Ring Chromosomes in Two Infants with Congenital Malformations*

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Ring chromosomes in association with congenital malformations have previously been reported in approximately 26 patients. The ring chromosome was apparently derived from one of the X chromosomes in 6 patients (Lindsten and Tillinger, 1962; Lüers, Struck, and Nevinny-Stickel, 1963; Hustinx and Stoelinga, 1964; Bain, Gauld, and Farquhar, 1965; Pfeiffer and Büchner, 1965; Bishop *et al.*, 1966), and was assigned to the A group in one instance (Gordon and Cooke, 1964), and to the B group in another (Rohde and Tompkins, 1965). Three examples of C group ring chromosomes (Smith-White *et al.*, 1963; Atkins *et al.*, 1966a); 8 of the D group (Wang *et al.*, 1962; Bain and Gauld, 1963; Turner, 1963; Reisman, Darnell, and Murphy, 1965; Adams, 1965; Teplitz *et al.*, 1967; Sparkes, Carrel, and Wright, 1967; Gerald *et al.*, 1967), 4 of the E group (Wang *et al.*, 1962; Genest, Leclerc, and Auger, 1963; Lucas *et al.*, 1963; Gropp, Jussen, and Ofteringer, 1964), and one of the G group (Hoefnagel, Schroeder, and Benirschke, 1967) have been reported. Two other infants with ring autosomes of unknown origin have also been described (Fisher, 1965; Atkins, Sceery, and Kennan, 1966b).

This paper presents two examples of infants with ring chromosomes. In one instance the ring chromosome replaces a chromosome of the D group (13-15); the other is a mosaic with one line consisting of normal 46,XY plus a ring chromosome of unknown derivation. The latter case is rare, since, to our knowledge, only one other instance of a trisomic state in which one of the chromosomes is a ring has been reported (Atkins *et al.*, 1966b).

Case Reports

Case 1. This Negro female infant was born at term to a 29-year-old woman who had three other apparently

normal children. Her pregnancy was complicated by hypertension. No history of consanguinity or irradiation was obtained. Birthweight 2268 g., length 45 cm., head circumference 29 cm., chest circumference 28 cm. At birth the infant failed to breathe for approximately 2 minutes, but responded to routine resuscitation procedures. A systolic murmur grade 2/4 was audible at the apex. The ears were noted to be low set with large pinnae. 'Café au lait' spots were noted over the back and ankles. The rest of the physical examination was non-contributory.

When she was 15 months old she was readmitted because of respiratory distress. At this time she weighed 4648 g., her height was 61 cm., and head circumference 37.2 cm. (Fig. 1). According to the mother the

FIG. 1. Case 1 at 15 months, weight 4648 g., height 61.0 cm. Note small stature, alert expression, and low-set ears.

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infant suffered from repeated colds and had had bilateral pneumonia at age 5 months. She was thought to be mentally retarded. Pneumonia was diagnosed clinically and radiologically. She was treated with antibiotics and discharged asymptomatic.

Several days later she again developed acute respiratory distress and was rushed to the hospital where she was pronounced dead on arrival.

Pertinent necropsy findings were as follows: the heart weighed 78 g. There was marked right ventricular hypertrophy and dilatation. The right ventricular wall measured 0.6 cm. in thickness compared to the left ventricular thickness of 0.4 cm. An interventricular septal defect was present in an unusual location, high in the posterior portion of the interventricular septum, 1 cm. from the posterior wall of the left ventricle and 1 cm. below the aortic cusps; it was roughly triangular in shape and measured 3 cm. in greatest diameter. Also present was an interatrial septal defect of the ostium primum type. This measured 0.8 cm. in diameter. The right atrium was also moderately dilated. The valves showed no abnormalities.

The lungs together weighed 110 g., and showed severe chronic passive congestion and oedema. The intrinsic pulmonary arteries in some sections of lung showed intimal thickening with fibrosis and accumulations of lipophages in the intima. Acute and chronic bronchitis and bronchiolitis were also present. The larynx and epiglottis were hyperaemic and oedematous. The liver weighed 220 g. and showed moderate chronic passive congestion. The spleen, pancreas, kidneys, and gastro-intestinal tract were not remarkable.

