An Unstable Ring Chromosome in a Female Infant with Hypotonia, Seizures, and Retarded Development

LEONARD ATKINS, ROBERT T. SCEERY, and MAURICE E. KEENAN

From the Departments of Pathology and Pediatrics, Harvard Medical School; the James Homer Wright Pathology Laboratories, the Joseph P. Kennedy, Jr. Laboratories of the Department of Neurology, and the Children's Services, Massachusetts General Hospital, Boston, Massachusetts, U.S.A.

The occurrence of a ring autosome in human cells has been described in association with congenital abnormalities and mental retardation (Turner, Jennings, Den Dulk, and Stapleton, 1962; Wang, Melnyk, McDonald, Uchida, Carr, and Goldberg, 1962; Genest, Leclerc, and Auger, 1963; Lucas, Kemp, Ellis, and Marshall, 1963; Bain and Gauld, 1963; de Grouchy, Lévêque, Debauchez, Salmon, Lamy, and Marie, 1964; Gropp, Jussen, and Ofteringer, 1964; Gordon and Cooke, 1964). A ring X chromosome has been reported in a few cases of ovarian agenesis (Lindsten, 1963; Lüers, Nevinny-Stickel, and Struck, 1964), and ring chromosomes have been noted in neoplasms (Levan, 1956) and in irradiated cells (Bender and Gooch, 1962).

The present report describes an infant who was found to have mosaicism in cultured blood and skin cells. One cell line was normal and another line contained a ring chromosome in addition to the normal complement.

Case Report

This female infant was admitted to the hospital 7 days after birth because of apnoeic spells associated with cyanosis starting at 10 hours of age. The pregnancy was uneventful with a normal delivery, birth weight 6 lb. 9 oz. (2976 g.) The parents were unrelated and normal, the father being 28 and the mother 27 years old. There were two normal brothers of the proposita, aged 5 and 3. There was no history of irradiation exposure.

Physical examination showed a hypotonic infant with an occasional high-pitched cry. The ears were large with tufts of hair protruding from the midportion of each helix. The finger- and toe-nails were poorly developed. Otherwise, the examination was negative.

The white cell and differential counts were normal, as

was a routine urinalysis. The serum calcium was 10.2 mg., phosphorus 5.7 mg., and blood sugar 63 mg./100 ml. A lumbar puncture yielded normal cerebrospinal fluid.

The first four days in the hospital were uneventful. On the fifth day there was another apnoeic spell associated with cyanosis and lasting about 2 minutes. There were no unusual movements. An electroencephalogram, an electrocardiogram, and X-rays films of the skull were normal. Subsequently, there were no further apnoeic spells, and the patient was discharged on the 11th hospital day.

Although phenytoinum sodium and phenobarbitonum were administered, apnoeic spells continued with tonic seizures during several febrile illnesses associated with coryza. During the seizures the eyes rolled back, she became rigid, and, after 1-2 minutes, cried out, became limp, and then went to sleep.

Motor development during the first year of life was slow. She smiled at 3 to 4 months and could raise her head at 4 months. At the age of 10 months she could roll over, sit up with support, crawl, and say 'da, da'. She was very hypotonic but jabbered and appeared alert but 'lazy'. The first tooth erupted when she was 8 months old. From the 11th to 14th month there were no further seizures, despite stopping phenytoinum sodium and phenobarbitonum therapy and the occurrence of two febrile illnesses during this time.

Examination at the age of 14 months showed a well-nourished, alert baby with marked hypotonia. The weight was 21 lb. (9.5 kg.), the height 35 in. (89 cm.) and the head circumference 48 cm. No tonic neck reflexes or Moro reflex were demonstrable. The plantar responses were extensor. There was no ankle clonus, bilaterally. The leg muscles were weak, and the patient was unable to sit without support, though she could crawl over obstacles. Speech consisted of jabbering. There was no mongoloid slant to the eyes. The eyelashes were straight and elongated, measuring 1.1 cm. in length. The irides were blue with Brushfield spots. There was an alternating esophoria. Seven teeth had erupted. The ears were enlarged and low-set; the previously noted tufts of hair were no longer present. The finger- and toe-nails were normal. Cutaneous dimples were present over the lateral condyles of the elbows and over both patellae. Examination of the chest

Received October 29, 1965.

* Supported by U.S. Public Health Service Research Grant No. NB-04662 from the National Institute of Neurological Diseases and Blindness.

