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Fragile Site X Chromosomes in Mentally Retarded Boys

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The fragile X syndrome is a common X-linked mental retardation and autism, affecting females as well as males. The fragile site X chromosomes were studied in a series of 153 mentally retarded boys of unknown etiology to determine the frequency of fragile X syndrome, and to assess the feasibility of making a clinical diagnosis of the fragile X syndrome in young boys before cytogenetic results were known. The 10 boys (6.4%) were positive for fra (X) (q27). The phenotype of fra (X) (q27) positive patients were typical except one who also had sex chromosomal mosaicism. There were three pairs of siblings among the fra (X) (q27) positive patients. Frequency of expression of the fragile site was in 10 to 47 per cent of cells. In addition, 19 boys showed a previously unsuspected chromosomal abnormality. The frequency of the fragile X syndrome in the present study is not significantly different from those in Caucacians and Japanese population. The fragile X syndrome can be recognized by noting key aspects of family history as well as the clinical features in mentally retarded boys.

Key Words: *Fragile site, Mental retardation, Fragile X syndrome, Marker X chromosome, X-linked mental retardation.*

INTRODUCTION

Fragile sites of chromosomes are visible as elongated, lightly staining regions between two chromosome bands which are normally adjacent only in metaphases of cells that have been subjected to various treatments known or suspected to interfere with DNA replication (Jordan 1987). Much of the interest is focused on the site close to the end of the long arm of the X chromosome at Xq 27.3 because of its association with the X-linked mental retardation syndrome (Harrison et al 1983).

It has been known for decades that a disproportionate number of mentally retarded individuals were male. Martin and Bell (1943) first reported a pedigree of X-linked mental retardation. Lubs (1969) first detected the marker X, or the fragile X chromosome in two brothers, their mother, and three additional relatives. Sutherland (1977) reported that the demonstration of the fragile X chromosome was dependent on the use of folate-deficient tissue culture media. The Martin-Bell pedigree was later shown to demonstrate the fragile X chromosome (Richards et al 1981). Recently, a gene designated "FMR-1" has been isolated at the fragile-X locus (Verkerk et al 1991).

The classic triad of physical findings in the fragile X syndrome in adult males consists of a long face with prominent jaw, large, prominent ears, and macroorchidism (Hagerman et al 1983). Fragile X syndrome is the most common familial form of mental retardation and autism (Sutherland 1985). It is the second most frequent chromosome anomaly associated with mental retardarion, after trisomy 21. The incidence of the fragile X syndrome is approximately one in 1000 (Webb et al 1986). However, most individuals with this disorder have not been diagnosed.

The purposes of this study were to detect fragile X chromosome in intellectually handicapped boys for the fragile X syndrpme, and to assess the feasibility of making a clinical diagnosis of the fragile X syndrome in young boys.

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SUBJECTS AND METHODS

The subjects studied were 153 boys with idiopathic mental retardation who consented cytogentic analyses for fragile X chromosome at Seoul National University Children's Hospital from March 1985 to February 1992. Excluded were patients with Down syndrome and other chromosomal abnormalities previously diagnosed.

Clinical examination included measurements of height, weight, head circumference, ear lobe length, ane testicular volume using Prader orchidometer. The intelligence quotients were evaluated by Korean Wechsler Intelligence Scale or Draw-a-Man test.

A case was diagnosed as fra (X) (q27) positive based on criteria described by De Arce et al (1986); more than two metaphases with fra (X) (q27) chromosome must be observed in 100 metaphases from the boy being investingated.

The ear lobe was defined as long when its length is over +2 SD by standard of ear size (Han et al 1992). Macroorchidism was defined testicular volume equal or over 4mL in prepubertal boy. The long face was judged subjectively.

Cytogenetic studies

Ten ml of venous blood was collected into sodium heparin, and each specimen was cultured in TC 199 medium (Gibco) with 15% fetal calf serum (Gibco) and phytohemaggluitin (Gibco) for three or four days at 37°C, and treated with methotrexate 24 hr before harvesting to a finsal concentration in the culture medium 0.01mg/ml. A minimum of 100 cells were examined per specimen, and the presence of the fragile sites at band q27 on the X chromosome was confirmed by Giemsa banding.

