Original Investigations

The Fragile Site on Chromosome 16 (q21q22)
Data on Four New Families

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Summary. The significance of the fragile site on 16 (q21q22) has not yet been fully evaluated. New data will contribute to the understanding of this cytogenetic finding. Therefore we report on four families where a chromosome 16 with fragile site was segregating and such problems as infertility, abortions, malformations, and aneuploidy were present. The hypothesis that this fragile site is a site of viral modification (or integration?) is considered.

Introduction

There have been several reports on individuals showing a chromosome 16 with a break-prone site (at q21q22) (Day et al., 1967; Magenis et al., 1970; Avirachan and Kajii, 1973; Giraud et al., 1976; Côté et al., 1978; Sele et al., 1979; Sørensen et al., 1979; Turleau et al., 1979). According to Magenis et al. (1970) this is a 'minor chromosome variation' inherited by simple Mendelian dominant transmission with full penetrance. Having been found in normal and healthy individuals, this 'fragile site' on chromosome 16 was believed to be harmless, and its presence has probably sometimes been overlooked or unreported. However, Sørensen et al. (1979) pointed out that it may increase the risk of chromosomal abnormalities in the offspring, as indeed previous reports had suggested (Day et al., 1967; Avirachan and Kajii, 1973; Giraud et al., 1976; Côté et al., 1978). Other cases have been relevant to the problem of fetal wastage (Sele et al., 1979; Turleau et al., 1979).

The significance of the fragility and the genetic risk for people having this 'fragile' chromosome have not yet been fully evaluated. Therefore we wish to report on four new families where such a variant was segregating.

Methods

Peripheral blood for routine cytogenetic studies was cultured in Medium 199 enriched with 5% fetal calf serum. In selected cases skin biopsies were also cultured in Ham's Medium F10. R, G and C banding was performed on the chromosomes. The location of the breakpoints was determined according to the Paris Conference (1971), Supplement (1975). Once the presence of the fragile site was established at least 100 cells were examined, to evaluate the frequency of its presence. Only cases with a frequency of more than 10% were considered in this report.

As the &-globin chain structural gene was known to have been allocated to chromosome 16 (Deisseroth et al., 1977) hemoglobin (Hb) electrophoresis was performed to detect any &-chain mutations or deficiencies. Erythrocytes were incubated with brilliant cresyl blue and checked for the presence of inclusion bodies (Hb H, tetramere of &-chain whose formation indicated &-chain deficiency).

Family I

An anencephalic baby and its mother (of Eastern European extraction) were cytogenetically examined immediately after the child's birth. Both the mother and the child presented a normal karyotype with a triple-satellited chromosome 21 (Fig. 1) and a fragile site on 16 (q21q22) in 48% and 17%, respectively, of the cells studied. The fragility varied from monochromatid gaps to dichromatid breaks or fragments that were completely detached and lying free (Fig. 2). We found no triradial figures or multiplication of the fragments. The general breakage rate was definitely increased over the normal for our laboratory.

Besides chromosomes 16, D- and C-group chromosomes were also significantly damaged (usually with monochromatid gaps). A mild virus infection in the second month and a febrile viral illness in the sixth month of pregnancy were reported. The father was not available for study and nor were the parents of the mother. She has latent diabetes mellitus. She gave birth to a normal child before and a normal child after the anencephalic baby.

Two years later the cytogenetic analysis was repeated. The fragile site on 16q occurred with a frequency of 20%. Hb electrophoresis did not reveal any abnormalities at that time but 5% of the erythrocytes presented brilliant cresyl blue inclusion bodies.
A couple was referred by the infertility clinic because of repeated abortions in the first trimester. The wife was gynecologically normal, the husband was moderately oligospermic. Both were of Yemenite origin, but not related as far as they knew. Husband and wife each had a normal karyotype (the wife with a 'partial pericentric inversion' in one 9, presenting constitutive heterochromatin in both the short and the long arm). As compared with the normal range accepted in our laboratory, both presented an increased breakage rate. Groups D and B were preferentially damaged. In addition, a fragile site at 16 (q21q22) was noted in 12% of the cells studied in the wife and in 20% of the cells studied in the husband. The fragility was apparent in mono- and dichromatid gaps, breaks and fragments. Multiplication of the fragments and triradial figures were also found (Fig. 3). Repeated cultures at intervals of several months constantly revealed the fragile site at 16 (q21q22) and a general significantly increased breakage rate, although in slightly different proportions.

Hemoglobin electrophoresis did not reveal any abnormalities either in the wife or in the husband, but 5% and 15%, respectively, of their erythrocytes had inclusion bodies after incubation with brilliant cresyl blue. Immunoelectrophoresis revealed normal values of immunoglobulins in the wife, and very low, absolutely abnormal levels of IgG, IgA, and IgM in the husband.

**Family III**

A young man was referred by the male infertility clinic for suspected Klinefelter's syndrome. Cytogenetic analysis of peripheral blood lymphocytes and skin fibroblasts confirmed the diagnosis, showing a karyotype with 47,XXY, and a variant chromosomes 17 with increased centromeric heterochromatin and satellited appearance in all the cells studied. The parents were also examined to investigate the inheritance of the 17 variant. Both of them came from Czechoslovakia. The variant was inherited from the mother, who also presented a fragile site at 16 (q21q22) in the form of mono- and dichromatid gaps and breaks in 12% of cells (Fig. 4). Re-evaluation of the slides of the index patient also revealed the fragile site at 16 (q21q22) in 8% of the blood cells but not in skin fibroblasts. The general chromosomal breakage rate was slightly increased in mother, father (smoking 30 cigarettes per day), and son.

**Case IV**

A severely oligospermic man of Eastern European extraction presented a normal karyotype and a fragile site on chromosome 16 at q21q22, which was apparent in 10% of cells as dichromatid gaps and breaks. Duplication of the fragment was also found (Fig. 5). The patient's parents and sibs were not available for study.