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Multibranched Chromosomes 1, 9, and 16 in a Patient with Combined IgA and IgE Deficiency

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Summary. Instability of the centromeric region of chromosome 1 and multibranched configurations formed by different numbers and combinations of arms of chromosomes 1, 9, and 16 were found in cultured lymphocytes of a 12-year-old male with combined IgA and IgE deficiency. No chromosome abnormalities were found in fibroblast cultures from the patient or in blood cultures from his parents.

A possible effect on the frequency of the abnormalities of the almost continuous antibiotic treatment received by the patient was found both in vivo and in vitro, but no abnormalities were found in blood cultures from control subjects who received similar treatment. Interphase association of chromosomes 1, 9, and 16 and a high frequency of interchanges among the centromeric regions of these chromosomes due to the presence of a fragile site is assumed to be the cause of the abnormalities.

Introduction

Instability of a specific region of a human chromosome resulting in chromatid breakage and in duplication of the chromosome fragment distal to the fragile site in a proportion of cells, was first described by Lejeune et al. (1968) in chromosome 2. Since then similar observations on other chromosomes have been reported, and the abnormal configurations resulting from the duplicated chromosome segment have been described in turn as selective endoreduplications, branched chromosomes, or triradials (see Fraccaro et al., 1972; Ford and Madan, 1973; Quack et al., 1978 for reviews). Apparent independence from phenotypic abnormalities was common to all these cases, and in many of them a Mendelian segregation of the fragile site was observed. We described a case of instability of

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the centromeric region of chromosome 1 showing, at variance with those previously reported, multibranched configurations involving various combinations of arms of chromosomes 1, 9, and 16.

Case Report

V.G., a male born at term in April 1966, was hospitalized in our clinic from September to October 1977 and from February to March 1978. He died in June 1978, from severe respiratory failure. He was the seventh and last child of healthy unrelated parents. Their third and sixth children, a male and a female, died in infancy. The cause of death of the third child is unknown, whereas the sixth was reported to have had recurrent bronchitis and pneumonic infections. Birth weight was 3400 g. From 3 months of age he showed poor weight gain and developmental retardation; he was not able to walk alone until 3 years of age. About two or three times a year he had to be hospitalized for severe lower respiratory tract infections. When admitted to our clinic the patient was 11½ years old; height (120 cm) and weight (17 kg) were both below the third percentile. On examination he showed severe psychomotor retardation, was not able to walk, and had an ataxic gait, dystonia, and choreoathetoid movements. On close examination of skin and bulbar conjunctiva no telangiectases were seen. The edge of the liver descended 3 cm below the right costal margin; the spleen was not felt. Lymph nodes were present but rather small and difficult to palpate. Otoscopy revealed chronic ear infection.

Laboratory Investigations

Blood urea and glucose, prothrombin time, serum protein, serum α-1 antitrypsin, and sweat electrolytes were normal. The hematocrit was 44%; WBC 16,500 with 88% neutrophils; platelets 300,000. Gas analysis indicated severe respiratory failure. Prick skin tests to 16 common allergens were negative. A sputum culture yielded an abundant growth of *Pseudomonas aeruginosa.* Electroencephalography revealed severe immaturity. X-ray films of the chest showed diffuse hyperaeration and an irregular, patchy area of consolidation in the right lower lobe. Computerized axial tomography showed widespread cerebral atrophy.

Serum immunoglobulin levels were: IgA = 3 I.U./ml; IgG = 170 I.U./ml; IgM = 386 I.U./ml; IgE = 5 I.U./ml. E rosettes were 80% (normal values 55%—75%). No autoantibodies were detected; antistreptolysin test for antibodies was very low. An inguinal lymph node biopsy after DPT vaccination showed severe lymphocyte depletion and no mass of proliferating lymphocytes was present.

Diagnosis. Combined IgA and IgE deficiency with recurrent pulmonary infections and progressive neurologic degeneration.

Antibiotic Treatment

First hospitalization (weight 17 kg): Tobromycin 6 mg/kg/day from 12/9 to 21/9, chloramphenicol 60 mg/kg/day from 22/9 to 3/10, Streptomycin 30 mg/kg/day from 18/9 to 25/10. Second hospitalization (weight 19.5 kg): chloramphenicol 60 mg/kg/day from 27/2 to 9/3, streptomycin 30 mg/kg/day from 27/2 to 9/3.

