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Ring chromosome 4 : Wolf syndrome and unspecific developmental anomalies

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A new case of ring chromosome 4 in a 18-month-old girl is described. The patient presented extreme growth failure, psychomotor retardation, and some features of 4p deletion or Wolf syndrome. No significant loss of genetic material could be seen by G-banding technique (breakpoints p16q35). The ring was found to be unstable both in lymphocyte and fibroblast culture and a substantial proportion of aneuploid cells containing derivatives of the ring could be observed.

An increased cell death-rate could be detected by cell viability determination with trypan blue in the first subculture of skin fibroblasts. It is suggested that this finding is a consequence of behavioural instability of the ring at mitosis existing probably in vivo as well.

The clinical and cytogenetic findings in this patient were compared with those in the other 16 cases with ring 4 published so far. It is suggested that the phenotype in patients with this chromosomal anomaly is a mixture of phenotypic abnormalities generated by both the chromosomal deletion prior to ring formation (features of Wolf syndrome) and the behavioural instability of the ring at mitosis (unspecific developmental anomalies).

A ring chromosome is generally believed to be the result of breakage in each of the terminal segments of a chromosome, the broken ends joining together to give a continuous ring. This mechanism presumes the loss of some genetic material from the end segments of a chromosome, leading to monosomy for the short and/or long arms.

Ring formation of chromosome 4 has been observed up to now in at least 16 cases [1, 2, 4, 5, 7—11, 13, 16—19, 21, 22]. The phenotypic abnormalities in some of these cases formed a clinical picture which resembled the 4p- or Wolf syndrome. Other patients had only a few features of Wolf syndrome, moreover, patients were reported who had practically ne features of the syndrome. Lack of uniformity of the phenotype changes in different cases may be caused by variations in breakpoints and the amount of deleted genetic material lost during ring formation. Since it is difficult precisely to locate the breakpoints in a ring chromosome, attempts to correlate the clinical picture to deletion of specific segments have been made only in 7 out of the 16 cases. Analysis of the clinical and cytogenetic data of these cases suggests that a phenotype resembling Wolf syndrome is present when the distal band of the short arm of

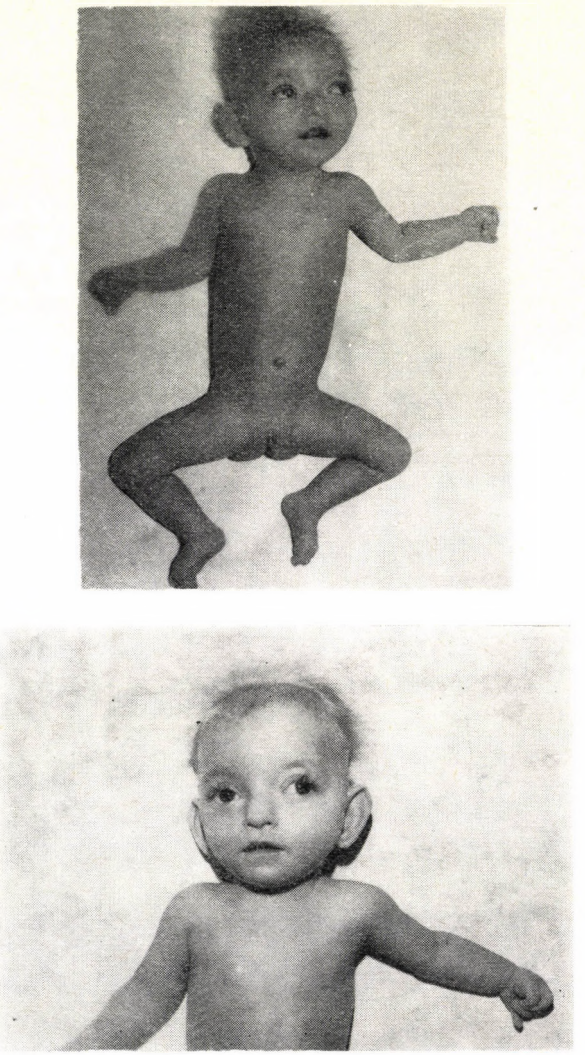

FIG. 1. The proposita at 15 months of age

chromosome 4 (band p16) is lost [8, 10].