RESULTS

Among 153 mentally retarded boys karyotyped, 10 boys (6.5%) were found to be fra (X) (q27) positive (Table 1). All fra (X) (q27) positive patients were presented initially with severe mental retardation, speech delay and in most of them with behavioral problems (Table 2.). The cytogenetic and main clinical findings of these 10 boys are summarized in Table 3. A typical facial appearance of the fragile X syndrome (patient No. 3), and general appearance and the fra (X) (q27) positive karyotype of patient No. 5 are shown in Figs. 1, 2 and 3.

One of the fra (X) (q27) positive patients (No. 6) was atypical for the fragile X syndrome in clinical features and showed sex chromosomal mosaicism, 45, X/46,

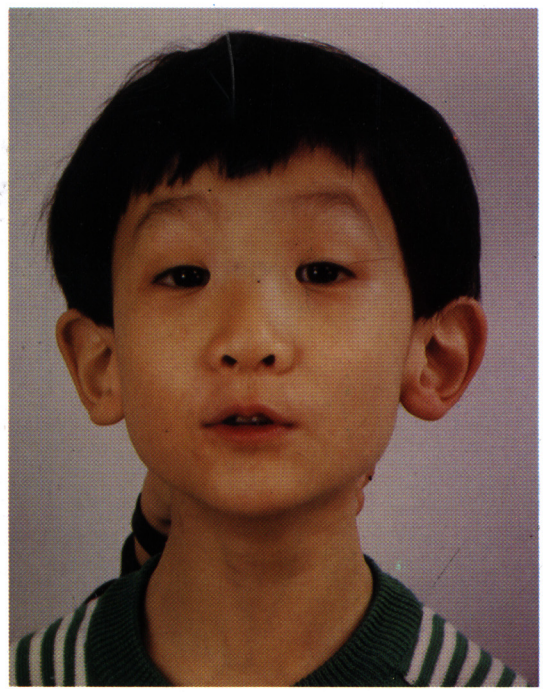

Fig. 1. Fragile X syndrome in 6.4-year-old boy (patient No. 3) with typical facial appearance.

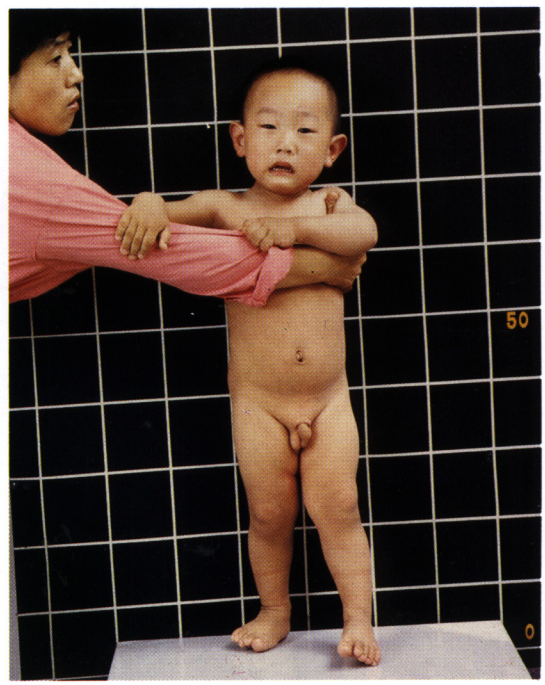

Fig. 2. General appearance of 2-year-old boy (patient No. 5). Note long face, high forehead, long and prominent ears and macroorchidism.

Table 1. Results of Cytogenetic Studies for the Fragile X Syndrome in 153 Boys with Mental Retardation of Unknown Etiology

Total no. karyotyped	153	
Positive for fra (X) (q27)	10	
Negative for fra (X) (q27)	143	
Other chromosomal abnormalities	20	
Autosomal*		16
Sex chromosomal†		4

*2p-, 3p-, 5p-, +8 (mosaic), inv (9) 3; 9qh+, 10p− (mosaic), 13q-, 18p+, 18p-, t (7;14), +mar, 3.
†: XXY, XXXXY, X/XY/XYY, Y−.