Chromosome Investigations

During the two periods of hospitalization, five blood samples were cultured for chromosome analysis (Table 1). In all these cultures we found anomalies consisting of chromatid and isochromatid breaks in the centromeric regions, deletions of whole chromosome arms, interchanges at the centromeric regions

Table 1. Frequencies of cells with abnormalities in the various blood samples cultured for 72 h

Sample	Date	Treatment	No. cells with abnormalities per 100 cells scored
1	30. 9. 77	10 days TOBR followed by 9 days CAP + STR	55
2	11. 10. 77	4 days CAP + STR followed by 9 days TOBR	36
3	24. 10. 77	10 days of TOBR followed by 4 days without treatment	37
4	22. 2. 78	Last 30 days without treatment	20
5	18. 3. 78	11 days CAP + STR followed by 9 days without treatment	47

TOBR = tobromycin; CAP = chloramphenicol; STR = Streptomycin

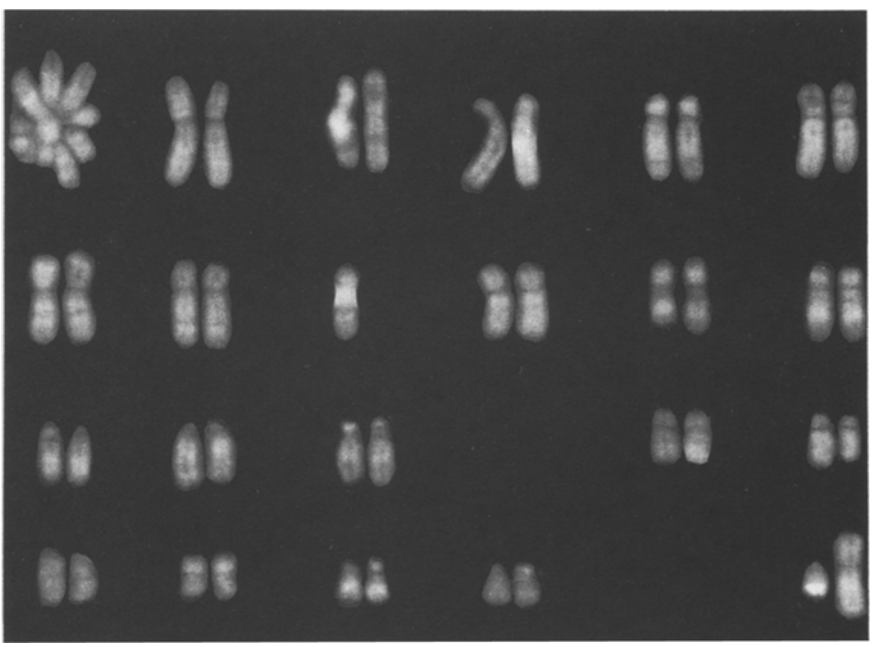

Fig. 1. Karyotype of a cell with 41 chromosomes and one multibranched configuration in which two chromosomes 1, one 9, two 16s and one 16q are involved

among entire homologous or nonhomologous chromosomes, multibranched configurations formed by up to ten arms of the same or of different chromosomes (Figs. 1 and 2). The overall frequencies of the anomalies ranged from 20% to 55% (Table 1).

The abnormal configurations found in at least three cells from blood cultures 1, 2, 3, and 5 are represented, with their frequencies, in Figure 3. In addition we scored more than 50 different combinations of anomalies, each present in less

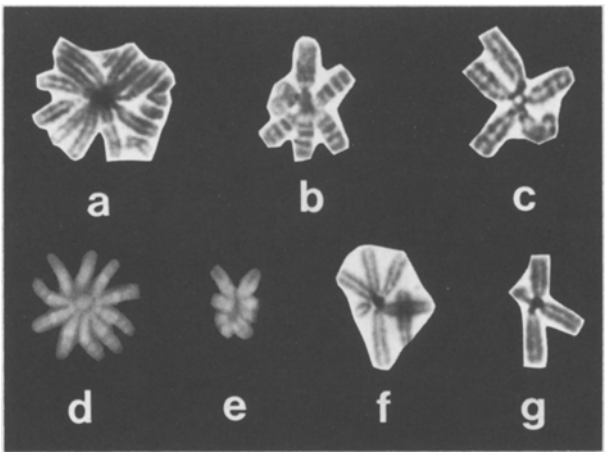

Fig. 2. Multibranched configurations involving different numbers and combinations of arms of chromosome 1 (a, orcein; c, G banding; d, Q banding), 1 and 16 (b, G banding), and 1, 9, and 16 (e, Q banding). f and g (C banding) show continuity of centromeric heterochromatin

than three cells. In all abnormal configurations only chromosomes 1, 9, and 16 were involved.