Microscopical examination of the ovaries showed a moderate reduction of primordial follicles compared to that usually seen in ovaries of this age-group. An unusual finding was bilateral hypertrophy of the adrenal medullae. The medullary tissue was estimated to be at least three times as great as that normally seen in this age-group. The adrenal cortex was not unusual.

Cytogenetic studies. During life, chromosomes were studied on three peripheral leucocyte cultures and on a culture of skin fibroblasts (Fig. 2). The results are shown in Table I.

TABLE I
CHROMOSOME COUNTS OBTAINED FROM CELLS
OF CASE 1

Examina- tion	< 44	45	45R	46R	47R	92	Total Cells Counted	No. of Karyo- types Studied
Blood 1	4	1	24	108	1	2	140	13
Blood 2	4	0	8	137	0	1	143	16
Blood 3	1	0	10	138	0	1	150	12
Skin	2	2	7	38	1	0	50	11

A ring chromosome was present in all cells with a count of 46 chromosomes but was variable in shape and size, resembling a figure eight in some metaphases, a doughnut in others, and interlocked circles in some others (Fig. 3). In the cells containing a ring, only 5

morphologically normal large acrocentrics were found. Metaphases with only 45 chromosomes including a ring were found to be broken cells with a random loss of a chromosome. Only 3 metaphase plates were found in which the ring appeared to be the lost chromosome. No cells with two rings were observed.

Buccal smears were positive for sex chromatin. The mother's chromosome study revealed a normal 46,XX pattern. The father did not consent to chromosome studies.

FIG. 3. One D group chromosome and the ring chromosome from each of 8 karyotypes from Case 1. Note that there is considerable variation in the size and shape of the ring in different cells. Satellite association is shown between the large acrocentric chromosome and the ring chromosome at the top of the first column; these two chromosomes are shown in the same relation that they bore to each other in the metaphase plate, and this association is seen at the point where the chromosomes appear to be in contact.

Dermatoglyphs. Analyses of the hand and sole prints, kindly performed by Dr. H. Warner Kloepfer, are expressed in the following formulations:

Palms:

Rt. 9.7.5'.1.O.A'. O.O.O.L 30:34:32
7.5.12

Lt. 7.5.3.1.t.L'. O.O.O.L 37-29.34 3.13.0.20

Soles:

Rt. L^d.L^p.K^p.L^p

Lt. L^d.L^p.L^p.L^p

Ulnar loops were found on all digits except the thumb which had a whorl on each hand.

Case 2. This male Negro infant was born to a 27-year-old mother, para 4, gravida 4. There was no history of consanguinity or irradiation. The father's age was 21. Gestation time 7 months, and birthweight 1785 g. General condition at birth appeared good but various anomalies were noted. The ears were low set and large, with pendulous lobes and a pre-auricular

FIG. 2. Metaphase plate and karyotype from skin fibroblast culture in Case 1 showing the ring in the 13-15 group. The two X chromosomes in the lower line at the extreme right are not labelled.

FIG. 4. Case 2 at age 3 months. Note abnormal ear lobes.

sinus on the left side (Fig. 4). Micrognathia and hypertelorism were noted, and the feet had a rockerbottom appearance. There were 5 nipples: 3 in the left and 2 in the right mammillary line. The right testicle was undescended, and a grade I hypospadias was present.

At the age of 5 weeks a harsh systolic murmur grade 4 was noted; the heart rate was 200/min.; and clinical diagnosis of pulmonary stenosis was made.

At the age of 3 months development and progress were considered excellent. The baby's weight at this time was 4280 g., height 56.2 cm., head circumference 39.2 cm.; chest circumference 34.5 cm. (Fig. 5). However,

days. Fibroblasts were cultured from a skin biopsy specimen obtained at age 3 months.