Table 2. Presenting Parental Complaints in Boys with Fra (X) (q27) Chromosome

Complaints	Frequency (%)
Mental retardation, severe	100
Speech delay	100
Hyperactivity	90
Short attention span	80
Delayed motor development	80
Autistic-like behavior	60
Difficulty in discipline	50
Shyness	30
Hypotonia	20

Table 3. Cytogenetic and Clinical Features of Boys with Fra (X) (q27) Chromosome

Patient No.	Age (Y)	% Fra (X) (q27) Expression	IQ*	Presence (+) or absence (−) of physical triad		
				Long face	Long ears (> +2 SD)	Macroorchidism (≥4ml)
1	10.2	46	44	+	+	+
2	8.0	36	40	+	+	+
3	6.4	24	37	+	+	+
4	9.0	18	34	+	+	+
5	2.0	13	30	+	+	+
6	7.0	27	38	−	−	−
7	2.6	20	49	+	+	−
8	6.8	11	53	+	+	+
9	5.9	21	36	+	+	−
10	4.4	15	35	+	+	+

*Korean Wechsler Intelligence Scale or Draw-a-Man Test.

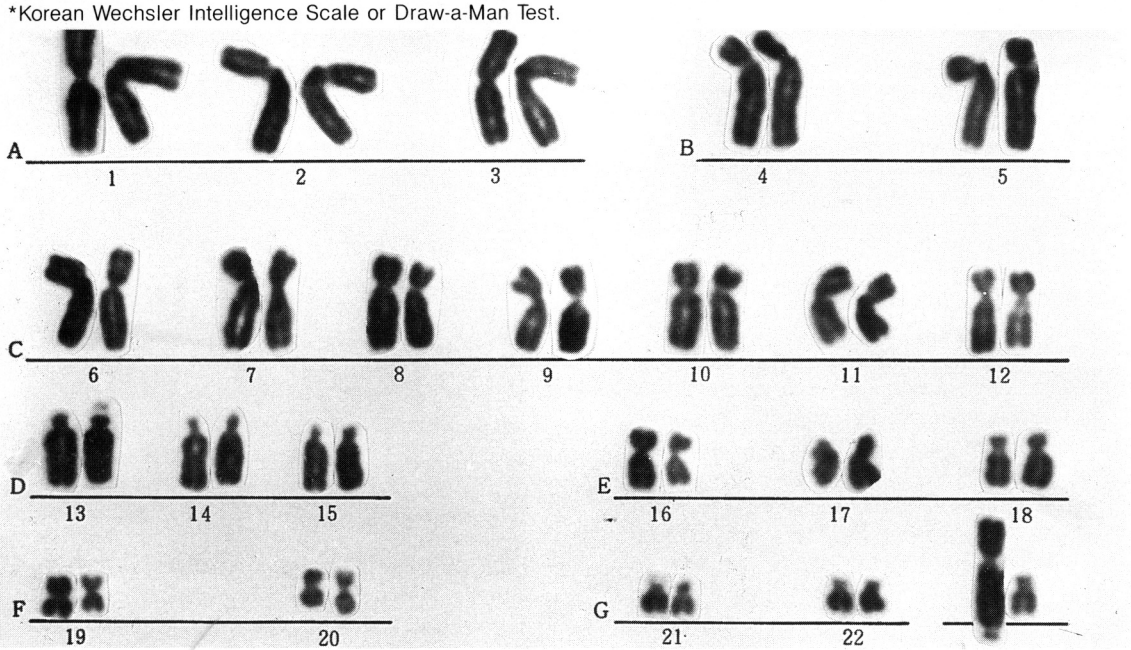

Fig. 3. Karyotype of patient No. 5 showing the fra (X) (q27) chromosome.

XY/47, XYY with 1, 20 and 6 fra (X) (q27) positive cells out of 3, 45, X; 82, 46, XY; and 15, 47, XYY metaphases, respectively. Additionally, 19 boys (12.4%) were found to have a previously unsuspected chromosomal abnormality, and in three of the nineteen, the abnormality involved the sex chromosomes.

In the fra (X) (q27) positive boys, the percentage of cells expressing the fra (X) (q27) varied from 10% to 46% with a mean of 22%. The IQs of the fra (X) (q27) positive patients ranged from 30 to 53 with a mean of 40. There were three pairs of sibships, patients Nos. 1 and 2; 3 and 4; and 9 and 10. The triad of physical findings in the fragile X syndrome was present in 70% of fra (X) (q27) positive boys.

DISCUSSION

Fragile X syndrome, associated with a rare fragile site at Xq27.3, is the most common familial form of mental retardation (Sutherland 1985).