We limit our description to the more frequent classes of abnormalities. Chromosome 1 often showed either stretching of or a break at the centromeric region, and in some cells two chromosomes 1 were closely associated by or interchanged at their centromeric regions (Fig. 4a). An extreme degree of stretching resulting in a long, despiralized, "moniliform" appearance was seen for chromosome 1 in an occasional cell, such as that shown in Figure 4b.

Associations or interchanges were found also between 1 and 9, 1 and 16, 9 and 16, 9 and 9, 16 and 16, and 1, 9, and 16, although the latter two chromosomes never showed stretching at the centromere. Fragility at the centromere of chromosome 1 led relatively frequently to the separation of the two arms into two centric fragments, 1p and 1q (Fig. 3), which were found alternatively in the cells analyzed, 1p more frequently than 1q. The centric arms 1p and 1q were found concurrently in only one cell, which also contained a normal chromosome 1. In many cells there were multibranched configurations formed by three or more arms of chromosomes 1, 9, and 16 joined at the centromeres in different numbers and combinations. In some cells, when stained for C-bands, each arm participating in these abnormal configurations appeared to maintain at least a portion of its centromeric heterochromatin (Fig. 2f and g).

Chromosomes 1 and 16 were involved in the multibranched configurations with an even or uneven number of their short and/or long arms. In contrast, chromosome 9, when involved in the configurations, was always present as an entire chromosome, and in no cell were losses or gains of chromosome 9 material observed. Therefore our assessment of the overall gains and losses was confined to chromosomes 1 and 16. Table 2 gives the balance of the arms of these chromosomes for each of 128 cells with anomalies. The diploid arm number is indicated as 2pq and the positive or negative deviations from it are entered in the table independently of the type of anomaly(ies) resulting in imbalance. Inspection of Table 2 reveals that there is a prevalence of chromosome loss: 69 cells are deficient for one or more arms of chromosome 1 while 34 have an excess of these

