Fragile (X)(q27) sites in a pedigree with female carriers showing mild to severe mental retardation

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SUMMARY A pedigree showing the fragile site at Xq27 in a severely retarded female and in other less retarded carriers is described. Two of the four moderately retarded males with the fra(X)(q27) show macro-orchidism, and a variety of other features usually used to support the effects of the fra(X)(q27) are also inconsistent. A second fragile site at (10)(q23) is also present and in the two oldest females its frequency is not decreased, whereas the fra(X)(q27) is not detectable in these females although probably present. It is concluded that pedigrees showing mentally retarded females and probable X linkage should be included in studies of the fra(X)(q27).

The development of the topic of fragile (X)(q27) sites and associated X linked mental retardation has recently been well summarised by Gerald.¹ Recently, interest has shifted from affected males to the effect of the fra(X)(q27) in heterozygous females who may be normal or 'dull', but it has been suggested that about 20 to 30% of carriers are clinically detectable as retarded.²

We report a family (fig 1) which was initially excluded from a study of fra(X)(q27) because it showed some mentally retarded females and so did

Received for publication 6 March 1981

not conform to a recessive mode of X linked inheritance. However, the proband showed macro-orchidism which has been strongly associated with the fra(X)(q27) by some authors,³⁻⁶ but not by others.⁷ On investigation the pedigree was found to involve both the fra(X)(q27) and the fra(10)(q23) sites.

Methods

For the subjects in this family it was not possible to measure mental abilities in a uniform fashion because

FIG 1 Family pedigree.

the members are widely dispersed, with retarded members in a variety of institutions. Because of this, retarded subjects are largely classified on the basis of clinical assessment, supported by psychological testing in certain critical cases. The classification of retardation is that of the World Health Organization⁸ with IQ ranges: mild 50–70, moderate 35–49, severe 20–34, and profound 0–19.

For chromosome studies whole blood was cultured in TC 199 using methods which are well documented for the detection of fragile sites.⁹ Initially, 5% calf serum was used but an increase to 14% was found to give an improved yield of metaphases without affecting the frequency of fragile sites.

A minimum of 50 metaphase plates were analysed for each patient, with more cells analysed to confirm low or zero scores. Apart from two cases investigated early in the study all cases were scored from G banded slides following the discovery of a lesion at 6q26 which looked very similar to the fra(X)(q27) in plain stained preparations.¹⁰ In some cases both methods were used.

The normal testes of two of the mentally retarded males were measured with an orchidometer. In the two mentally retarded males with macro-orchidism the testicular volumes were calculated from the length (l) and width (w): the volume being $\pi lw^2/6$.¹¹ The two males with macro-orchidism had mean testicular volumes well in excess of 23 ml (90th centile).³

Results

MALES WITH fra(X)(q27)

All four males with the fra(X)(q27) also carry the fra(10)(q23) and they are all assessed as being in the lower range of moderate mental retardation.

The proband (III·1) and his maternal uncle (II·3) have similar unusual facies with a prominent mandible, long narrow face, broad solid nose, and large ears (figs 2, 3). Individual unusual

FIG 3 Male II·3.

characteristics of III·1 include bright blue irides, rather hyperkeratotic skin with an unusual rash across the cheeks, nose, and chin, and pes planus. II·3 has hazel eyes, brachycephaly, and hepatomegaly. However, the most striking difference between these two males is the size of the testes: III·1 has macro-orchidism, whereas II·3 has testes with a normal volume (table).

The two brothers II·6 and II·7 also show a marked difference in testis size: II·6 has normal testes and II·7 shows extreme macro-orchidism (table). II·6 (fig 4) is quite unlike II·3 and III·1. He has a long narrow face with a normal chin, large ears with unrolled helices, and an antimongoloid slant to his brown eyes. He shows mild gynecomastia and has received hormone treatment, but his testes, penis, beard, and pubic hair are normal. II·7 has not been seen by us but has been examined at our request. He has brown eyes, normal chin, no gynecomastia, and large ears (75 mm long).

MALES WITHOUT THE fra(X)(q27)

The intellectual normality of these males is indicated by their normal progress in school and in some cases

FIG 2 The proband III·1.

