY, two cases, 45,X/46,X,r(Y), 45,X/46,X,del(X)(q21), and 45,X/46,XX/46,X,r(X)). Four of these five cases were mentally retarded. The skin in each showed a linear streaked or whorled pattern of hyper- or hypopigmentation which in one case was thought to resemble incontinentia pigmenti and in two hypomelanosis of Ito. The female with a 45,X/46,X,r(Y) karyotype had hypertelorism and a broad nasal bridge, otherwise there is little comment on the facial appearance of these patients.

A female with a 45,X/46,X,r(X) karyotype reported by de Grouchy et al. was thought to have incontinentia pigmenti with evolving skin lesions, mental retardation, and short stature, but not microcephaly. She was omitted from the review by Thomas et al. Her facial appearance is similar to that of our cases 2 and 3 and patients 2, 5, 6, and 7 of van Dyke et al. This patient was thought to indicate a juxta-centromeric location on the X chromosome for the incontinentia pigmenti gene. Familial incontinentia pigmenti (IP2) is now known to map to Xq28.17 Several patients have, however, been described with incontinentia pigmenti and X-autosome translocations involving Xp11.21. Whether these all disrupt the same locus (IP1) is unclear since Gorski et al. showed that the breakpoints were spread over 1250 kb and the genetic basis of IP1 remains problematical.

It therefore appears that there is a syndrome, usually associated with mosaicism for small ring (X) chromosomes but sometimes with ring (Y) and sometimes with 45,X/46,XY mosaicism, of growth deficiency, mental retardation, seizures, a facial appearance reminiscent of Kabuki make up syndrome, in some cases streaky hypo- or hyperpigmentation of the skin, and syndactyly or other limb defects. This syndrome accounts for some cases of hypomelanosis of Ito and possibly some cases diagnosed as incontinentia pigmenti. It is not yet clear to what extent these features are specific to the cytogenetic abnormality. The similarities between the clinical effects of small ring (X) and ring (Y) chromosomes, and of diploid/triploid mosaicism, suggest that the syndrome may be a non-specific effect of mosaicism for a lethal or near lethal cell line, although why a small ring (Y) should be so deleterious is unclear. Failure of small ring (X)s to be inactivated because of the deletion of the X inactivation centre, thought to correspond to the XIST gene located at Xq13, provides an attractive hypothesis for the severe effects seen in patients with a small r(X). It would also explain why only small rings are associated with severe effects.

De Grouchy et al. reported that in their patient the ring (X) did not appear to be late replicating, while Beckmann et al. 14 found that the ring (X) in both of their patients appeared to be early replicating in approximately 30% of the cells in which it was present while Grompe et al. found that the ring (X) was not late replicating in any of the 50 cells examined, and similar observations were made by van Dyke et al. in three of their eight patients. However, a cytogenetic judgement on the replication status of small rings is very difficult and we believe that such observations must be viewed with caution. In the majority of cases where XIST expression has been studied, including case 2 in the present report, it was present, so failure of X inactivation does not appear to explain the severe phenotype. Furthermore, hypotheses based on constitutive activity of ring (X)s would not explain the small number of cases where similar effects have occurred with ring (Y) chromosomes.

The existence of this syndrome has implications for genetic counselling when a r(X) or r(Y) is found at prenatal diagnosis. The survey carried out by van Dyke et al. would suggest that the risk of mental retardation is very high in the presence of a small r(X). We surveyed 22 patients known to us with an X or Y derived ring chromosome for whom information on the size of the ring was available. The rings were classified as small, (< than the size of an F group) or large (> than an F group chromosome). Excluding the three probands described above, clinical information was available on 15 of the remaining 19 patients, eight of whom were examined by one of us. Three of these patients were mentally retarded (IQ 70 or less), one with a large r(X), one with a small r(X), and a female with a small r(Y). Three had minor learning difficulties (IQ approximately
70 to 85), two of whom had large r(X)s and one a small r(X). The remaining nine patients were of normal intelligence (two large r(X), five small r(X), and two small r(Y)). Thus our data suggest that the risk of mental retardation is considerably less than suggested by van Dyke et al. However, it is not possible to assess the risk accurately at this time.

There is now a need both for the collection of unbiased data on the phenotype of patients with r(X) or r(Y) chromosomes and also for the molecular characterisation of such rings in patients with and without severe clinical abnormalities.

Cell lines
Lymphoblastoid cell lines were established from cases 2 and 3 but when these were examined no cells containing the r(X) were found. A fibroblast derived cell line is available from case 2 which contains approximately 60% r(X) cells. It is available from the European Collection of Animal Cell Cultures, PHLS, Porton Down, Salisbury, Wiltshire SP4 0JG, UK, catalogue number DD964.

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