Both blood and skin studies showed mosaicism, with a cell line having 47 chromosomes including a ring (Fig. 6), and another cell line with 46 chromosomes (Fig. 7). The chromosome counts are shown in Table II. No

TABLE II
CHROMOSOME COUNTS OBTAINED FROM CELLS
OF CASE 2

Examination	< 46	46R	46	47R	92	< 92	Total Cells Counted	No. of Karyotypes Studied
Blood 1	16	2	59	23	0	1	101	13
Blood 2	7	0	54	29	0	0	90	11
Skin	2	1	46	32	2	0	83	13

significant difference in the percentage of cells with a ring was found in either of the blood studies or in the fibroblast culture studies. The ring chromosome showed variation in shape and size. It appeared very rarely as a perfect ring and more often resembled a figure eight stained intensely throughout. Its size in most metaphases was comparable to the chromosomes of group F (19-20) (Fig. 8). Metaphase plates with a count of 46 including a ring chromosome, and those with less than 46 with no identifiable ring, were found to be broken cells with a random loss of a chromosome. No cells with two rings were detected.

Chromosome studies on cultures of peripheral blood leucocytes of the patient's mother showed a normal female complement. The infant's father was not available for study.

Dermatoglyphs. Patterns were analysed by Dr. H. Warner Kloepfer.

Palms:

Rt. 7(8) .5" (6) 5'1.t.L^r O.O.O.L
Lt. 7.5" .3^h .2.t.L^r L/O.O.L

FIG. 5. Case 2 same age. Note accessory nipples, two on the left and one on the right, hypertelorism, and micrognathia. An umbilical hernia is also present.

ocular hypertelorism was conspicuous and a left divergent strabismus was noted.

Cytogenetic Studies. The patient's buccal smear was chromatin negative.

Two cultures of peripheral leucocytes for chromosome studies were done at the ages of 9 days and 32

FIG. 6. Metaphase plate and karyotype from skin fibroblast culture of Case 2. The X and Y chromosomes are not labelled. The ring is shown at bottom right. This represents a cell from one of the two cell lines in this mosaic.

FIG. 7. Case 2: Normal male metaphase plate and karyotype representing the second cell line in the mosaic. The X and Y chromosomes are not labelled.

Soles:
Rt. L^p.O.O.L^d
Lt. L^p.O.L^d.L^d

Among the 6 digits in which the pattern could be determined, there was an ulnar loop on digits 1 and 3 of the right hand and 1 and 4 of the left. There was a whorl on digit 4 of the right hand and on digit 3 of the left.

FIG. 8. An F group chromosome and the ring chromosome from each of 8 karyotypes of Case 2. The selection of a chromosome of the F group was a random choice and serves only to indicate the relative size of the ring.

Discussion

In Case 1 there is little doubt that the ring is one of the chromosomes of Group D. A summary of the clinical features of the other cases of ring D reported and of our patient is given in Table III. It is now possible with autoradiography to attempt to separate members of the D group. Of the 8 cases reported in the literature with ring D chromosomes only 2 have had autoradiographic studies (Sparkes *et al.*, 1967; Gerald *et al.*, 1967). In the case reported by Sparkes *et al.*, the ring chromosome was identified as D₂. The absence of thumbs and other anomalies common to his case and two others (Adams, 1965; Bain and Gauld, 1963) suggest that the ring chromosome was also a D₂ chromosome in the latter two cases. In the case of Gerald *et al.* the D₁ chromosome was thought to be the chromosome affected.

Episodes of respiratory infections, 'cafe au lait' spots, low-set protruding ears, mental retardation, and failure to thrive were found in both the case reported by Teplitz *et al.* (1967) and in our case. In the cases of Wang *et al.* (1962) and Reisman *et al.* (1965) somewhat similar findings were noted, though 'cafe au lait' spots and respiratory infections