TABLE Frequencies of the fra(X)(q27) and fra(10)(q23) sites

Person	Sex	Year of birth	fra(X)(q27) (%)	fra(10)(q23) (%)	Mean testis volume (ml)
I.1	F	1903	0	19	
I.2	F	1905	0	15	
II.1	F	1934	12*, 6	18*, 15	
II.3	M	1940	52	34	17.5
II.4	F	1943	32	0	
II.5	F	1945	42	0	
II.6	M	1942	6	17	20
II.7	M	1945	14	20	71
III.1	M	1959	35	27	59
III.2	F	1960	34	0	
III.3	F	1965	18*, 19	10*, 11	
III.4	M	1970	0	18	
III.8	F	1962	46	0	

*Percentages derived from plain stained preparations.

FIG 4 Male II·6.

by clinical assessment. IV·1 is only 6 months old but is developing normally. The male in generation I was intellectually normal and died aged 68. Only one normal male is carrying the fra(10)(q23). The rest of them are not at risk for inheritance of this fragile site.

RETARDED FEMALES WITH THE fra(X)(q27)

There is a large range of mental abilities in females in the pedigree who carry the fra(X)(q27). The most retarded carrier is II·5 (fig 5) who is functioning intellectually at the lower limits of severe mental retardation as assessed clinically and psychologically (IQ less than 30, mental age 3·5 years). She is emotionally disturbed and cries easily. She has few self-help skills, poor personal hygiene, a short attention span, and is capable of only simple tasks. She has similar facies to her brother II·3, with hazel eyes, a very prominent jaw, and broad nose. Characteristics which are not shared with her brother include a divergent squint, poor teeth, and mild obesity. Along with three of five members of her sibship, II·5 has not inherited the fra(10)(q23) from her mother.

III·2 (fig 6) is regarded as physically very similar

FIG 5 Female II·5.

FIG 6 Female III·2.

to her aunt II·5. However, psychological testing gave a result in the upper moderate range. She is not permanently confined but works in a sheltered environment. She speaks in short sentences and cries readily. During psychological testing she was notably shy, embarrassed, and distracted by the male assessor. She has a prominent jaw and bright blue irides like her brother III·1, and she has a high arched palate but no other dysmorphic features. She is mildly obese.

III·8 has a very prominent jaw and shows similar shy and embarrassed behaviour to her cousin III·2. She has been psychologically assessed as mildly retarded. She reached middle secondary school and will probably gain employment in a sheltered environment. This girl had fra(X)(q27) sites visible in 46% of her lymphocytes, slightly higher than her aunt II·5 (table). These are the highest frequencies yet recorded in carriers, the next highest frequency being 38%.⁵

OTHER FEMALE CARRIERS OF THE fra(X)(q27)

It is likely that all but one of the carrier females in this pedigree have some intellectual impairment, probably resulting from the fra(X)(q27). III·3 has blue eyes and a prominent jaw. She has an arched palate but no other dysmorphic features. She was clinically assessed as dull normal—mildly retarded (borderline). Her own doctor describes her as highly emotional. She reached middle secondary school with difficulty and her part-time employment involves simple work.

The carrier mothers in the pedigree are socially well adjusted and some of them are coping with economically deprived situations. II·1 only reached the end of primary schooling and she was clinically assessed as dull normal—mildly retarded at the same time as her daughter III·3. Her sister II·4 was more successful in her schooling but is described by the family physician as slower than II·1. However, for want of further information we must regard II·4 as dull normal.

The intellectual capacity of I·1 is regarded as difficult to assess by her family physician, who has known her for only 12 months. This is because of age-related physical deterioration. However, her normal daughter (II·2) gave a description of her mental capacities at a younger age which indicates that she was at least mentally normal and probably better than average. I·2 has been known to her family physician for 30 years and is regarded by the doctor and by her normal niece as intellectually dull. It is interesting to note that in these old sisters the fra(X)(q27) cannot be detected, although it must be presumed present. However, the frequency of the fra(10)(q23) is not affected by their age (table). Turner *et al*⁵ found the fra(X)(q27) in 2% of cells in a 65-year-old obligate carrier, the oldest female to show the site, but it is often not detectable in obligate carriers at a much younger age. Therefore, it is unlikely that the fra(X)(q27) could be detected in I·3. For social reasons I·3 and her family cannot be tested.

FEMALES WITHOUT THE fra(X)(q27)

These are all normal. III·2 is less than 2 years old but she has reached her milestones at expected ages. The evidence on the normality of the family of I·3 is heresay but it is on reasonable grounds.

