THE FRAGILE X SYNDROME: FIRST FAMILY REPORTED IN MALAYSIA

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SYNOPSIS

An Indian family with all 3 sons having the fragile X syndrome is reported. The frequency of fragile X cells observed ranged from 4-16%. The phenotypically normal mother, although an obligate carrier, did not express any fragile X chromosomes in her lymphocyte cultures. The range of mental retardation in affected hemizygous males and heterozygous females as well as the significance of the fragile X chromosome in prenatal diagnosis are discussed.

INTRODUCTION

The fragile X syndrome is a recently described clinical entity distinguished by mental retardation and a cytogenetically detectable marker X chromosome. It is estimated that one third of families with X-linked mental retardation are affected with this syndrome (1). Because of its familial nature, this genetic defect, with the exception of Down's syndrome, is the most common chromosome abnormality associated with mental retardation in the males (2).

Affected males can present with a moderate to severe mental handicap. Although earlier reports noted a lack of major defects in such affected males, many investigators have since found that some forms of physical abnormalities do exist. These frequent but not invariable characteristics include bilateral testicular enlargement/macro-orchidism (1, 3, 4, 5,), autism (6), severe impairment of verbal abilities and unusual facies (7). The latter include high forehead, high arched palate, large simple ears, broad nose, hypoplasia of the maxilla, and prominent large mandibles with facial asymmetry.

Among female heterozygotes, the range of intellectual capacity is even more varied. Most female carriers are phenotypically normal. Only about 20 to 30% of the carriers are clinically detectable as retarded, the retardation ranging from borderline ("dull") or mild to severe retardation (8, 9). In a few cases, slight facial changes, as in males, could be seen in the retarded females.

The fragile X syndrome actually derives its name from a fragile site at region 2 band 8 on the long arm of the X chromosome (Xq28). Scanning electron microscopy has since allowed a more precise location of the fragile site to the Xq27.3 region (10). This site appears as a constriction or break in a single chromatid or chromosome, or as a triradial (11).

Although the fragile site on the X chromosome is the cytogenetic marker for the fragile X syndrome, its expression in short term lymphocyte culture is highly dependent on its culture conditions. It expression requires a folate deficient medium like medium 199, a slightly alkaline pH and a low serum content (12, 13). Its frequency can however be enhanced by addition of fluorodeoxyuridine (FUdR) (14, 15), methionine (16) and methotrexate (17). Even then, the frequency of fragile X positive cells in lymphocyte cultures of affected males varies from less than 8% to 50% or more (7, 17, 18, 19, 20, 21).

In female heterozygotes, the range of fragile X positive cells is even more variable; ranging from 2 to 36% (8, 20). In several instances too, the fragile X chromosome is not seen at all in lymphocytes from many obligate carrier females even when inducing agents like FUdR are used (1, 7, 20, 22). This is especially so in carriers over the age of 30 (23).

MATERIALS AND METHODS

In 1982, a mentally retarded Indian boy aged 10 years was referred to us for chromosome analysis. Tracing of his family history revealed that his two elder brothers were also mentally retarded but to different degrees. Their parents who are not consanguineous, are phenotypically normal (Fig 1a). Relatives of both parents are in India and there is no history of mental retardation in them. An X-linked form of mental retardation was suspected and this initiated the screening of the whole family for the fragile X chromosome.

Blood specimens were cultured for 71 hours at 37°C in medium 199 (GIBCO) with 5% foetal bovine serum, phytohaemagglutinin and antibiotics. After routine preparation, the slides were stained very briefly with Giemsa and photographed. The representative C group chromosome which had a fragile site at the end of its long arm was confirmed as an X chromosome by destaining the slide and G-banding. At least 50 metaphases were analysed from each patient. 4% of cells expressing the fragile site at Xq28 was taken as the lower limit for fragile X positivity (24).

Fig 1a Family members

TABLE
FREQUENCY OF FRAGILE X CHROMOSOME IN FAMILY MEMBERS

	Age (yrs)	No. of fra (x) / No. of metaphases	% fra (x)	Mental Status
II.1	16	4/50	8	Moderate MR
II.2	14	2/50	4	Severe MR
II.3	12	8/50	16	Mild-Moderate MR
Mother I.1	31			
1st culture		0/50	0	Normal obligate
2nd culture		0/70	0	carrier
Father I.2	56	0/50	0	Normal

MR: mental retardation

Case History

The family pedigree is shown in Fig 1b.