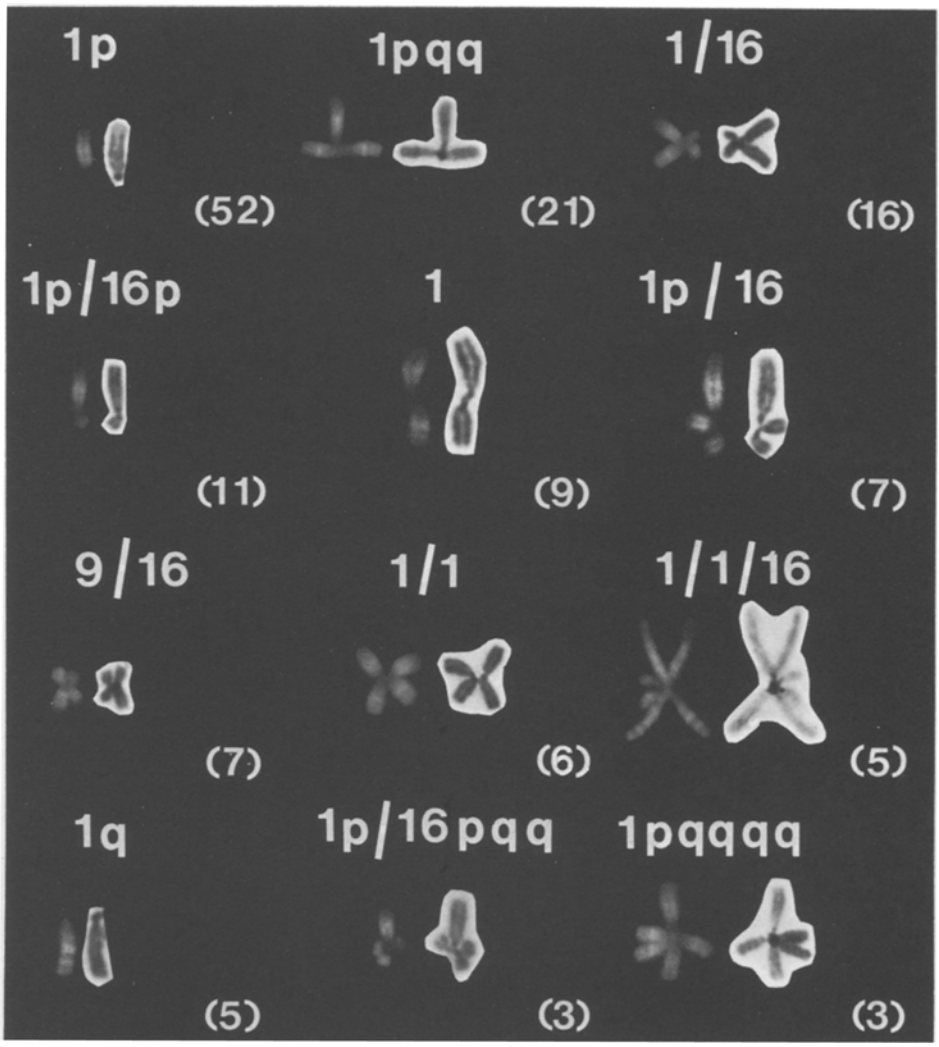

Fig. 3. Abnormalities found, alone or with others, in at least 3 of the 400 cells scored. The frequency of each abnormality indicated by figure in brackets

arms. The same tendency was observed for chromosome 16, with 22 and 7 cells, respectively. Combined deficiencies for both chromosomes 1 and 16 were observed in 16 cells, while combined excess was found in only one cell.

No anomalies were detected in cultured fibroblasts from a skin biopsy of the patient. Blood cultures from his parents revealed a normal karyotype in all cells. It was not possible to investigate other members of the family.

Blood cultures of sample 2 of Table 1 were incubated in the presence of ^{3}H-TdR for the last 6 h of culture. Autoradiography revealed no abnormal pattern of DNA replication in 264 labeled cells. In one cell, however, there was evidence of

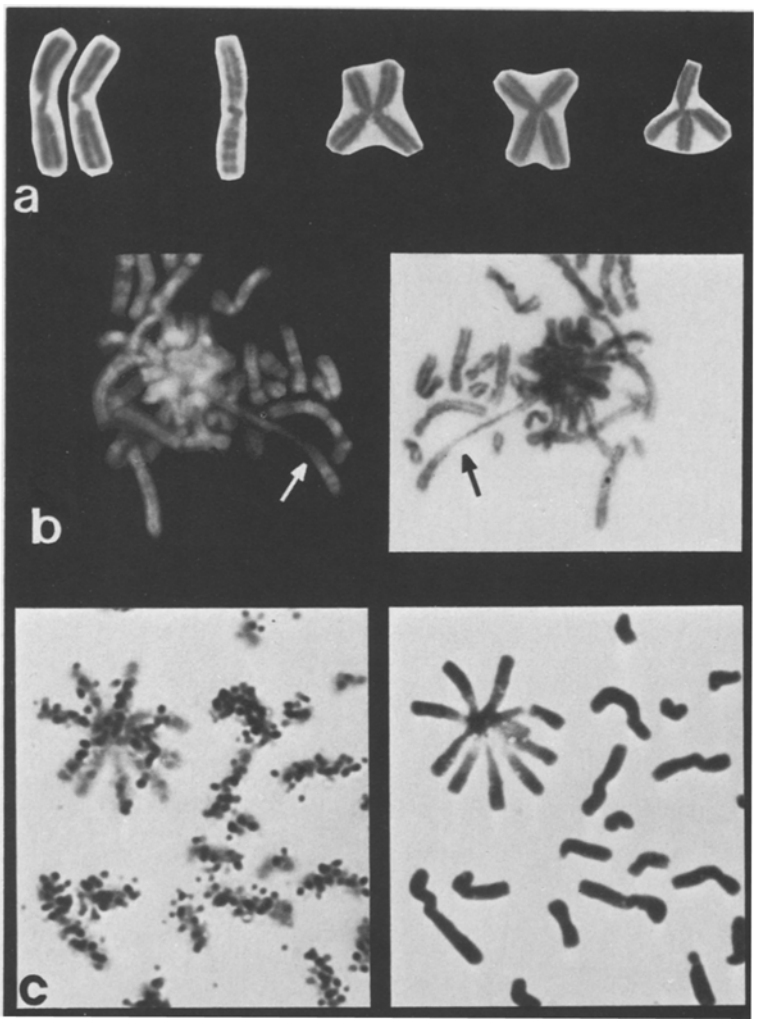

Fig. 4. **(a)** Various degrees of stretching or breakage and of association or interchange of chromosome 1. The first two chromosomes on the left are homologues from the same cell; **(b)** Complex multibranched configuration from which an extremely stretched chromosome 1 emerges (arrows). The two photographs are mirror-image-like, left stained for Q- and right for G-bands; **(c)** Multibranched configuration with asynchrony of DNA replication among arms. Autoradiography after 6 h contact with ^{3}H-TdR (left) and the same detail after removal of the grains

