Texas Children's Hospital, Houston, Texas

Human Ring Chromosome Syndromes
An "E" Ring Associated with an Abnormal Phenotype *

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The significance of ring chromosomes in maize (McClintock, 1938) and Drosophila (Morgan, 1933) is well known to cytogeneticists. In man it has recently been realized that like other forms of chromosomal aberrations, chromosomes showing ring configuration may as well be associated with anomalous phenotypes. The occurrence of ring type aberrations in humans was first reported by Levan (1956) in neoplastic tissues.

Reports are now available in cytogenetic literature to suggest that this type of morphological alteration may involve a majority of human chromosome groups. In most instances the carriers of ring chromosomes were phenotypically deficient. It is reasonable to suppose that they represent the type of structural changes classically known as deletions, even though cytologically detectable deletions are commonly seen in a linear form.

The chromosomal analysis undertaken in cultured leukocytes of a sixteen month old girl exhibiting a certain degree of phenotypic defects revealed the presence of a ring chromosome replacing an E group member.

Case report

The patient was born in July, 1967 after a normal pregnancy but with delivery complicated by a placenta praevia. The infant had an episode of staphylococcal enteritis at the age of one month, but she responded adequately to antibiotic therapy.

At the time of birth the father was 27 and the mother was 23. The only sibling

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was a normal male infant born in 1961. The only positive family history for congenital malformations was a maternal cousin with a harelip and a cleft palate.

Further examinations were not carried out until the age of 16 months when the infant was seen by her physician, and pediatric, ophthalmologic and neurosurgical consultants because of slow development.

Physical examination at this time revealed the infant to be apathetic, dull, and slow to respond to stimuli. She was unable to stand or walk. She barely crawled or rolled over. Muscle control and general developmental level were interpreted as at the sixth to seventh month. She had a vocabulary of only a few words and had a throaty laugh.

She weighed 17½ lbs. Her head was small and the nasal bridge was wide. Epicanthal folds were prominent. There was weakness of the lateral rectus muscle of both eyes. No Brushfield spots were identified. The tongue was large and thick. There were seven teeth. The skin was dry and scaly and there was a mild carotenemia. In each hand, there was a dual palmar crease bilaterally and the fifth finger was extremely short.

By neurological examination the cranial nerves were intact. There was good muscle strength although there was a hyperextendibility of all joints and a generalized hypotonia, particularly of the lower extremities. Reflexes were equal and normal. There was no evidence of an increased intracranial pressure. Ophthalmic examination revealed no evidence of congenital glaucoma.

An ECG was interpreted as sinus tachycardia. An EEG was interpreted as within normal limits but suspicious because of bursts of high voltage with slow wave activity.

X-rays of the skull, chest, and a bone survey were normal. Laboratory data including blood count, urinalysis, blood urea nitrogen, blood sugar, calcium, phosphorus, alkaline phosphatase, total protein, and PBI were within normal limits.

The parents and the sibling of the propositus were not available for their chromosomal analysis.

Chromosome preparation technique

Temporary chromosome preparations were made by utilizing a modification of the standard technique of peripheral blood culture (Moorhead *et al.*, 1960). The leukocytes were allowed to incubate in the presence of phytohemagglutinin at 37° C. On the third day, the cultures were treated with colchicine. After four hours, the cells were transferred to 1% sodium citrate and fixed in acetic alcohol (1 : 3). The cells were then washed with 45% acetic acid and resuspended in a mixture of acetic acid and alcohol. The suspended leukocytes were flame-dried on slides and stained with 2% Orcein in 45% acetic acid. Well-spread metaphases were photographed at a final magnification of 100X.

Result and discussion

Of the metaphase figures that were photographed before counting the chromosome number, 25 cells were considered as of unbroken complement. Karyotyping of these intact figures suggested the diploid chromosome number as 46; XX female distribution. However, in each of these figures, a regular E group member was represented by an aberrant chromosome appearing as a ring (Figs. 1 to 5). Such a ring chromosome was present even in those cells that were considered incomplete. The ring chromosome was noted in 100% of the recorded cells, and its size in individual metaphases remained fairly constant (Fig. 6).