II.1, now aged 16 years, is moderately retarded with a history of delayed milestones and fits. He started walking at the age of 2 years, spoke at 5 years and was toilet trained at 8 years. He was sent to a normal school but was unable to cope. Since then he has stayed at home. He can follow instructions and is able to help around in the house. On clinical examination, he is found to have a small head, strikingly large ears, micrognathia, high arched palate, over-crowding teeth and is constantly smiling. His arm span is wide, but his chest wall is small. His speech is incomprehensive and he has involuntary movements of hands and legs. Both his testes are larger than normal (Fig. 2).

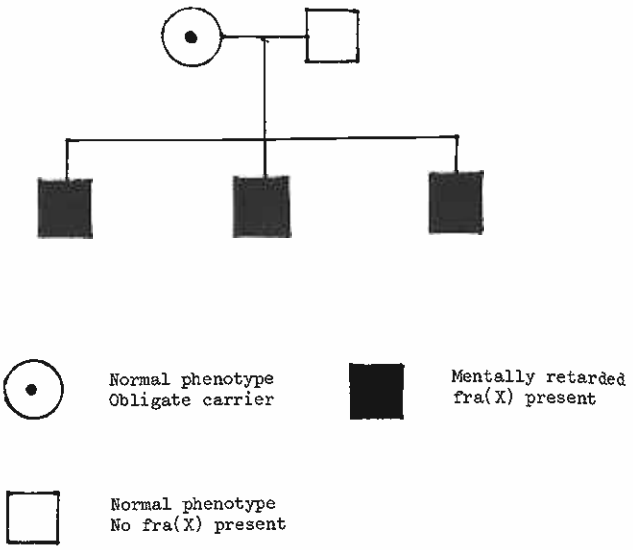


Fig 1b Pedigree of family

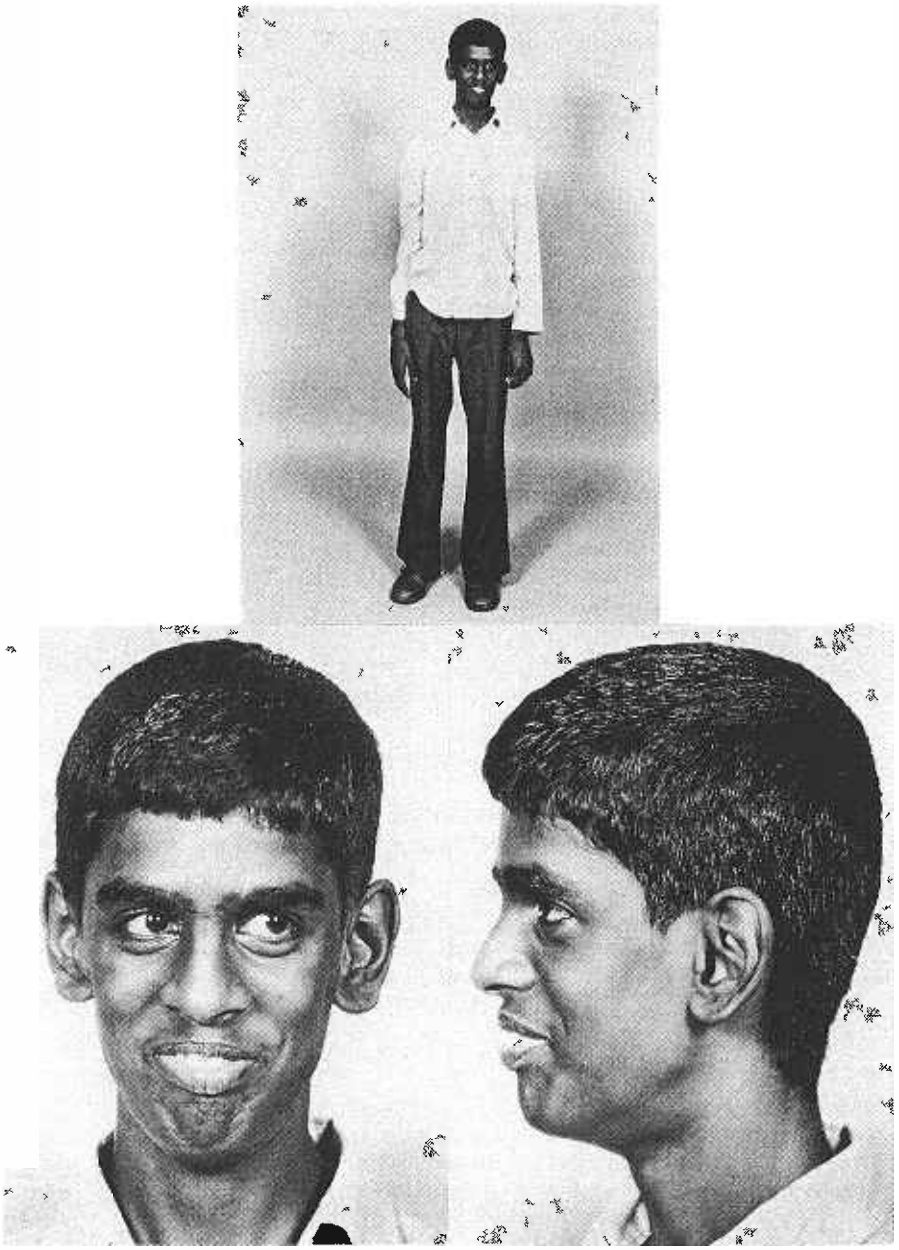


Fig 2 II.1

II.2 At age 14, this second son is severely retarded. He is unable to take care of himself, is not toilet trained and needs to be guarded and kept in the house. He is not able to speak, is very noisy, violent and restless. He salivates excessively and keeps sticking out his tongue. His gait is unsteady and needs to be supported. He also has a history of fits. He has an expressionless facies with large ears and a divergent squint of the left eye. He has "knock" knees and involuntary movements of head, hands and legs (Fig. 3). Because of the violent and uncontrollable behaviour of this boy, clinical examination of his genitals was not possible.

II.3 is aged 12 years. He is mildly to moderately retarded, is very shy and quiet. He smiles persistently. He has a history of delayed milestone: he started walking at 1½ years, talked at 3 years and was only toilet trained by 10 years. Presently he is attending a special day school for retarded children after dropping out from a normal school. He has no history of fits. His gait is steady but he has involuntary movements of hands and legs. His head is small with large ears, high arched palate and crowding of teeth. His chest wall is small and the lower epiphysis of the major joints are prominent. His testes are of normal size (Fig. 4).

