

## Cytogenetics of Fragile X Chromosome: Autosomal Sites as Potential Markers for Fragile X Chromosome Analysis

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**KEY WORDS** Fragile X chromosome; autosomal mimics; autosomal indicators; differential diagnosis.

**ABSTRACT** Fragile X chromosome screening was undertaken among 100 non-specific mentally retarded group from the Indian population. 14 subjects from 10 families showed the presence of fragile X chromosomes in 4 to 40% of cells. Various 'C' group autosomes showed the fragile sites at the telomeric regions of long arm in low percentages, which is well enough to be confused with fragile X chromosome manifestation in unbanded chromosome preparations. The necessity of analysing good G-banded preparations and scoring more number of cells for the confirmation of fragile X syndrome cytogenetically has been stressed. The presence of constitutive fragile site at 3p14 and polymorphic 9qh+ in high percentages among fragile X positive subjects, made these markers a potential indicators in the diagnosis of fragile X syndrome. The implications of the autosomal mimics and indicators in the differential diagnosis of fragile X syndrome has been highlighted.

### INTRODUCTION

Fragile X chromosome is the most common inherited cause of mental retardation with a population prevalence of 1/1000 males and 1/2000 females (Sherman et al. 1984, 1985). Its frequency among the mental retardates ranges between 6-10% (Blomquist et al. 1982; Webb et al. 1986; Bunday 1987). Cytogenetic visualization of fragile X chromosome is one of the main tools which helps in the diagnosis of fragile X syndrome (Sutherland 1977; Manjunatha et al. 1988; Reiss and Freund 1990; Sujatha et al. 1998). Because of the difficulties involved in culture methods for visualizing the fragile X chromosome and the interference of false posi-

tive and false negative results, the accuracy of the cytogenetic methods in the diagnosis of the syndrome may not exceed 90% (Wang et al. 1993). We have screened for the presence of fragile X chromosomes among non-specific mental retardates from the Indian population and noticed that some of the autosomal fragile sites mimic fragile X chromosome and some of the constitutive fragile sites such as fra 3p14 and 9qh+ serve as markers, which tend to occur at a higher frequency in the fragile X subjects. Here in, we have discussed the implications of these markers in the differential diagnosis of fragile X syndrome.

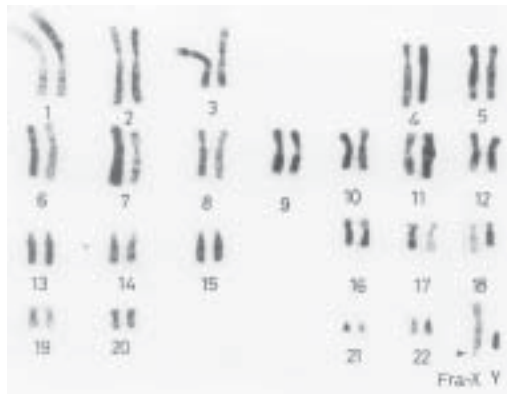
### MATERIAL AND METHODS

One hundred non-specific mental retardates attending the MR clinic at National Institute of Mental Health and Neurosciences, Bangalore, were selected for the study, following exclusion criteria where in subjects with Down's syndrome, congenital malformations and biochemical defects were excluded. Whole blood cultures were incubated for 72 hours using folic acid deficient TC 199 medium with low serum concentration (5%) and a high pH (7.4 to 7.6). Culture were also set up in serum rich (15%) RPMI medium with inducers like FudR or MTX for 96 hours. Chromosome preparation was done by standard methods. Atleast fifty unbanded metaphase plates for each culture were screened for the initial ascertainment. If a fragile site was observed at the tip of long arm of C group chromosomes, the screening of fragile X chromosome was extended to hundred or more metaphases. The slides were then de-stained and subjected for Giemsa-Trypsin-Giemsa (GTG) banding for the confirmation of fragile at Xq27.3 region.