Wang *et al.* (1962) originally discovered ring-shaped autosomes in non-neoplastic human cells. The propositus had a speech defect, deafness, bilateral epicanthal folds, and bilateral syndactyly of second and third toes. In all the cells with 46 chromosomes, one of the presumptive 18 homologs was replaced by a ring.

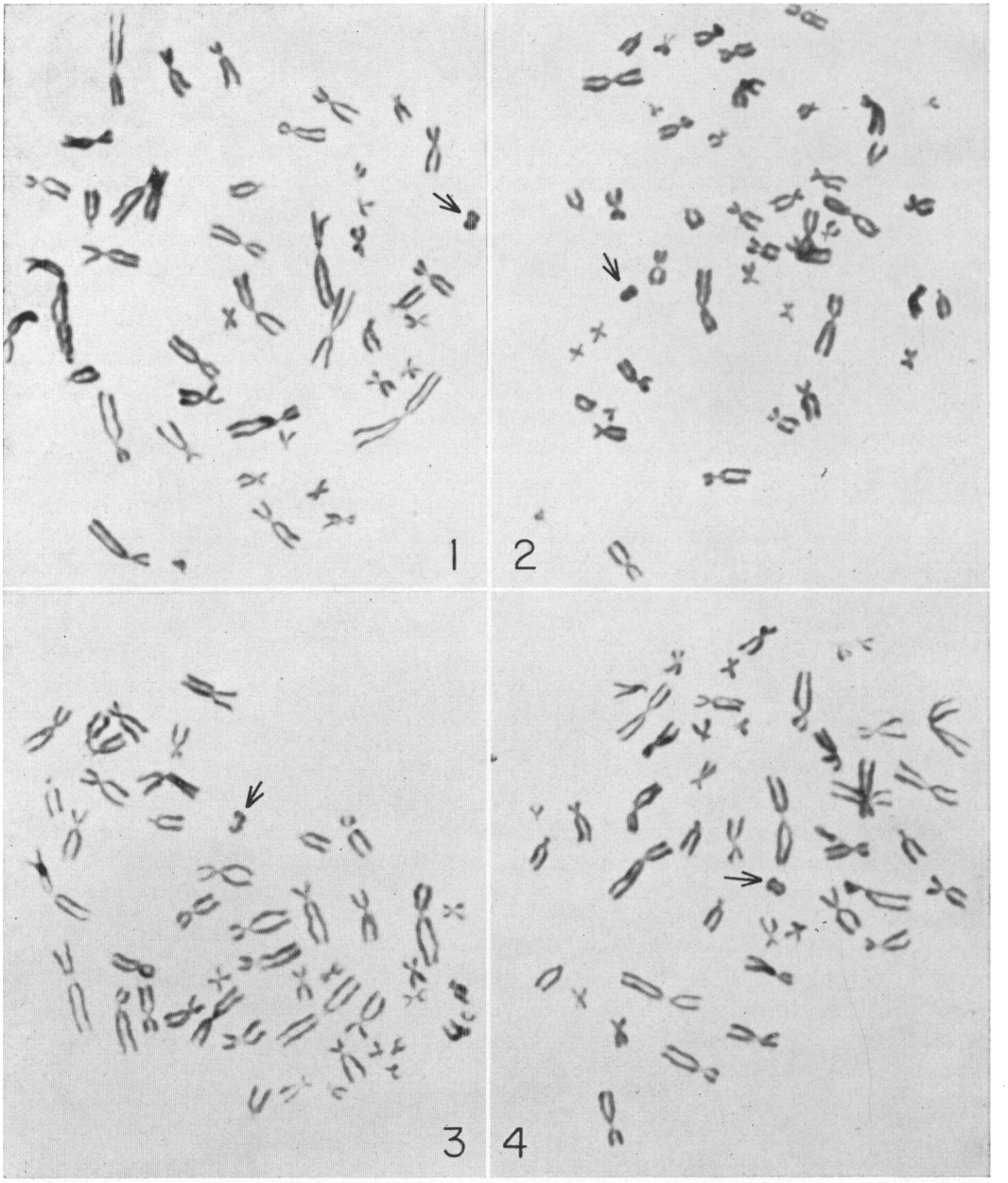
In the following year two female patients (Genest *et al.*, 1963; Lucas *et al.*, 1963) were separately reported with ring chromosomes replacing an E group member. The patient studied by Genest, *et al.* (1963) exhibited one of the D chromosomes with a somewhat elongated short arm in addition to an E group ring. This D-chromosome was presumed to carry the extra chromosomal material which had been deleted from the ring. The other chromosomes of the complement were normal. The clinical findings of the proband were not strikingly different from those described by Wang *et al.* (1962). However, the patient described by Lucas *et al.* (1963) had congenital anomalies limited to a small head and lower jaw, mid-line cleft palate, and dislocation of the left hip. Such a restricted number of birth defects were presumably due to an additional cell line with a normal chromosome complement.