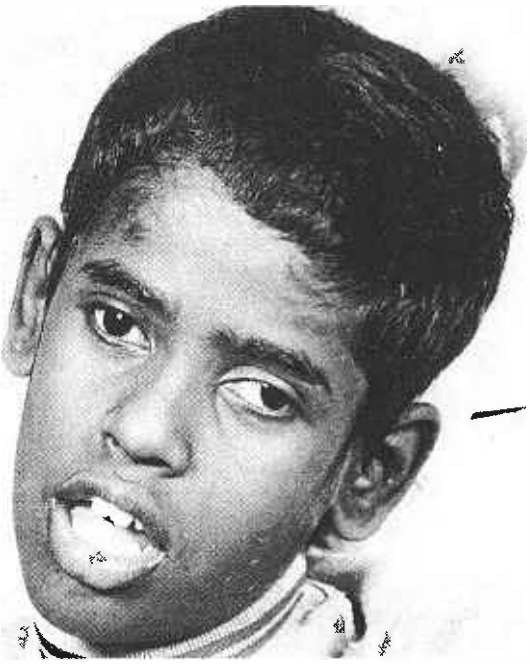


Fig 3 II.2

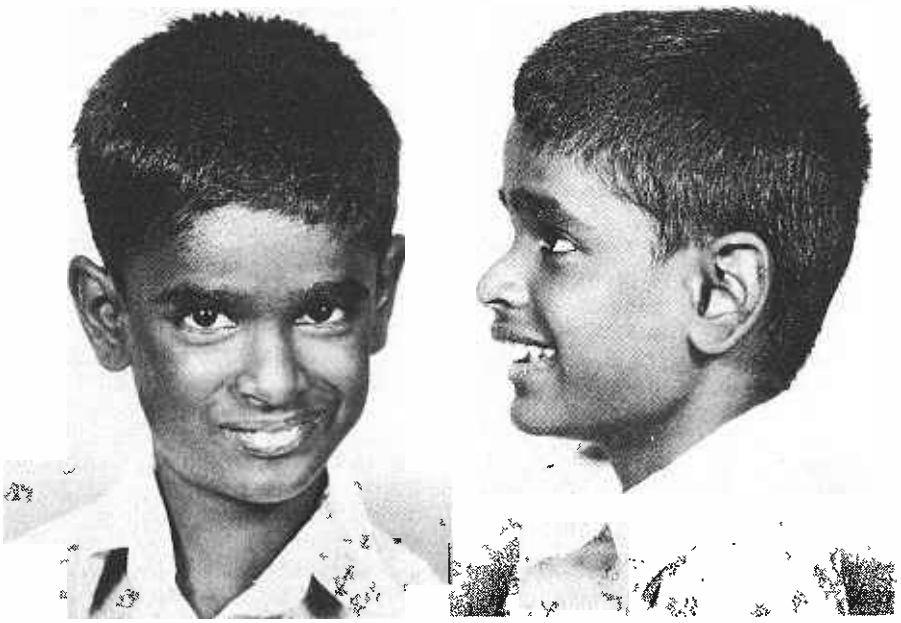
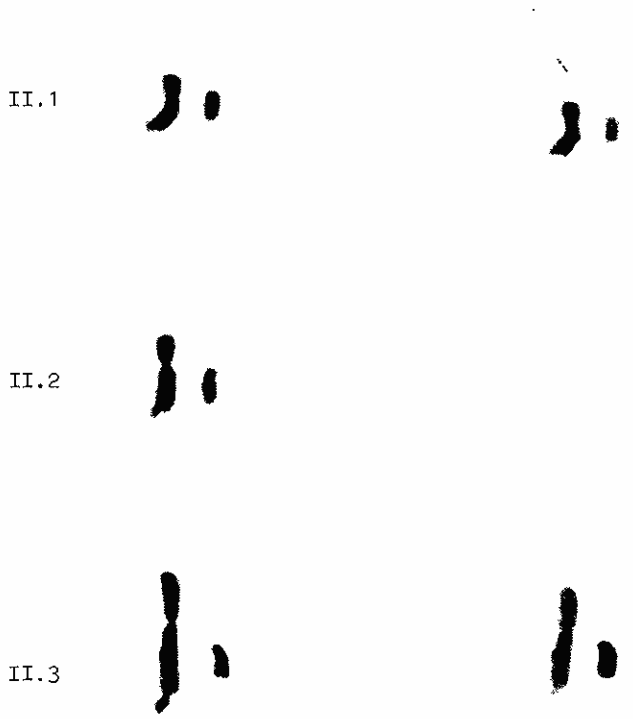


Fig 4 II.3

RESULTS

The fragile X frequencies are set out in the Table. All the 3 retarded sons studied had the fragile X chromosome (Fig. 5), at frequencies ranging from 4-16%. The phenotypically normal mother, although an obligate carrier, has no fragile X chromosome even though cultures were set up on two separate occasions. On both occasions, the lymphocyte cultures of the mother resulted in few metaphase spreads and were of poor quality. The father has a normal karyotype.



GIEMSA STAINING SHOWING THE 'SATELLITED' EFFECT AT Xq27-28.

G BANDING OF THE SAME FRAGILE X

Fig 5 Appearance of the fragile X in the three brothers

DISCUSSION

The frequency of fragile X positive cells in the three brothers is very low, ranging from 4-16%. The low frequency obtained is probably due to the suboptimal culture condition and preparation technique used. It is now an established fact that the number of fragile X cells is influenced by variation in the culture time, method and colcemid treatment (16, 22). However if all culture conditions are maintained, the frequency of fragile sites at the X chromosome for a given individual will remain constant even at different times of assessment (25). Although the severity of retardation in the three brothers varies, no correlation was seen between the frequency of the fragile X cells present and the level of their retardation. This observation is in accordance with the findings of other authors (21, 24, 25).