asynchrony of replication among the chromosome 1 arms involved in one multibranched configuration (Fig. 4 c).

The acrocentric chromosomes were seen to associate with the chromosomes 1, 9, and 16 involved in the abnormal configurations. We did not attempt a full quantitative analysis of these associations, but among 127 abnormal cells, one or more acrocentric chromosomes were associated 44 times with abnormal chromosomes 1, 9, and 16, while in the same cells they were associated 20 times with the normal chromosomes 1, 9, and 16.

Table 2. Chromosome-arm balance in 128 cells with anomalies of chromosomes 1 and 16. 2pq indicates complete diploid number of chromosome arms

		Chromosome 16													Total
		−p	−q	−pq	−2p	−2q	−p2q	2pq	+p−q	+p	+q	+pq	+2q	+p2q	+3q	
Chromosome 1	−p							1								1
	−q	1	5			3		44		1	3		1			58
	−pq							3								3
	−2p															—
	−2q		6			1										7
	−p2q															—
	2pq		1	1		1		19	1		1					24
	+p−q				1											1
	+p		1				1	5								7
	+q		1					20			1					22
	+pq							2								2
	+2q															—
	+p2q							1								1
	+3q							2								2
Total		1	14	1	1	5	1	97	1	1	5	—	1	—	—	128

Table 3. Effect of chloramphenicol (CAP) and streptomycin (STR) for 72 h in blood cultures

Antibiotic dose (μg/ml)		Cells scored	Cells with abnormalities (%)
CAP	STR		
—	—	100	20 (20)
0.12	0.06	34	14 (41)
0.25	0.125	50	29 (58)
0.50	0.25	50	31 (62)
1.2	0.60	34	18 (52)

Table 4. Frequency of cells with the abnormalities with increasing culture time (blood)

Hours of culture	Cells scored	Cells with abnormalities (%)
48	49	2 (4)
48	42	3 (7)
72	100	20 (20)
96	50	14 (28)

Effect of Treatment and of Time in Culture

An attempt was made to evaluate a possible correlation between the prolonged and almost continuous treatment of the patient with antibiotics and the frequency of chromosome abnormalities. As shown in Table 1, there was no clear-cut relationship between type and length of treatment and frequency of the anomalies. However, the lowest proportion of abnormal chromosomes was found after the longest period without treatment (sample 4 of Table 1).

We treated the patient's blood cultures with concurrent, increasing doses of chloramphenicol and streptomycin as shown in Table 3. The results indicate a possible effect of these antibiotics on the frequency of anomalies, although there was not a linear relationship between dose and effect. The same treatment on cultures from normal subjects did not induce abnormalities. Variation of the frequency of anomalies with time of culture was tested on blood samples processed after 48, 72, and 96 h. As seen in Table 4, the frequency of the abnormalities is much lower at 48 h than at 72 and 96 h. At 48 h complex rearrangements were almost absent and the most frequent anomalies consisted of chromosome 1 with various degrees of stretching, breaks at the centromere, and centromeric interchanges between two homologues. We examined the chromosomes of one child and of one adult, both of whom underwent an antibiotic treatment similar to that of our patient, and we found no abnormal chromosomes.

Discussion

The branched chromosomes found in our case differ from those previously described, mainly in the location of the fragile site, which is at the centromere

instead of along the chromatids; for the involvement of three different chromosomes in the formation of multibranched configurations; and for the high number of arms found in some of them. Two alternative hypotheses have been suggested for the formation of branched chromosomes: one by Lejeune et al. (1968), based on "selective endoreduplication" of a chromosome fragment, and the other one proposed independently by Ford and Madan (1973) and by Ferguson-Smith (1973), based on mitotic malsegregation of the chromosome segment distal to the fragile site.