After these reports, Gropp *et al.* (1964) found a ring chromosome replacing an E chromosome in a male child with more severe phenotypic defects.

Recently, Gripenberg (1967) studied an unusual behavior of an E-ring with regard to its distribution and size in the blood cells of a mentally retarded girl. Other clinical features of the patient were not mentioned. Although the majority of the cells were found with rings in place of an E-chromosome, 19% had only 45 chromosomes lacking the ring and an E group chromosome. In a few cells double rings were present, and in 9% of cells this ring chromosome was noticeably enlarged.

Considering the clinical features described in the above cases and those of the present case, it is obvious that they do not conform with a common pattern, even though an E-chromosome is involved in each case. There could be two possible reasons for this disparity. First, the abnormal E group chromosome associated with the different cases may not be the same one. Second, if the ring resulted from the same E group chromosome, the respective deletions of this chromosome must have occurred to variable degrees in the individual patients involved.

With the exception of the findings of Gripenberg (1967), none of the patients, including the present one, had a cell line monosomic for an autosomal homolog.



Figs. 1 to 4. Metaphase leukocytes of the patient showing the aberrant ring chromosome, indicated by arrows

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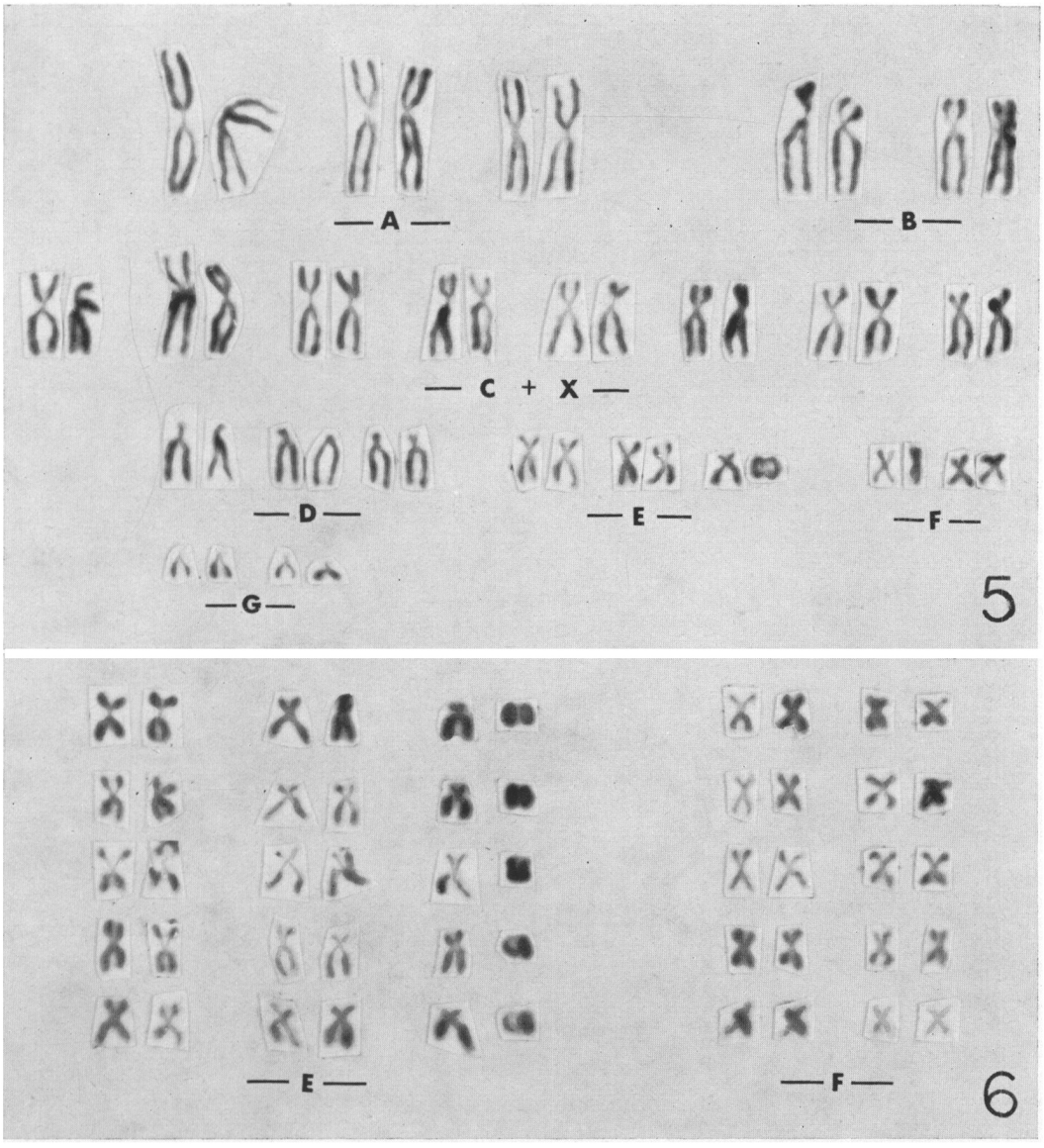