Phenotypically all the three brothers show most of the characteristic features of the fragile X syndrome such as large ears, speech impairment and unusual facies. Macro-orchidism has been noted to be one of the most readily identifiable feature of the syndrome, occurring with an incidence of greater than 75% (26). Brown et al (27) even suggested that the fragile X syndrome may be screened by testicular measurement alone. But as can be seen from this study, macro-orchidism is not a constant clinical feature.

The risk of a carrier female bearing a retarded son was given to be greater than 50% (22). In the pedigree study by McDermott et al (26), 16 of 27 sons born to obligate carriers were mentally retarded. In our present study, all 3 pregnancies of the carrier mother resulted in mentally retarded children.

Even though the mother is an obligate carrier (having contributed the sole X chromosome in her sons), no fragile X chromosomes were seen in both her lymphocyte cultures. This failure to detect fragile X cells could probably be due in part to the low quality of the metaphase preparation or the fact that the frequency of the fragile X cells is so low that it is undetectable considering that only 70 metaphase spreads were examined. In the study of an obligate carrier by van Roy et al (28), only 2 fragile X cells were picked up after screening 300 metaphase spreads (0.7%). On the other hand, several authors (1, 20, 22, 28, 29, 30) had also failed to detect any fragile X chromosome in their obligate carriers. These observations spurred Martin et al (31) to emphasize that the failure to detect the fragile X chromosome does not exclude the carrier state. In the review by Tariverdian and Weck (32), they summarized that 44 out of 85 reported obligate female carriers showed the fragile X in between 0.5 and 38% of the cells. One explanation put forward was that the frequency of fragile X chromosomes in women decreases with increasing age (20, 23). Others believe that besides age, intelligence may be a key factor, there being 2 types of family with the fragile X expression in heterozygous females. The female carriers of the first type of family are believed to be of normal intelligence and in these it is increasingly difficult to detect the fragile X chromosome with increasing age. In the second type, the female carriers may be mildly retarded and will show the fragile X chromosome regardless of age (33). The findings of both normal and mentally retarded heterozygous females in the families studied by several authors (8, 11, 20, 21) do not support this hypothesis. To date, there is still much uncertainty regarding this issue of diminuation with age in the number of fragile X in female carriers

Several theories have been proposed to explain the range of clinical findings in female heterozygotes, the most common being lyonisation (2, 8). The severity of mental retardation in female carriers is said to depend directly on the proportion of cells which by chance have the normal X inactivated in tissues critical to intellectual capacity. The basis of retardation in the dull female is the relatively high proportion of cells with the fragile X active in the relevant tissues, compared to a normal carrier who would have most cells having the normal X active in critical tissues. This proposal had been supported by the observation of a greater preponderance of cells with early replicating fragile X chromosomes (that is genetically active fragile X chromosome) in mentally retarded female carriers compared to carriers with normal intelligence (26, 34). Previous to this, Jacobs et al (24) and Schmidt (35), independently, found that the frequency of the fragile X in cultures is related to the mental status of the individual, having detected the aberration more readily in lymphocyte cultures from retarded female carriers than their normal counterparts. There is certainly a

need for more information on the effect of fragile X on the mental capacity of female heterozygotes.

It is also a mystery as to how the presence of the fragile site affects the mental development of the affected. Lubs (29) postulated that the fragile site may be the site of the mental retardation gene or that they are closely linked. Considering the fact that the fragile X syndrome is found in several ethnic groups, (mostly Caucasians, American Blacks, Australian aborigines, Filipino, Zulu, Cape coloured populations, and Indians) (36, 37, 38), Gardner et al (38) postulated that the gene controlling the phenotype and the fragile site are the same, or at least overlap.

This association of the presence of the fragile X chromosome and mental retardation has initiated a great deal of excitement among scientists and clinicians alike in view of its potential as an invaluable tool in prenatal diagnosis and in providing genetic counselling for families of the affected. Techniques have been successfully developed to detect the fragile X chromosome in cultured amniotic fluid cells (39, 40, 41) and fibroblasts (17, 42). Amidst these progress, a nagging doubt still prevails over the strength of association between the fragile X chromosome and mental retardation, especially among female carriers. This uncertainty is further aggravated by the finding of fragile X chromosomes in normal males (28, 37, 43, 44, 45). There is thus less than 100% penetrance of the fragile X site and this has casted a serious doubt over the validty of using the presence of the fragile X chromosome as an indication for termination of pregnancy with identified fragile X fetuses. Certainly more data is required to solve this puzzle before clinicians can confidently predict the phenotype in prenatally identified fragile X hemizygote males and heterozygote females.