The clinical phenotype comprises mental retardation, macroorchidism, and a typical but variable facial dysmorphy. The most special chracteristics, however, is expression of a fragile site at Xq 27.3 in the affected males and in a proportion of female carriers (Lubs 1969; Sutherland 1977). Cytogenetic detection of the fragile X site in lymphocytes requires culture in folic acid-free medium or by induction with 300mg thymidine/liter, 0.05mg FUdR/liter or 0.01mg methotrexate/liter (Sutherland et al 1990).

The prevalence rates for persons with all degrees of intellectual handicap and the fragile X syndrome in the public school population were 1:2610 for males and 1:4221 for females in an Australian population of 1.2 million (Turner et al 1986). There have been several reports on the prevalence of the fragile x syndrome in Caucasians showing different frequencies ranging from 6.2% to 13.6% (Carpenter et al 1982, Froster-Iskenius et al 1983, Kahkoner et al 1983, Bundey et al 1985). Blomquist et al (1983) suggested an ethnic factor as one of the possible causes of different prevalences among the mentally retarded population studied. Arinami et al (1986) reported the frequency of the fragile X syndrome in Japanese males without a specific cause of mental retardation as 8.6% suggesting that the prevalence of the fragile X syndrome in Japanese is not significantly different from that in Caucasians.

The fragile X syndrome can occur in patients with another condition such as sex chromosomal aneuploidy (Brondum-Nielsen 1986, Froster Iskenius et al 1982). For unknown reasons, there is an increased incidence of nondisjunction in the fragile X syndrome. Because of the facial appearance and or the clearly abnormal testicle size, fragile X syndrome was suspected prior to cytogenetic studies in the fra (X) (q27) positive patients except one who had sex chromosomal mosaicism. The clinical features of the boys positive for fra (X) (q27) were very similar to those in the literature, including severe or moderate mental retardation, thin and long face with large nose and ears and macroorchidism. Thake et al (1985) reported clinical features that helped to distinguish the boys with the fragile X chromosome from those without were head circumference over the 50th centile, postpubertal testicular volume over the 50th centile, and an IQ between 35 and 70. The prepubertal boys may demonstrate fewer obvious physical features. Macroorchidism was present in fewer than half of patients, and was more difficult to recognize when the testicular volume was small (Carpenter 1983). The normal testicle has a volume of 2 ml until the earlier stage of puberty at approximately 8 or 9 years of age, when it begins to increase in size. Recognition of macroorchidism (4 ml volume) can be difficult unless the testicle is measured with an orchidometer. Simko et al (1989) suggested the fragile X syndrome can be recognized in young children by noting key aspects of behavioral and family histories as well as the physical findings. Thus, patients with fragile X syndrome may be suspected from the clinical features of males with mental retardation of unknown etiology.

The locus of the abnormal gene is on the terminal portion of the long arm of the X chromosome at Xq27.3 and is associated with a fragile site. Not all individuals who carry the fragile X gene, however, demonstrate the fragile X chromosome in cytogenetic studies.

Approximately 20% of males and 40% to 50% of females who inherit the fragile X gene are unaffected by the syndrome and are fra (X) (q27) negative (Sherman et al 1984, 1985). There is evidence that the loss of function of the FMR-1 gene causes mental retardation and the clinical phenotype of the fragile X syndrome. Recently, Wohrle et al (1992) detected a molecular delection at Xq 27.3, including exons of the FMR-1 gene in a fra (X) (q27) negative mentally retarded male who presented the clinical phenotype of the fragile X syndrome.

Hitherto all patients with fragile X syndrome have been ascertained cytogenetically by detection of the fragile site. A molecular genetic analysis is required, however, for the patients who present the clinical phenotype of the fragile X syndrome, but there is no cytogenetic expression of the fragile site.

REFERENCES

Arinami T, Kondol I, Nakajima S: *Frequency of the fragile X syndrome in Japanese mentally retarded male. Hum Genet 73:309-312, 1986.*

Blomquist HK, Gustarson KH, Holmgren G, Nordenson I, Palssonstrae U: *Fragile X syndrome in mildly mentally retarded children in a Northern Swedish country. A prevalence study. Clin Genet 24:293-398, 1983.*

Brondum-Nielsen K: *Sex chromosome aneuploidy in fragile X carriers. Am J Med Genet 23:537-544, 1986.*

Bundey S, Webb TP, Thake A, Todd J: *A community study of severe mental retardation in the West Midlands and the importance of the fragile X chromosome in its aetiology. J Med Genet 22:258-266, 1985.*