TABLE III
SUMMARY OF CLINICAL FEATURES IN PATIENTS WITH A RING D

Authors	Sex	Age	Clinical Features
Wang <i>et al.</i> (1962)	M	5 mth.	Failure to thrive, odd facies, large abnormal ears, small mandible, epicanthic folds, inguinal and umbilical hernias, high-arched palate, simian crease, mental retardation
Bain and Gauld (1963)	F	Still-born	Absence of rt. thumb, rudimentary thumb on lt., talipes equinovarus, short hallux, fusion of 4th and 5th toes, simian creases, bilateral microphthalmus, ant. defect of falx cerebri, fusion of cerebral hemispheres, arrhinencephaly, interventricular defect, overriding aorta, hypoplastic kidneys, bicornuate uterus
Turner (1963)	F	30 yr.	Short stature, moderate mental retardation
Reisman <i>et al.</i> (1965)	M	20 mth.	Odd facies, large abnormal ears, diffuse eczematoid rash, hypospadias, bilateral undescended testes, mental retardation
Teplitz <i>et al.</i> (1967)	M	2½ yr.	Numerous episodes of pneumonia and upper respiratory infections since birth, failure to thrive, low-set and protruding ears, high-arched palate, 'cafe au lait' spots over chest, small genitalia, hypospadias, mental and motor retardation
Sparkes <i>et al.</i> (1967)	M	5 yr.	Mental and physical retardation, imperforate anus, hypospadias, trigonocephaly, abnormal ears, colobomata of irides, epicanthal folds, micrognathia, systolic murmur, ambiguous genitalia, absent thumbs, syndactyly of second and third toes
Gerald <i>et al.</i> (1967)	M	5 yr.	Systolic murmur, microcephaly, plantar valgus deformity of both feet, peculiar overriding toes, mental and growth retardation, anomalous inheritance of haptoglobin type
Adams (1965)	F	?	Microcephaly, holoprosencephaly, hypertelorism, ptosis of eyelids, epicanthal folds, malrotated rt. pinna, micrognathia, hypoplastic lt. nipple, interventricular septal defect, congenital hip dysplasia, aplasia of thumbs, failure to thrive
Present case	F	15 mth.	Failure to thrive, low-set ears with large pinnae, 'cafe au lait' spots over back and ankles, frequent respiratory infections, interventricular and interatrial defect, bilateral hypertrophy of adrenal medullae

are not recorded. In contrast to these 4 cases other patients with D rings have shown gross malformations, especially of the limbs.

The identity of the ring in our second case cannot be established from the available data. The line of cells containing the ring represents a partial trisomy. Since the buccal smears were negative for sex chromatin the ring is probably not derived from the X chromosome. The congenital malformations present indicate a probable derivation of the ring from an autosome rather than from the Y chromosome, since the XYY syndrome is usually accompanied by a more nearly normal phenotype.

Size of the ring chromosome itself cannot be used for identification, since the amount of chromosomal deletion associated with the formation of the ring chromosome is unknown. Comparison of the phenotype with that of other known trisomies might be useful, but in this case the findings include stigmata reminiscent of both the D and E trisomy syndromes. Furthermore, the presence of mosaicism makes comparisons still more difficult. No conclusion concerning the identity of the ring can be reached on clinical grounds.

The cytogenetic findings in our second case can be explained in several ways. If the abnormality were entirely postzygotic two errors could explain trisomy, ring formation, and mosaicism. Non-disjunction in a division following the zygotic cleavage could give rise to mosaicism with a normal cell or cells, a trisomic cell, and a cell which, lacking an autosome, would presumably therefore disappear: ring formation would then occur in one of the trisomic chromosomes. If abnormal gametogenesis is involved, three errors must be postulated: non-disjunction in a gamete to give rise to a trisomic zygote, ring formation in one of the trisomic chromosomes occurring either in the gamete or postzygotically, and mosaicism which must of course be postzygotic. In this latter explanation the order of the first two errors can be reversed, resulting in what seems the most likely sequence of events: ring formation→non-disjunction→postzygotic loss of the ring in some cells.

In the case reported by Atkins and collaborators, instability of the ring, with progressive loss of the ring, was observed in repeated chromosome studies over a period of 13 months and also in prolonged fibroblast cultures. No tendency to ring loss has been demonstrated in prolonged fibroblast cultures of the skin of our patient. A recent chromosome study of peripheral blood leucocytes at the age of 18 months indicated no significant difference in the percentage of cells containing the ring.

Summary

Two unrelated infants with multiple congenital malformations and ring chromosomes in peripheral leucocyte cultures and skin fibroblast cultures are reported. The first patient, a Negro female, who died at the age of 15 months, had failed to thrive since birth. She had low-set ears, repeated respiratory infections, 'café au lait' spots, and congenital heart defects. The chromosome complement was 46, with a ring replacing one of the chromosomes of the D group (13-15). Eight other cases of patients with ring D chromosomes are reviewed.

The second infant had abnormal ear lobes,

polythelia, unilateral cryptorchidism, micrognathia, hypertelorism, and congenital heart disease. This patient showed 46/47 mosaicism, with an extra chromosome which was a ring of unknown derivation.

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