This family represents the first kindred with the fragile X syndrome in Malaysia. We believe that there may be more cases that are yet to be detected. To do this may entail screening all cases of mentally retarded males and females for the fragile X chromosome. However, to incorporate this additional test into our routine chromosome analysis of all retarded cases may not be practical considering the cost, time-consuming nature of the test and the difficulty in demonstrating the fragile X in some cultures. Nevertheless, it is warranted in instances where an X-linked inheritance is suspected or when there is clinical justification to do so.

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REFERENCES

1. Turner G, Till R, Daniel A: Marker X chromosomes, mental retardation and macro-orchidism. N Engl J Med 1978; 299:1472.
2. Gerald PS: Editorial. X-linked mental retardation and an X-chromosome marker. N Engl J Med 1980; 303:696-7.
3. Turner G, Eastman C, Casey J, McLeay A, Procopis P, Turner B: X-linked mental retardation associated with macro-orchidism. J Med Genet 1975; 12:367-71.
4. Jacobs PA, Mayer M, Rudak E, et al: More on marker X chromosomes, mental retardation and macro-orchidism. N Engl J Med 1979; 300:737-8.
5. Hecht JT, Moore CM, Scott Jr CI: A recognizable syndrome of sex-linked mental retardation, large testes, and marker X chromosome. South Med J 1981; 74:1493-6.
6. Brown WT, Friedman E, Jenkins EC, et al: Association of fragile X syndrome with autism. Lancet 1982, i:100.
7. Howard-Peebles PN, Stoddard GR, Mims MG: Familial X-linked mental retardation, verbal disability, and marker X chromosome. Am J Hum Genet 1979; 31:214-22.
8. Turner G, Brookwell R, Daniel A, Selikowitz M, Zilibowitz M: Heterozygous expression of X-linked mental retardation and X-chromosome-marker fra(x) (q27).N Engl J Med 1980; 303:662-4.
9. Webb GC, Halliday JL, Pitt DB, Judge CG, Leversha M: Fragile (x) (q27) sites in a pedigree with female carriers showing mild to severe mental retardation. J Med Genet 1982; 19:44-8.
10. Harrison CJ, Jack EM, Allen TD, Harris R: The fragile X: a scanning electron microscope study. J Med Genet 1983; 20:280-5.
11. Carpenter NJ, Leichtman LG, Say B: Fragile X-linked mental retardation. Am J Dis Child 1982; 136:392-8.
12. Sutherland GR: Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium. Science 1977; 197:265-6.
13. Sutherland GR: Heritable fragile sites on human chromosomes. I. Factors affecting expression in lymhocyte culture. Am J Hum Genet 1979; 31:125-35.
14. Glover TW: FUdR induction of the chromosome fragile site. Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 1981; 33:234-42.
15. Tommerup N, Poulsen H, Brondum-Nielsen K: 5-flouro-2'-deoxyuridine induction of the fragile site on Xq28 associated with X-linked mental retardation. J Med Genet 1981; 18:374-6.
16. Howard-Peebles PN, Pryor J: Fragile sites in human chromosomes. I. The effect of methionine on the Xg fragile site. Clin Genet 1981; 19:228-32.
17. Mattei MG, Mattei JF, Vidal J, Giraud F: Expression in lymphocyte and fibroblast culture of the fragile X chromosome: A new technical approach. Hum Gent 1981; 59:166-9.
18. Howard-Peebles PN, Stoddard GR: X-linked mental retardation with macro-orchidism and marker X chromosomes. Hum Genet 1979; 50:247-51.
19. Sutherland GR: Heritable fragile sites on human chromosomes. II. Distribution, phenotypic effects, and cytogenetics. Am J Hum Genet 1979; 31:136-48.
20. Sutherland GR: Heritable fragile sites on human chromosomes. III. Detection of fra (x) (q27) in males with X-linked mental retardation and their female relatives. Hum Genet 1979; 53:23-7.
21. Herbst DS, Dunn HG, Dill FJ, Kalousek DK, Krywaniuk IW: Further delineation of X-linked mental retardation. Hum Genet 1981; 58:366-72.
22. Harvey J, Judge C, Wiener S: Familial X-linked mental retardation with an X chromosome abnormality. J Med Genet 1977; 14:46-50.
23. Tommerup N, Nielsen KB, Mikkelson M: Marker X chromosome induction in fibroblasts by FUdR. Am J Med Genet 1981; 9:263-4.
24. Jacobs PA, Glover TW, Mayer M, et al: X-linked mental retardation: A study of 7 families. Am J Med Genet 1980; 7:471-89.
25. Eberle G, Zankl M, Zankl H: The expression of fragile X