This report describes a new case of ring chromosome 4 and summarizes the 17 cases (including the present one) with this anomaly published so far. A new approach is also described which was planned to throw some light upon the non-specific developmental anomalies usually present in cases with ring chromosome.

CASE REPORT

The proposita was the first-born child of unrelated healthy parents. The mother was 20 and the father

25 years of age at the time of the birth. The patient was born after an uneventful pregnancy of 35 weeks. Her birthweight was 1240 g. During infancy she was seen several times in a local hospital (poor feeding, vomiting, loose stools, respiratory tract infections, febrile convulsion). She was sent to us for diagnostic investigation at the age of 12 months because of severe unexplained failure to gain weight. Physical examination revealed an infant with proportionate somatic retardation (weight 2780 g, height 53 cm, head circumference 32 cm). There were only few dysmorphic stigmata (Fig. 1): down-turned mouth, beaked nose, relatively simple large ears, and protruding big eyes with divergent strabismus. Psychomotor development was severely retarded (developmental age was calculated to be 1 month). There were no clinical abnormalities in the cardiovascular, respiratory, urinary and alimentary systems. Laboratory data were all within normal limits (growth hormone was not determined). Radiographs of skull, chest, abdomen, and long bones were normal. The bone age was estimated to be 3 months. In spite of great efforts, her weight reached only 3400 g by the age of 17 months (at this age height was 56 cm, head circumference 33 cm). She died of pneumonia at the age of 18 months. Autopsy showed no abnormalities of the internal organs except a small atrial septal defect.

Cytogenetics

Cytogenetic studies from peripheral blood culture (conventional 72-h culture) are shown in Table I. Of the cells, 87.6% were diploid and contained one ring, its size being more or less constant. G-band analysis showed that the ring consisted of an apparently complete chromosome 4, without clear evidence of deletion prior to formation of the ring (Fig. 2). Breakpoints were considered to be in or distal to the terminal bands of the short arm (p) and the long arm (q). Thus, the patient's karyotype was 46,XX,r(4)(p16q35). Of the cells, 3.6% showed monosomy 4, without ring. In 8.8 per-

TABLE I

Distribution of cells with various chromosome counts and derivatives of ring chromosome 4 in lymphocyte culture

Karyotype	No. of cells	per cent
45, XX, -4	6	3.6
46, XX, r(4)	148	87.6
46, XX, dic r(4)	8	4.7
47, XX, two r(4)	3	1.7
two interlocked rings	2	1.2
pulverized ring	2	1.2
total	169	100

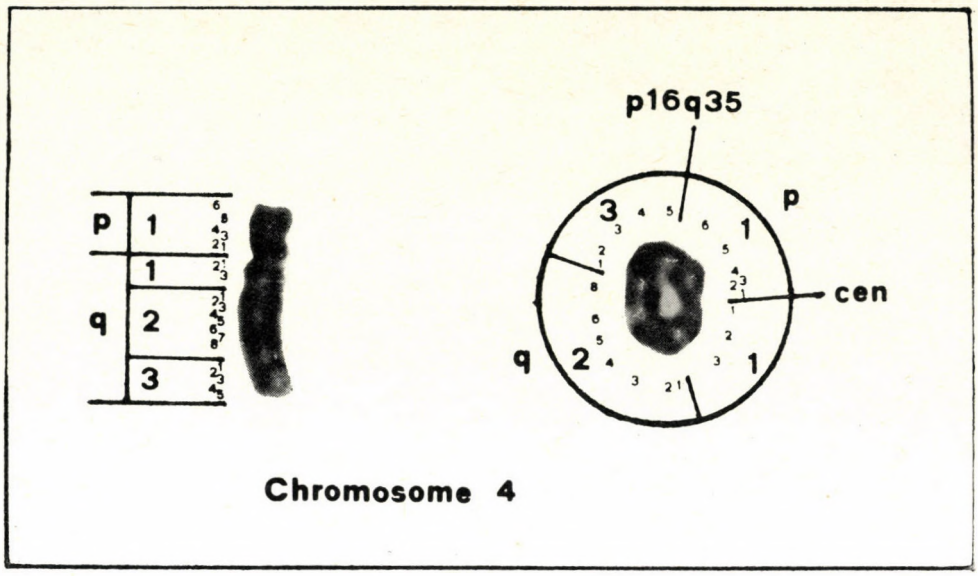

FIG. 2. Ring chromosome 4 and its homologue. *cen* indicates centromere. Ring is joined at p16q35