Carpenter NJ, Leichtman LG, Say B: *Fragile X-linked mental retardation. A survey of 65 patients with mental retardation of unknown origin. Am J Dis Child 136:392-398, 1982.*

Carpenter NJ: *The fragile X chromosome and its clinical manifestations, in Sandberg AA (ed): Cytogenetics of the Mammalian X Chromosome. Part B. New York, Alan R. Liss, P399, 1983 .*

De Arce MA, Hecht R, Sutherland GR, Webb G: *Guidelines for the diagnosis of fragile X. Clin Genet 29:95, 1986.*

Froster-Iskenius U, Schwinger E, Weigert M: *Replication pattern in XXY cells with fragile X. Hum Genet 60:278-280, 1982.*

Froster-Iskenius U, Felsch G, Schirren C, Schwinger E: *Screening for fra (X) (q) in a population of mentally retarded males. Hum Genet 63:153-157, 1983.*

Hagerman RJ, Smith ACM, Mariner R: *Clinical features of the fragile X syndrome, in Hagerman RJ, McBogg P (eds): The Fragile X Syndrome: Diagnosis, Biochemistry, and Intervention, Dillon, Colo, Spectra Publishing Co, Inc. pp17-53, 1983.*

Harrison OG, Jack EM, Allen TD, Harris R: *The fragile X: A scanning electron microscope study. J Med Genet 20:280-285, 1983.*

Han HS, Moon HR: *Standard of ear size in Korean children. J Korean Pediatr Assoc 35:1089-1095, 1992.*

Jordan BR: *Fragile sites still a mystery. Lancet i:497-498, 1987.*

Kähköner M, Leisti J. Wilska M, Varonen S: *Marker X-associated mental retardation. A study of 150 retarded males. Clin Genet 23:397-404, 1983.*

Lubs HA: *A marker X chromosome, Am J Hum Genet 21:231-244, 1969.*

Martin JP, Bell J: *A pedigree of mental defect showing sex-linkage. J Neurol Neurosurg Psychiatry 6:154-157, 1943.*

Richards BW, Sylvester PE, Brooker C: *Fragile X-linked mental retardation: The Martin-Bell syndrome. J Ment Defic Res 25:253-256, 1981.*

Sherman SL, Morton NE, Jacobs PA, Turner G: *The marker (X) syndrome: a cytogenetic and genetic analysis. Ann Hum Genet 48:21-37, 1984.*

Sherman SL, Jacobs PA, Morton NE, et al: Further segregation analysis of the fragile X syndrome with special reference to transmitting males. Hum Genet 69:289-299, 1985.

Simko A, Hornstein L, Sonkup S, Bagamery N: *Fragile X syndrome: Recognition in young children. Pediatrics 83:547-552, 1989.*

Sutherland GR: *Heritable fragile sites on human chromosomes. I. Factors affecting expression in lymphocyte culture. Am J Hum Genet 31:125-135, 1977.*

Sutherland GR: *The enigma of the fragile X chromosome. Trends Genet 1:108-112, 1985.*

Sutherland GR, Baker E: *The common fragile site in band q27 of the human X chromosome is not coincident with the fragile X. Clin Genet 37:167-172, 1990.*

Thake A, Todd J, Bundey S, Webb A: *Is it possible to make a clinical diagnosis of the fragile X syndrome in a boy? Arch Dis Childh 60:1001-1007, 1985.*

Turner G, Robinson H, Laing S, Purvis-Smith S: *Preventive screening for the fragile X syndrome. NEJM 315:607-609, 1986.*

Verkert AJMH, Pieretti M, Sutcliffe JS, Fu Y-H, Kuhl DPA, Pizzuti A, Reiner O: *Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905-914, 1991.*

Webb TP, Bundey S, Thake A: *The frequency of the fragile X chromosome among school children in Coventry. J Med Genet 23:396-399, 1986.*

Wöhrle D, Kotzot D, Hirst MC, Manca A, Korn B, Schmidt A, Barbi G, Rott H, Poustka A, Davies KE, Steibach P: *A microdeletion of less than 250 kb, including the proximal part of the FMR-1 gene and the fragile-X sites, in a male with the clinical phenotype of fragile-X syndrome, Am J Hum Genet 51:299-306, 1992.*