Short report

A new apparently folate sensitive fragile site, 5q35

R T Howell, A McDermott, Jane L Evans

A little over 100 fragile sites have now been found in the human karyotype, the majority being the common type occurring in a high proportion of persons, many of these being aphidicolin inducible. Rare fragile sites occur in a minority of persons and may be grouped into BrdU inducible, distamycin A inducible, or folate sensitive types.[1] Seventeen out of 26 rare fragile sites are classified as folate sensitive, having maximum expression under conditions of folic acid depletion or blocking of thymidilate metabolism by methotrexate, fluorodeoxyuridine, or excess thymidine.[2 3] This report concerns the observation of a new fragile site, probably of the folate sensitive type, close to the tip of the long arm of chromosome 5 at band q35 in two brothers.

The fragile site at 5q35 was ascertained during cytogenetic screening of a mentally retarded adult male referred as a suspected case of fragile X syndrome because of a strong family history of male mental retardation. The fragile chromosome 5 was confirmed on a repeat sample and subsequently found in the patient's clinically normal brother. Extensive screening failed to show the fragile site in the patient's mother or in her two mentally handicapped brothers. The patient's maternal grandfather was also negative. The patient's father was not available for study.

At referral the blood specimen of the proband was subjected to culture protocols in routine use for high resolution banding analysis and fragile X studies. Cultures for synchronisation were grown in McCoy's 5a medium supplemented with 10% fetal bovine serum and PHA. After 48 hours' incubation, methotrexate was added to a final concentration of 10^{-7} mol/l overnight. The methotrexate block was released with 10^{-5} mol/l thymidine for five hours 15 minutes before harvesting, colchicine being added for the last

SW Regional Cytogenetics Centre, Southmead Hospital, Bristol BS10 5NB.
R T Howell, A McDermott, J L Evans
Correspondence to Dr Howell.

Received for publication 19 February 1990.
Accepted for publication 20 March 1990.

Levels of fragile 5 expression in the two brothers in different culture conditions. (MTX=methotrexate, TdR=thymidine.)

Culture protocol	Proband at ascertainment	Proband repeat	Brother
TC 199 + 5% FBS	9/50	11/53	10/100
McCoy's/ MTX/TdR	8/15	12/35	9/50
McCoy's/ MTX/BrdU	Not done	12/50	Not done
Ham's F10	Not done	1/50	Not done
Ham's F10 TdR block	Not done	Failed	0/50

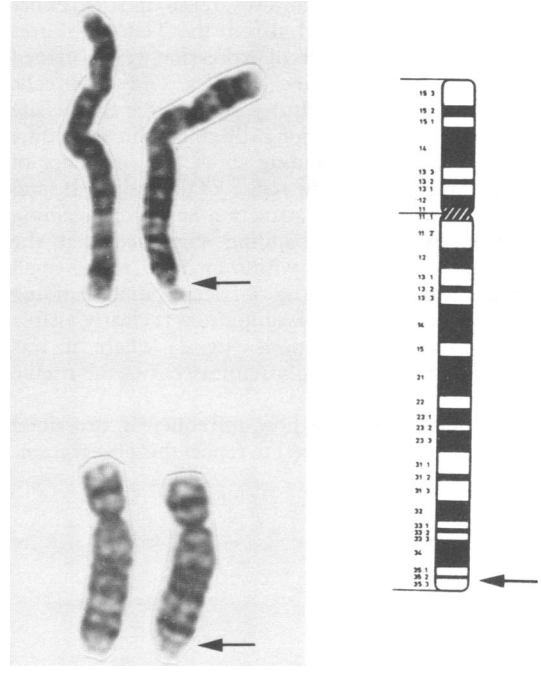

Figure 1 G banded preparations and ideogram illustrating the location of the fragile site close to the tip of the long arm of chromosome 5.

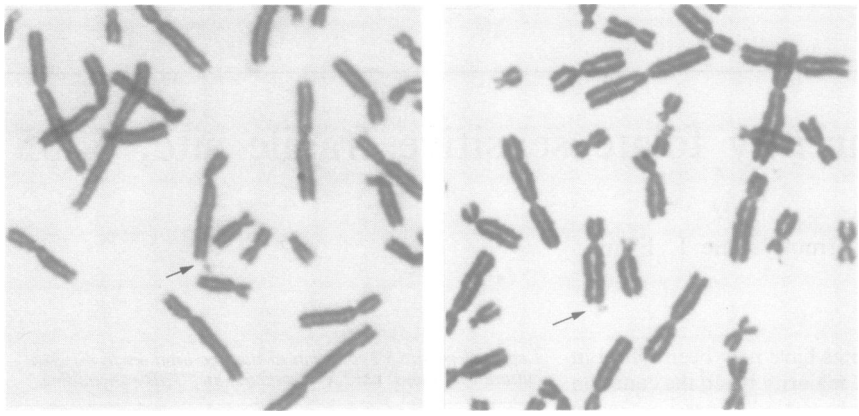

Figure 2 The fragile 5 in non-banded preparations.

30 to 60 minutes. Cultures for fragile X induction consisted of TC199 with 5% fetal bovine serum and PHA, or Ham's F10 with PHA and 10% serum to which was added 600 mg/ml thymidine for the final 18 hours of incubation. On the repeat study of the proband, cultures were also grown in Ham's F10 plus 10% serum for three days with no blocking, and BrdU released, methotrexate blocked cultures were used to investigate replication banding patterns. Culture duration was three days in all cases.

The fragile site at 5q35 was ascertained in the methotrexate blocked cultures released with either thymidine or BrdU, and also in the TC199 cultures (table). The highest levels of expression were obtained in methotrexate/thymidine cultures. Only 1/50 cells from the Ham's F10 cultures showed the fragile site and there was no expression in 50 cells from thymidine blocked cultures. C banding showed no evidence of differential staining in the region of the fragile site and there was no satellite attraction or silver staining associated with it. G banding suggested that the fragile locus was located within or distal to the small dark sub-band 5q35.2 (fig 1). Replication banding was uninformative. The fragile site was clearly visible in non-banded preparations (fig 2) where it was sometimes morphologically reminiscent of the fragile X.

New fragile sites are now infrequently described and it is therefore of interest to report this observation. The fra(5)(q35) is considered to be a folate sensitive site as its expression was extremely low in standard Ham's F10 medium, a formulation containing appreciable levels of folic acid and thymidine. However, the lack of expression after blocking with excess thymidine is most unusual and not typical of folate sensitive fragile sites such as the fragile X.[3]

The expression of the fragile locus in the methotrexate blocked, thymidine released or BrdU released cultures is compatible with folate sensitivity, as addition of the releasing agent would inhibit the fragile site only if added before its replication. Fragile site expression is considered to be S dependent for fragile X.[4]

The occurrence of the fragile chromosome 5 in the clinically normal brother indicates that this fragile site is coincidental to the strong history of male mental retardation in this family and has no direct clinical consequences. Possibly it was inherited from the father who was not available for study.

1 Hecht F. Rare, polymorphic and common fragile sites: a classification. *Hum Genet* 1986;**74**:207–8.
2 Sutherland GR. Ledbetter DH. Report of the committee on cytogenetic markers. Human Gene Mapping 10. *Cytogenet Cell Genet* 1989;**51**:452–8.
3 Sutherland GR, Hecht F. *Fragile sites on human chromosomes*. Oxford: Oxford University Press, 1985.
4 Savage JRK, Fitchett M. When does the fra(X) event occur? *J Med Genet* 1989;**26**:285A.