chromosomes in members of the same family at different times of examination. Hum Genet 1982: 61:254-5.

26. McDermott A, Walters R, Howell RT, Gardner A: Fragile X chromosome: clinical and cytogenetic studies on cases from seven families. J med Genet 1983; 20:169-78.
27. Brown WT, Mezzacappa PM, Jenkins EC: Screening for fragile X syndrome by testicular size measurement. Lancet 1981; ii:1055.
28. Van Roy BC, De Smedt MC, Raes RA, Dumon JE, Leroy JG: Fragile X trait in a large kindred: transmission also through normal males. J Med Genet 1983; 20:286-9.
29. Lubs, HA: A marker X chromosome. Am J Hum Genet 1969; 21:231-44.
30. Kahkonen M, Leisti J, Wilska M, Varonen S: Marker X-associated mental retardation. A study of 150 retarded males. Clin Genet 1983; 23:397-404.
31. Martin RH, Lin CC, Mathies BJ, Lowry RB: X-linked mental retardation and marker X chromosome. Am J Med Genet 1980; 77:433-41.
32. Tariverdian G, Weck B: Nonspecific X-linked mental retardation — A Review. Hum Genet 1982; 62:95-109.
33. Howard-Peebles PN: Fragile sites in human chromosomes. II. Demonstration of the fragile site Xq27 in carriers of X-linked mental retardation. Am J Med Genet 1980; 7:497-501.
34. Howell RT, McDermott A: Replication status of the fragile X chromosome, fra(x) (q 27), in three heterozygous females. Hum Genet 1982; 62:282-4.
35. Schmidt A: Fragile site Xq27 and mental retardation. Clinical and cytogenetic manifestation in heterozygotes and homozygotes of five kindreds. Hum Genet 1982; 60:322-7.
36. Venter PA, Gericke GS, Dawson B, Op't Hof J: A marker X chromosome associated with nonspecific male mental retardation. The first South African cases. S Afr Med J 1981; 60:807-11.
37. Rhoads FA, Oglesby AC, Mayer M, Jacobs PA: Marker X syndrome in an oriental family with probable transmission by a normal male. Am J Med Genet 1982; 12:205-17.
38. Gardner RJM, Smart RD, Cornell JM, Merckel LM, Beighton P: The fragile X chromosome in a large Indian kindred. Clin Genet 1983; 23:311-7.
39. Jenkins EC, Brown WT, Duncan CJ, et al: Feasibility of fra X chromosome prenatal diagnosis demonstrated. Lancet 1981; ii:1292.
40. Shapiro LR, Wilmot PL, Brenholz P, et al: Prenatal diagnosis of fragile X chromosome. Lancet 1982; ii:99-100.
41. Schmidt A, Passarge E, Seemanova E, Macek M: Prenatal detection of a foetus hemizygous for the fragile X-chromosome. Hum Genet 1982; 62:285-6.
42. Jacky PB, Dill FJ: Expression in fibroblast culture of the satellited X chromosome associated with familiar sex-linked mental retardation. Hum Genet 1980; 53:267-9.
43. Daker MG, Chidiac P, Fear CN, Berry AC: Fragile X in a normal male: a cautionary tale. Lancet 1981; i:780.
44. Webb GC, Rogers JG, Pitt DB, Halliday J, Theobald T: Transmission of fragile (x) (q 27) site from a male. Lancet 1981; ii:1231-2.
45. Fryns JP, van den Berghe H: Transmission of fragile (x) (q27) from normal male(s) Hum Genet 1982; 61:262-3.