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## RESULTS AND DISCUSSION

Out of the one hundred subjects from 82 families studied, 14 subjects from 10 families showed the presence of fragile X chromosome (Fig.1) in 4-40% of the cells. 8 subjects out of 100 subjects showed various C group autosomal sites in 1-3% of the cells (Table 1 and Fig.2) and 6 of the 14 fragile X positive subjects showed fragile 3p14 (Fig.3) and/or 9qh+ (Fig.4) in 10-60% of the cells (Table 2).

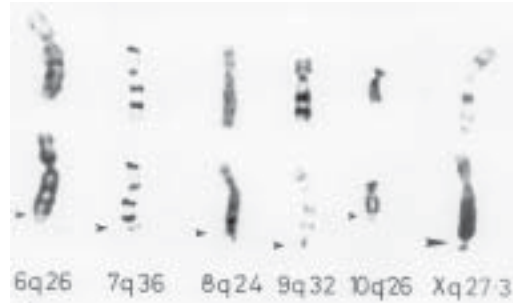


**Fig. 1.** Karyotype showing fragile X chromosome in one of the positive subjects (arrow)

**Table 1:** Various autosomal fragile sites seen in telomeric regions among C group chromosomes

S. No.	Fragile Sites	No. of Subjects	Male/Female	% of Expression
1	6q26	3	2/1	1-3
2	7q36	1	1/0	1
3	8q24	1	1/0	1
4	9q32	2	1/1	1-2
5	10q26	1	1/0	2
Total		8	8	1-3

Genetic factors contribute about 15-30% of etiology for mental retardation (Russel 1985). The contribution of fragile X chromosome to this group began to unravel with the observation of fragile X chromosome in a family with mental retardation by Lubs (1969) and its expression was first observed in specific culture medium by Sutherland (1979). Since then, many geneticists affirmed the importance of cyto-

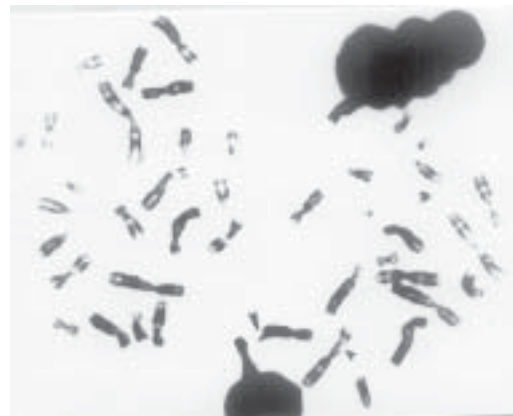


**Fig. 2.** Cut out of normal G-banded 6, 7, 8, 9, 10 and X chromosome in lane 1 and the respective homologues with fragile sites (small arrow) mimicking the fragile X site (big arrow) in lane 2

**Table 2:** Markers serving as indicators for screening positive fragile X chromosomes, such as fragile site 3p14 and polymorphic 9qh+ among fragile X positive subjects

Fra (X) Subject	% of Fra 3p14	% of 9qh+
1	30	30
2	20	45
3	10	-
4	-	60
5	50	-
6	30	50
Total	10-50	30-60

netic methodologies in the diagnosis of fragile X syndrome and greatly improved the techniques for the accurate diagnosis (Glover 1981; Tommerup et al. 1981; Mattei et al. 1981). However, difficulties in the culture protocol for the



**Fig. 3.** Metaphase plate showing fragile X chromosome (big arrow) and fragile site at 3p14 (small arrow)



**Fig. 4. Metaphase plate showing fragile X chromosome (big arrow) and secondary constriction on chromosome 9 (small arrow)**

induction of fragile X expression, the interference of false negative and false positive results in the ascertainment of fragile X site, low percentage of fragile X expression and its genetic heterogeneity give the cytogenetic analysis only 90% accuracy. Although all affected mentally retarded males express fragile X, only a proportion (50%) of females express fragile X cytogenetically. As there are normal transmitting males without fragile X expression and normal transmitting females with fragile X expression, the diagnosis of the fragile X syndrome becomes complicated (Sherman et al. 1984, 1985).