Discussion

The most important result in this investigation was the finding of a range of intellectual capacity in female carriers of the fra(X)(q27) with the most extreme being severely retarded. In previous investigations, female carriers of the fra(X)(q27) have been reported with degrees of retardation up to the mild level (Turner *et al*² and other papers listed by them). The Lyon hypothesis has been used to explain a range of retardation in carriers of the fra(X)(q27).^{1,2} Gerald¹ suggested that the severity of expression of mental retardation in these females might depend directly on the proportion of cells which by chance have the normal X inactivated in tissues critical to intellectual capacity. The finding of a severely retarded carrier is compatible with this argument and suggests that in such a case the relevant tissues might have a preponderance of cells with the normal X inactive and the fra(X)(q27) active. The alternative extreme, with most cells having the normal X active in critical tissues, should pertain in carriers who are normal. These carriers might even have greater than average intelligence and I·1 could be in this category.

Another hypothesis, put forward by Gerald,¹ is that carrier mothers of affected sons with the fra(X)(q27) represent a population selected for their

closeness to intellectual normality. Superficially our results appear to agree with this hypothesis but there is no reason to suppose that III·2, III·3, and III·8 could not bear children.

It is clear that the presence of retarded females in a pedigree should no longer be taken as a reason for not searching for the fra(X)(q27) in retarded males and their female relatives. What is not clear is a set of indications which will always lead to detection of the fra(X)(q27).

Although macro-orchidism initially led us to this family, normal sized testes were also found in males with the fra(X)(q27). Furthermore, this variation was observed within one sibship. Another such finding was family A in the report of Harvey *et al*¹² after later measurement of some of the testicular volumes by Sutherland *et al*.⁶ Four of the six mentally retarded males with the fra(X)(q27) in this family were measured, three showed macro-orchidism, and one had normal testicular volumes. Sutherland and Ashforth³ described a solitary 65-year-old male with fra(X)(q27) and small (12·5 ml) testes, who was the only post-pubertal male who did not have macro-orchidism in a sample of males with the fra(X)(q27). Testicular atrophy was suggested in this male by Sutherland and Ashforth, but a drastic reduction in size must have taken place if this male showed macro-orchidism at a younger age. While macro-orchidism is probably common among males with the fra(X)(q27), the finding of normal testis volumes among such males may not be rare, as illustrated in the pedigree we have described. Indeed, of six families with pedigrees of X linked mental retardation described by Jacobs *et al*,⁷ three showed the fra(X)(q27) and no macro-orchidism, one had no fra(X)(q27) but showed macro-orchidism, and the remaining two had neither fra(X)(q27) nor macro-orchidism. It does appear that there is a relationship between macro-orchidism, fra(X)(q27), and mental retardation, but elucidation of the exact interrelationship of the factors concerned will probably require much more information from pedigrees.

Pale blue irides are not seen in all males and females with the fra(X)(q27) in our pedigree, although in the index case they were seen as a supportive feature. In the affected members of the pedigree with hazel and brown eyes, genes for brown irides appear to be dominant to blue eyes. Mild gynaecomastia has been previously reported in some mentally retarded males with macro-orchidism who by now might have been analysed for fra(X)(q27).^{13,14} However, II·6 is the first male reported with the combination of gynaecomastia, fra(X)(q27), mental retardation, and normal testes. In the same male the

usual prominence of the jaw is absent. In one girl (III·9) without the fra(X)(q27), a prominent jaw was noted so this feature might be under some familial influence in this pedigree.

The variability of males and females carrying the fra(X)(q27) supports the recommendation that a medium suitable for demonstration of fragile sites should be routinely used in diagnostic laboratories (Sutherland editorially supported by Hecht and Kaiser-McCaw⁹). The finding of fra(X)(q27) may be an unexpected result in cases of mental retardation. For example, in our laboratory we have detected the fra(X)(q27) in prepubertal males with the provisional diagnoses of 'developmental delay' and 'XYY'.

The occurrence of the fra(10)(q23) in the pedigree and its resistance to the aging effect which applies to the fra(X)(q27) is of cytogenetic interest. The males carrying both of these sites should yield some interesting observations on the behaviour of the sites during male meiosis if a biopsy can be obtained.

We thank Dr J G Rogers, Dr S Wiener, Professor D M Danks, Jan Schofield, Susan Pavey, and Lynda Green for advice and help. The donation of a photomicroscope by the Fund for Research and Rehabilitation of Retarded Children is gratefully acknowledged.

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