TABLE I
CHROMOSOME COUNTS OBTAINED FROM CELLS OF PROPOSITA

	44	45	46	47	48	92 (4n)	94 (4n)	Total	% Cells with Ring Chromosome
Blood I, age 11 dy.	—	2	102	29	—	—	1	134	22.4
Blood II, age 18 dy.	—	2	112	41	1	2	3	161	26.7
Skin, age 18 dy.	1	3	136	14	1	4	1	160	10
Blood III, age 7 mth.	—	4	88	20	—	2	—	114	17.5
Blood IV, age 13 mth.	3	3	252	26	—	—	—	284	8.4

and abdomen was negative, and the external genitalia were normal. An electroencephalogram at this time was normal.

Cytological Data. One normal-sized sex chromatin body was present in 36% of buccal mucosa cells and in 23% of cultured skin cells.

Four leucocyte cultures for chromosome studies were done by the method of Moorhead, Nowell, Mellman, Battips, and Hungerford (1960) at the ages of 11 days, 18 days, 7 months, and 13 months. A skin biopsy specimen obtained from the patient when she was 18 days old was cultured by the method of Harnden (1960). Both blood and skin showed mosaicism with one normal cell line having 46 chromosomes and another line with 47 chromosomes, including a ring chromosome in addition to a normal complement (Fig. 1). The chromosome counts and the percentage of cells with a ring chromosome are shown in Table I. A progressive loss of the ring chromosome in the four leucocyte cultures occurred so that from about 25% shortly after birth there was a fall to 8.4% in the latest culture done when the proposita was 13 months old. Although the total data obtained from cultured skin showed a much lower percentage of cells with a ring chromosome than a leucocyte culture done at the same time, earlier harvestings showed a percentage similar to the first two short-term leucocyte cultures with a subsequent gradual elimination of the ring, so that after two months it was no longer present in the culture. The ring chromosome showed some variation in size, though in most cells its diameter was about equal to the length of a chromosome in Group 19-20 (Fig. 2). No cells were found to have 2 ring chromosomes.

Chromosome studies on cultured leucocytes of both parents and the two sibs were normal (Table I).

Autoradiographic studies with H_3 -thymidine were

carried out to determine whether the ring chromosome replicated late as an X chromosome. No late-labelling pattern was found. Although certain regions of the late-replacing X tend to complete DNA synthesis before others (Atkins, Santesson, and Voss, 1965), and hence might cause some difficulty in identification in the case of a ring X, one would expect to find at least some of the late-replicating Xs to show diffuse heavy labelling. The latter was not found in this case.

Dermatoglyphic Patterns. A simian line was present in both palms. The *atd* angle was proximal, bilaterally. The fingerprints showed the following patterns bilaterally: I arch, II radial loop, III ulnar loop, IV ulnar loop, and V ulnar loop.

Erythrocyte Antigens. The family data are summarized in Table II. No unexpected combinations were found.

Discussion

The identity of the ring chromosome in this case cannot be established from the available evidence. Since the sex chromatin pattern was normal and the autoradiographic studies were negative, the ring was probably not derived from an X chromosome. Because ring chromosomes are known to be unstable, they can be of different size and contain different amounts of chromosome material in different cells. Therefore, size itself cannot be used as a criterion for identification. In some cases, the phenotype of the patient may give the clue to the identity of the ring chromosome that is present. In

TABLE II

	ABO	Rh	Fy(a)	K	M	N	P	Le ^a	Le ^b	S	s	Wra	Jk ^a	Jk ^b	V
Proposita	o	CDe/cde	+	—	—	+	+	+	—	—	+	—	+	—	—
Mother	o	CDe/cde	—	—	—	+	+	—	+	—	+	—	—	—	—
Father	A ₁	CDe/cde	+	—	—	+	+	+	/	—	+	—	/	/	—
Brother (aged 3)	o							Insufficient sample							
Brother (aged 5)	o	CDe/cde	+	—	—	+	—	—	+	—	+	—	+	—	—

FIG. 1. Metaphase plate and karyotype prepared from a cultured leucocyte. The ring chromosome (R) is placed to the right of the 13-15 group.

FIG. 2. Metaphase plates of cultured leucocytes showing some variation in the size and configuration of the ring chromosome (indicated by arrows). Two rings can be seen in the figure at lower right, in which most isochromatids are separated at the centromere.

the present case, though phenotypically few stigmata associated with the known trisomy syndromes were present, the possibility of the ring being derived from a No. 18, 21, or the large acrocentric chromosome associated with the D¹ trisomy syndrome cannot be excluded. Naturally, the ring may have arisen from any other autosome. Mosaicism with the presence of a normal cell line probably accounts for the comparatively few clinical abnormalities observed.