cent of the cells analysed various configurations of the ring chromosome could be found including dicentric rings of various sizes, interlocked dicentric rings, two rings, and pulverization of the ring. Micronuclei and nuclear projections were present at a frequency of 3.6% in lymphocyte culture (as compared to 0.18% in a control culture).

The karyotypes of the parents were normal.

A fibroblast culture was initiated from a skin specimen taken one hour after death and maintained in RPMI 1640 medium (GIBCO) with 20% fetal calf serum. Cultured fibroblasts were harvested for chromosome analysis after the first subculture. Six of the 29 cells analysed were aneuploid because of various ring derivatives.

Cell viability (in vitro experiment)

It was noted that the fibroblast culture grew poorly compared to normal ones. In an attempt to clarify the cause of poor growth, cell viability was determined. Cells of first subcultures from the patient and a matched normal control were stained in situ with 0.5% trypan blue and the rate of unstained (living) cells was determined by counting at least 500 cells in each subculture.

Cell viability of the patient was 72%, while that of the control was 93%. (Standard viability in the author's laboratory is 92 $\pm$ 5% in control cultures.) This result suggested an increased cell-death in the first subculture of our patient compared to controls.

Discussion

The main clinical symptoms of our patient were a very severe growth failure with proportionate somatic retardation and a profound psychomotor retardation. Apart from these non-specific features she had only few dysmorphic stigmata which belonged to the features of the 4p- or Wolf syndrome. Out of the 7 cases with ring chromosome 4 in whom the breakpoints are known (Table II), the patients described by Niss and Passarge [17], McDermott et al [16], del-Mazo et al [8], and Finley et al [10] showed few signs of the 4p deletion syndrome, while the patient of Chavin-Colvin et al [5] had practically none of the main features. All these cases including the present one had no obvious loss of terminal band on the short arm (p16) of chromosome 4. The patients of Perez-Castillo and Abrisqueta [19] and Friasse et al [11] in whom the entire p16 band (breakpoints in band p15) was missing, showed many of the features of Wolf syndrome. Only one case [5] had a detectable deletion on the long arm of chromosome 4. Since this patient showed no specific dysmorphic features, the role of the missing q34-35 terminal regions is difficult to determine.

These observations suggested that the terminal region of the short arm of chromosome 4 (p16) is significant in the final manifestation of Wolf syndrome. The fact that some features of the syndrome could be seen even in cases with no obvious loss of band p16 may be explained that even with high-resolution banding technique it is not possible to exclude the loss of some chromosomal material.

Many authors agree that at least some abnormalities of the phenotype in cases with ring chromosome are due to the specific behaviour of the ring in mitotic anaphase. Without sister chromatid exchanges (SCE) ring chromosomes can behave normally as long as the two chromatids can separate freely. However, ring chromosomes are usually subject to various difficulties at mitosis as a result of the normal occurrence of SCE. The behavioural instability of rings is reflected in the number of various ring configurations and of aneuploid cells, promoted by the peculiar ring mechanics. These cells are less likely to survive [3] and a certain proportion of cells with ring chromosome will presumably be lost at subsequent cell divisions.

In our patient, a high proportion of the cells with abnormal ring configuration was found in both lymphocyte and fibroblast cultures. In addition, we observed an increased cell death-rate in the first subculture of fibroblasts as compared to controls. This observation suggests that cells containing these abnormal configurations do not survive in vivo, and their elimination at subsequent divisions is likely to end with a reduction in the total number of viable cells. This assumption was proposed by Kjessler et al [15], Cote et al [6], and Jansen et al [14] as well studying cases with ring chromosome 1, 2, and 2, respec-