Fig. 5. Karyotype prepared from Fig. 4. Note the presence of a ring-shaped chromosome replacing an E group member

Fig. 6. Cut-out metaphase chromosomes representing E and F groups from additional 5 cells

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Gripenberg had classified a high percentage of cells (19%) as monosomic for an E group chromosome.

The presence of these monosomic cells further strengthens the concept that under *in vivo* conditions a certain number of leukocytes is able to exist with less than diploid autosome number (Sinha, 1967). In the peripheral blood of a phenotypically normal mammal, the somatic chromosome number may be reduced to near-haploid in an appreciably large number of leukocytes (Sinha, 1967). One should, however, bear in mind that the cytological events responsible for the occurrence of chromosomally aberrant leukocytes in the individual case may not be alike.

Thus far, none of the parents contributing offspring with ring chromosomes has been found to be a carrier of the ring. Unfortunately the parents of the present case were not available for chromosomal analysis. Considering the parents to be cytologically normal, abnormal replication responsible for ring chromosome formation must either occur in the parental meiotic division or during early embryogenesis of the proband (Fig. 7).

Considerable variations in the size of the E rings have been noted by Lucas *et al.* (1963) and Gripenberg (1967). Based on McClintock's principle of " breakage-

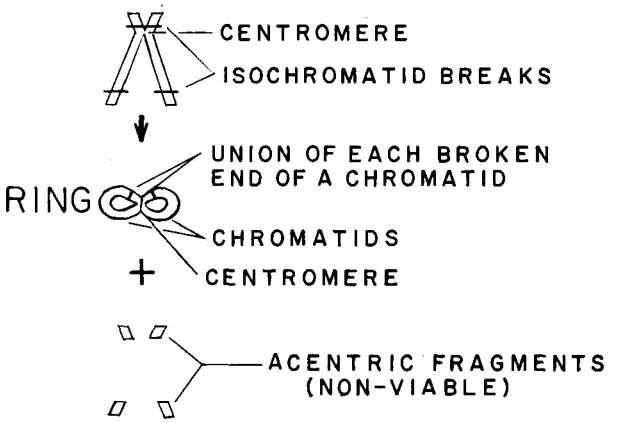

Fig. 7. A tentative scheme showing cytological steps in the formation of an E-ring chromosome

fusion-bridge cycle " for elimination of ring chromosomes in maize (McClintock, 1938), it might be reasonable to suppose, as indicated by Lucas *et al.* (1963) that the population of cells with the ring will gradually decrease as the patient advances in age. On the contrary, the sizes of the rings in the leukocytes of the present case and those studied by Wang *et al.* (1962) were within the normal range of variation. In addition to the consistent size of the rings, 100% of the cells studied in both cases contained the ring chromosome. These pieces of evidence indicate that the ring in these patients will continue replication along with the normal chromosomes during

the entire life-time of these persons. Jacobson (1966) has recently reported a D-ring in an abnormal human male aged 41 years. These human rings presumably have the self-replicating properties similar to those of the X-chromosome rings which have become the permanent part of the complement in certain stocks of Drosophila (Morgan, 1933).