The cytological findings can be explained by a number of possibilities. Formation of the ring through loss of the distal ends of the arms of a chromosome with union of the broken ends of the centric fragment could have occurred in a parental gonad or during an early stage of the embryo. Subsequently, non-disjunction in meiosis or mitosis would give rise to a cell line with 47 chromosomes. Non-disjunction could have preceded the formation of a ring either in a parental germ cell or in the embryo. A formally possible, though much less likely, situation would involve ring formation in one parental gonad, and non-disjunction of the homologous chromosome leading to a gamete with 24 chromosomes in a gonad of the other parent before zygote formation. Gradual elimination of the unstable ring chromosome resulted in a predominant normal cell line and a diminishing line with 47 chromosomes. In maize, ring chromosomes are unstable with a tendency for small rings to be lost in mitosis and for large rings to show frequent changes in size as a result of breakage-fusion-bridge cycles (McClintock, 1938). In the case under discussion the instability of the ring chromosome was apparent over the course of 13 months. Eventual complete loss of the ring seems likely, and further follow-up studies are planned to confirm this. It is of interest that the ring was also gradually eliminated from skin cells *in vitro*. Lucas *et al.* (1963) noted a tendency for the ring in their case to be lost *in vitro* as the cultures aged. In the latter case the ring was relatively small, being derived from a No. 18 chromosome.

Summary

A 14-month-old female infant with muscular hypotonia, motor retardation, and seizures, is

reported. Cytogenetic studies showed 46/47 mosaicism with an extra chromosome in the form of a ring. The percentage of cells with a ring chromosome decreased from about 25% shortly after birth to 8.4% at age of 13 months, and prolonged culture of skin fibroblasts was associated with a gradual disappearance of the ring.

We wish to thank Misses Ida Leone, Mary Hauck, and Susan Fletcher for technical assistance. The erythrocyte antigen studies were done by Miss Susan Jago of the Blood Bank of the Massachusetts General Hospital.

REFERENCES

- Atkins, L., Santesson, B., and Voss, H. (1965). Partial deletion of an X chromosome. *Ann. hum. Genet.*, **29**, 89.
- Bain, A. D., and Gauld, I. K. (1963). Multiple congenital abnormalities associated with ring chromosome. *Lancet*, **2**, 304.
- Bender, M. A., and Gooch, P. C. (1962). Persistent chromosome aberrations in irradiated human subjects. *Radiat. Res.*, **16**, 44.
- Genest, P., Leclerc, R., and Auger, C. (1963). Ring chromosomes and partial translocation in the same cell. *Lancet*, **1**, 1426.
- Gordon, R. R., and Cooke, P. (1964). Ring-1 chromosome and microcephalic dwarfism. *ibid.*, **2**, 1212.
- Gropp, A., Jussen, A., and Ofteringer, K. (1964). Multiple congenital anomalies associated with a partially ring-shaped chromosome probably derived from chromosome No. 18 in man. *Nature (Lond.)*, **202**, 829.
- de Grouchy, J., Lévêque, B., Debauchez, C., Salmon, C., Lamy, M., and Marie, J. (1964). Chromosome 17-18 en anneau et malformations congénitales chez une fille. *Ann. Génét.*, **7**, 17.
- Harnden, D. G. (1960). A human skin culture technique used for cytological examinations. *Brit. J. exp. Path.*, **41**, 31.
- Levan, A. (1956). Self-perpetuating ring chromosomes in two human tumours. *Hereditas (Lund)*, **42**, 366.
- Lindsten, J. (1963). *The Nature and Origin of X Chromosome Aberrations in Turner's Syndrome*. Almqvist & Wiksell, Stockholm.
- Lucas, M., Kemp, N. H., Ellis, J. R., and Marshall, R. (1963). A small autosomal ring chromosome in a female infant with congenital malformations. *Ann. hum. Genet.*, **27**, 189.
- Lüers, T., Nevinny-Stickel, J., and Struck, E. (1964). Ring chromosomes bei Gonadendysgenese. *Geburtsh. u. Frauenheilk.*, **24**, 173.
- McClintock, B. (1938). The production of homozygous deficient tissues with mutant characteristics by means of aberrant mitotic behaviour of ring-shaped chromosomes. *Genetics*, **23**, 315.
- Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A. (1960). Chromosome preparations from leukocyte cultures from human peripheral blood. *Exp. Cell Res.*, **20**, 613.
- Turner, B., Jennings, A. N., Den Dulk, G. M., and Stapleton, J. (1962). A self-perpetuating ring chromosome. *Med. J. Aust.*, **2**, 56.
- Wang, H. C., Melnyk, J., McDonald, L. T., Uchida, I. A., Carroll, D. H., and Goldberg, B. (1962). Ring chromosomes in human beings. *Nature (Lond.)*, **195**, 733.