Noel et al. (1977) supported the second hypothesis after studying the number of duplications undergone by triradials in cells treated with BUdR. Recently Stahl-Maugé et al. (1978) postulated that a third mechanism, namely homologous triradial chromatid translocation, may account for some of such abnormal configurations. None of these hypotheses can explain all our findings.

A fragile site located at the centromere of a chromosome 1 can cause a centromeric malsegregation during anaphase, with the migration of only one half-chromatid of the short arm to one pole while the remnant three half-chromatids migrate jointly to the other pole. This type of malsegregation resulting in 1pqq and 1p, which are the most frequent configurations (Fig. 3), is not the only one that occurs. Indeed other anomalies, such as 1q and 1ppq, are found, although with lower frequencies. To correlate the presence of the fragile site with centromeric malsegregation, we may assume that during DNA duplication cross-links or interchanges preventing the normal segregation of the chromatids are induced by the presence of the fragile site itself at the centromeric region. The origin of the more complex configurations involving two or more chromosomes 1, 9, and 16 could be explained by considering that the centromeric regions of these chromosomes are characterized by the preferential location of satellite II DNA (Jones and Corneo, 1971; Gosden et al., 1975) and are likely to be associated in interphase (Jones and Corneo, 1971). At duplication, because of this association, a fragile site may cause a high frequency of different interchanges among the closely associated and identical repetitive DNA sequences resulting in the observed combination of chromosome 1, 9, and 16 arms rearranged at the centromere. We found by C banding that these abnormal configurations are all apparently centric and can therefore pass through successive duplications, increasing in this way the number of their arms with the possibility, at each DNA duplication, of new and more complex rearrangements. We found indeed an increase in the complexity of the abnormal configurations at each DNA synthesis as indicated by the fact that the number of the more complex rearrangements increased from 48 to 72 h. Incidentally, our observations confirm that also human centromeres, in addition to those of several other species, contain a longitudinal structure, which can be subdivided into two functional halves, as already indicated by the few reported cases of centric fission in man (see Dallapiccola et al., 1976, for a review).

Not all the abnormal chromosomes 1, 9, and 16 possible were found in our material, and those observed are present with different frequencies. This can be due to selection against cells containing special abnormal configurations and/or to the preferential formation of some of the rearrangements. The latter possibility could derive from an ordered and fixed localization of each chromosome in the interphase association favoring, by physical contiguity, some of the possible

exchanges and/or from differences in the time of duplication of the associated centromeric regions. The fact that chromosome 9 is frequently involved in associations with 1 and 16 but never structurally rearranged could be explained by this hypothesis. The overall tendency towards chromosome loss, in absence of a significant number of acentric fragments, is indication of a relevant role of negative selection.

The data we were able to collect before the patient's death are inadequate to establish a possible correlation between antibiotic treatment and frequency of abnormal configurations. However, we found no abnormalities in control subjects with similar antibiotic treatment and a possible effect of chloramphenicol and streptomycin on the patient's blood cultures. It is noteworthy that there is a striking similarity between the abnormal configurations found in our case and those induced by mitomycin C in lymphocyte cultures. Centromeric regions of chromosomes 1, 9, and 16 are also preferentially involved in the aberrations induced by this antibiotic (Morad et al., 1973). The mechanism of action of mitomycin C involves DNA alkylation, either with attachment to a single base or cross-linking the two DNA strands. This last possibility, together with preferential distribution of mitomycin C to the centromeric regions of chromosomes 1, 9, and 16 (Brøgger and Johansen, 1972) would induce chromosome instability similar to that postulated in our case, resulting in the same type of aberrations.

From the clinical point of view our case is very similar to that described by Amman et al. (1970), with combined IgA and IgE deficiency, but for which no chromosome analysis was reported. Recently Hultén (1978) described a patient with combined IgG, IgA, and IgM deficiency and a fragile site on band q12 of one chromosome 1. In blood culture of this patient chromosome abnormalities similar to those found in our case were present.

From all the available data we conclude that multiple chromosome anomalies including multibranched configurations, similar to those described in our case, are probably increased by treatment with specific antibiotics and are expected whenever a fragile site is present in the centromeric region of a chromosome 1 or, possibly, of a 9 or 16.

Only the study of further cases will reveal whether this type of abnormality is directly related to the immunodeficiency or whether the need for continuous antibiotics treatment of these patients makes obvious the consequences of a fragile site.

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