Summary

In peripheral blood cultures of a phenotypically abnormal Caucasian girl, a ring-shaped chromosome was observed in place of an E group member. The rest of the chromosomes of the complement did not show any obvious structural anomaly. The ring chromosome was noted in 100% of the recorded cells, and its size in individual metaphases remained fairly constant. It is suggested that this ring chromosome has become a permanent part of the complement of the patient.

Reference

Genest P. *et al.* (1963). Ring chromosome and partial translocation in the same cell. *Lancet*, **2**: 1426.

Gripenberg U. (1967). The cytological behavior of a human ring-chromosome. *Chromosoma*, **20**: 284.

Gropp A. *et al.* (1964). Multiple congenital anomalies associated with a partially ring-shaped chromosome probably derived from chromosome No. 18 in man. *Nature*, **202**: 829,

Jacobsen P. (1966). A ring chromosome in the 13-15 group associated with microcephalic dwarfism, mental retardation and emotional immaturity. *Hereditas*, **55**: 188.

Levan A. (1956). Self perpetuating ring chromosomes in two human tumours. *Hereditas*, **42**: 366.

Lucas M. *et al.* (1963). A small autosomal ring chromosome in a female infant with congenital malformations. *Ann. Hum. Genet.*, **27**: 189.

McClintock B. (1938). The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. *Genetics*, **23**: 315.

Moorhead P. S. *et al.* (1960). Chromosome preparations of leucocytes cultured from human peripheral blood. *Exp. Cell Res.*, **20**: 613.

Morgan L. V. (1933). A closed X-chromosome in Drosophila melanogaster. *Genetics*, **18**: 250.

Sinha A. K. (1967). Spontaneous occurrence of tetraploidy and near-haploidy in mammalian peripheral blood. *Expt. Cell Res.* **47**: 443.

Wang H. C. *et al.* (1962). Ring chromosomes in human beings. *Nature*, **195**: 733.

RIASSUNTO

In culture di sangue periferico di una ragazza caucasica fenotipicamente anormale, è stato osservato un cromosoma ad anello al posto di un membro del gruppo E. Il resto del complemento cromosomico non presentava evidenti anomalie strutturali. Il cromosoma ad anello è stato riscontrato nel 100% delle cellule esaminate, e la sua grandezza nelle singole metafasi si è rivelata praticamente costante. Si ipotizza che tale cromosoma ad anello sia divenuto un elemento permanente nel complemento cromosomico della paziente.

RÉSUMÉ

Des cultures de sang périphérique d'une fille caucasienne, phénotypiquement anormale, ont démontré un chromosome annulaire à la place d'un membre du groupe E. Le rest du complément chromosomique n'a pas démontré d'évidentes anomalies structurelles. Le chromosome annulaire a été remarqué en 100% des cellules éxaminées, et ses dimensions dans les métaphases individuelles sont résultées pratiquement constantes. L'on suggère que ce chromosome annulaire soit devenu un élément permanent du complément chromosomique de la patiente.

ZUSAMMENFASSUNG

Bei einem phänotypisch anomalen kaukasischen Mädchen wurde in den Kulturen von peripherem Blut anstelle eines Angehörigen der Gruppe E ein ringförmiges Chromosom beobachtet. An den übrigen Chromosomen liessen sich keine Strukturanomalien feststellen. Das ringförmige Chromosom fand sich bei 100% der untersuchten Zellen; seine Grösse blieb praktisch bei den einzelnen Metaphasen konstant. Man vermutet, dass dieses ringförmige Chromosom ein permanentes Element im Chromosomensatz der Patientin geworden ist.

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