TABLE II

Comparison of physical features and chromosomal breakpoints

	Carter et al.	Dallaire	Hecht	Faed et al.	Bobrow et al.	Surana et al.	Bofinger et al.
Sex	♂	♀	♂	♂	♂	♀	♂
Age		16y			4y	5y2m	
Early death	+			+			+
Low birth weight	+	++	+	+++	++	+	++
Growth failure		+	+		+++	++	
Mental retardation		−	+		+	−	
Retarded bone age					+	+	
Beaked/broad nose							+
Down-turned mouth							+
Large simple ears							+
Short philtrum							+
Hypertelorism					+		
Abnormal thumbs and/or radii	+	+		+			+
Cleft lip/palate	+						+
Heart malformation	+		+				+
Sacrococcal dimple					+		
Hypospadias	+						+
Ring unstable	?	?	?	?	+	+	?
Breakpoints							

tively. Cote et al [6] found that these abnormal products were found after two or more cell cycles but not after one cycle in lymphocyte culture, suggesting that these anomalies do not survive in vivo. We think that our results with the cell viability testing in fibroblast culture have given further support to this assumption and are in favour of the opinion that aneuploid cells found in cases with ring chromosome are derived *de novo* in each instance and not perpetuated in a clonal manner proposed by the majority of authors observing "mosaicism" in cases with ring chromosome.

Concerning the extreme somatic retardation and severe growth failure, we are of the opinion that these symptoms are direct consequences of the persistent generation of cells with various aberrant configurations induced by ring mechanics. This process, followed by the elimination of aneuploid cells in vivo, will result in the long run in a reduction of the total number of viable cells expected for a given interval of proliferation and is

in 17 patients known to have ring chromosome 4

Parker et al.	Niss, Passarge	Perez-C., Abrisqueta	Mc-Dermott et al.	Fraisse et al.	Chavin-C. et al.	del Mazo et al.	Young, Zalneraitis	Finley et al.	Present case
♀	♂	♂	♂	♂	♀	♀	♀	♂	♀
9y	11y		4y	8y	5y		6y	2m	1y2m
		+				+			
+	+	++	++	+	++	++		+	+++
++	+	+	++	+++	++			+	+++
+	+		+	+	?		+	+	+
+			+	+	+				+
				+	+			+	+
		+		+					+
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	+			+				+	
?	+	+	+	+	+	+	?	+	+
	p16 q35	p15 q35	p16 q35	p15 q35	p16 q33	p16 q35		p16 q35	p16 q35

thought to account for the marked reduction of body mass in these patients. Extreme somatic retardation was found in cases with ring chromosome 4 both with and without features of Wolf syndrome (see Table II) indicating that this degree of growth failure is less related to the deletion of genetic material than to the specific behaviour of the ring during cell division.

Microcephaly is usually mentioned in the reports of cases with ring chromosome 4 as a dysmorphic stigma. In relation to their body size, however, the patients cannot be considered microcephalic. The head circumference found to be small on percentile graph is simply a manifestation of the proportional somatic retardation. Hence, "microcephaly" cannot be regarded as a distinct feature in cases with ring chromosome.

Analysing other features of the 17 cases with ring chromosome 4 (Table II), abnormal thumbs and/or radii have been observed in more than half of the patients. Although Haspes-

lagh et al [12] have recently reported on limb malformations in a case with 4p deletion, the other known cases with this chromosomal defect did not show abnormal thumbs and/or radii, and these malformations do not belong to the features of 4q deletion, either [20]. Thus, it is not unrealistic to assume that this limb defect, too, is caused primarily by ring mechanics, possibly reducing the number of viable cells at a critical point of morphogenesis rather than by terminal deletion of chromosome 4. That these features are less specific to chromosome 4 than to the ring per se, can be inferred from the fact that they occur also in cases with ring formation of other chromosomes.

Conclusion

Our observations and analysis of the clinical and cytogenetic findings in the 17 known cases with ring chromosome 4 suggest that the clinical picture consists of a mixture of anomalies originated from both the chromosomal deletion prior to ring formation (it is frequently undetectable even with fine techniques) and the behavioural instability of the ring chromosome. The former brings about symptoms of 4p deletion or Wolf syndrome depending on the lost amount of genetic material, while the latter generates unspecific anomalies like growth failure (occasionally very extreme) including "microcephaly", developmental delay, and supposedly some other developmental anomalies (perhaps limb defects).

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