Fragile X site is made to express in folate deficient medium and the folate antagonists like methotrexate (MTX) and inducer 5 fluoro-deoxy uridine (FudR) can be used for the enhanced expression of fragile X chromosome under different culture conditions. Lymphocyte culture method with TC199 for the induction of fragile X expression is simple and highly reliable. Even under optimal conditions, using above method, the expression of fragile X will never exceed 50% (Turner and Jacobs 1983; Hecht and Sutherland 1984). Diagnostic problems arise in the patients showing low percentages of fragile X expression because of the interference of autosomal telomere fragile sites, especially of the 'C' group chromosomes (Leversha et al. 1981; Soudek and McGregor 1981; Vekemans et al. 1983; Deniel et al. 1984 and Webb 1985). Autosomal fragile sites are seen with a frequency of 0.2% in general population. The presence of fragile sites in high percentages has been corre-

lated with certain types of cancer, recurrent abortion, fertility failure and mental retardation (Ying and Donnell 1983; Hecht and Hecht 1984; Yunis and Soreng 1984; Sutherland and Hecht 1985).

We have observed that, 8 out of 100 study group showed fragile sites at 6q26, 7q36, 8q24, 9q32 and 10q26 (see Fig.2) in folate deficient medium in low percentages (1-3%), which is well enough to be confused with fragile X chromosome expression in unbanded preparations. This observation emphasises the necessity of G-banding for the accurate diagnosis of fragile X syndrome, as recommended by Webb (1985). As these fragile sites are expressed in low percentages, a cut off point of 4% fragile X expression to be taken as positive for both the male and female subjects has been recommended (Jacobs et al. 1980; Howard Peebles 1981). The total number of cells to be screened should be increased in the low expressing subjects and should be extended to 100 or more cells for each culture for the female relatives of the patients, who have a very good chance of carrying the fragile X chromosome.

The fragile X subjects from our study showed 4 to 40% fragile X expression in different culture conditions. The constitutive fragile site at 3p14 (Fig. 3) and a polymorphic marker 9qh+ (Fig. 4) was seen to express in high percent (10-60%) in the fragile X patients. 6 out of 14 positive subjects showed these markers. Three subjects showed both the parameters in their cells, where as other three subjects showed one of the two markers.

Fra 3p14 was seen to express among normal individuals as well as in the mentally retarded group in 20-80% of the cells in TC199 medium and using inducer like aphidicolin. It is thought to be a constitutive fragile site without any clinical significance (Markkanen et al. 1982; Luthardt 1982; Glover et al. 1984). Zhou et al. (1984) have shown that the fra 3p14 expresses in high percentage in patients with reduced serum folic acid content. The in vitro effect due to the depletion of folic acid in TC199 and MTX or FudR induced culture conditions could be a possible explanation for the presence of fra 3p14 in the fragile X positive subjects. Another polymorphic marker 9qh+ is seen in 0.1% of normal population and is also seen in low percentages in

patients with congenital defects and in mentally retarded group (Palmer et al. 1971; Nielsen et al. 1974; Howard Peebles and Stoddard 1979; Christian et al. 1980; Soudek and McGregor 1981). The significance of these markers associated with fragile X has to be delineated for better understanding of their implications. However, the presence of these markers in the fragile X families could be well used as good indicators for the presence of fragile X chromosome.

The reliability of cytogenetic methods in the diagnosis of fragile X syndrome can be increased by minimising the false positive and false negative ascertainment, through proper chromosome banding methods and scoring hundred or more number of cells per culture. We recommend the analysis of fragile X by two different researchers independently to rule out biased ascertainment. The use of two polymorphic markers viz., fra 3p14 and 9qh+ in the diagnosis of fragile X syndrome has been highlighted and they may serve as markers for fragile X chromosome diagnosis in certain percentages of subject groups. While making the accurate diagnosis of fragile X syndrome, one must bear in mind the scoring of metaphase plates with well G-banded preparations to rule out the possible inclusion of autosomal mimics of fragile sites, especially the 6,7,8,9 and 10 chromosomes at the telomeric ends.

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