Twelve families with fragile X(q27)

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SUMMARY Through a community study of boys requiring special education for the severely mentally retarded, 12 families were ascertained in which the fragile X was found to be segregating. By assiduous follow up of these families, it was found that in only four of them could male transmission be ruled out from the grandparents' or great grandparents' generation and that the segregation ratios are disturbed.

In the course of a population study of X linked mental retardation, 14 boys who were receiving special education at schools for the severely mentally subnormal were found to carry the fragile X(q27.3).¹ There were two sets of brothers so a total of 12 families in which the fragile X was segregating had been ascertained in a completely unbiased manner. The pedigrees are shown in the figure.

In view of the reported unusual segregation pattern of the fragile X syndrome, in that one-third of the heterozygous females can be affected² and that the disease can be transmitted through a normal male,³⁻⁵ it was decided to follow up all family members of either sex, both affected and unaffected. In this way it was hoped to determine whether the unusual segregation and inheritance patterns found in the fragile X syndrome could be partially the result of bias in ascertainment of the families studied so far, or of partial follow up of affected and unaffected subjects in fragile X families.

Methods

A population study of boys who were receiving special education for the severely mentally handicapped has been previously described.¹ Before the results of the cytogenetic investigation became known, each of the 156 boys who entered the study was visited at home and a pedigree constructed. A clinical assessment of each child was also undertaken.

Any information gained was not imparted to the cytogeneticist who undertook the chromosome studies 'blind'.

When a fragile X(q27.3) was detected, permission was then sought to study the relatives of such

probands through further home visits in which blood samples were taken and a clinical examination made of each family member. Efforts were made to contact each member of the family pedigree so that a complete cytogenetic study could be made of the extended family.

CYTOGENETIC STUDIES ON FAMILY MEMBERS

Chromosome studies were carried out as described previously, with the addition that further treated cultures were maintained for each family member, as the heterozygote is more difficult to diagnose than her hemizygous affected male relative, particularly if she is of normal intelligence.

For each subject, six cultures were established. Day 1. (1) TC199+2% fetal calf serum (FCS). (2) TC199+2% FCS+10⁻⁷ mol/l methotrexate (MTX). (3) Medium M+2% FCS. Day 2. (1) TC199+2% FCS. (2) TC199+2% FCS+10⁻⁷ mol/l FUDR. (3) Medium M+2% FCS+10⁻⁷ mol/l FUDR.

Lymphocyte cultures were always established on two separate occasions using two separate batches of TC199 in case the first culture failed to grow. After 72 hours at 37°C, chromosome harvesting, slide making, staining, and banding were all carried out by routine methods. At least 100 cells were studied for each subject at risk. Where possible this total included 25 cells from an MTX⁶ containing culture and 25 from a FUDR^{7 8} containing culture. On occasions, however, these antagonists were found to lower the mitotic index to such an extent that 25 mitoses were not available. Further mitoses were then studied from another culture to make up the deficiency.

Three slides were made from each culture. The first was stained with Leishman and the others were GTG banded. Despite controversy as to whether banded or unbanded slides should be used for the detection of the fragile X, the initial examination was always

made with unbanded slides. If no fragility of a C group chromosome was detected in the unbanded mitoses, then it was reasonably certain that the marker X was not being missed. Any detection of fragile X(q27.3) was always confirmed using banded slides, but it was found that much time was spent agonising over 'woolly ends' when quantitative results were being compiled. It is for this reason that unbanded slides were used for the initial screening.

During home visits to individual family members, the IQ range was assessed. When the females were divided into retarded and normal, the type of schooling received by the subject was used as the criterion to aid this assessment.

Results

In all, 163 family members (excluding the 14 probands) from the 12 families cooperated in this study. Their ages ranged from 0 to 76 years and their IQs from normal to severely retarded for both sexes. Of the 163 subjects, 95 were female and 68 male. In all, 63 members in addition to the 14 probands were found to carry the fragile X(q27.3): 51 females and 12 males. All but one of the males with the marker were mentally retarded, one being apparently normal (III.1 from family 7). Of the 24 mentally retarded males the numbers of fragile X present ranged from 0 to 40% with an average of 16%. Seventeen of the 51 fragile X positive females were mentally retarded (33%).

Of the 20 obligate heterozygotes studied only 17 could be shown to carry the fragile X. Thus three of 20 or 15% of the obligatory heterozygotes were missed. The range of fragile X found in this group ranged from 0 to 29% with an average of 6% and their ages ranged from 17 to 75 years.

Of the 20 obligate heterozygotes, six were retarded. If the remaining 14 are considered separately, then three of 14 or 21% obligatory heterozygotes of normal IQ were not found to carry the fragile X and were thus missed. The range of fragile X found was from 0 to 11% with an average of 3.3%. For the six retarded obligate heterozygotes the range of fragile X found was from 7 to 29% with an average of 15%.

To test the reproducibility of the detection in 'low level' females, several family members were retested after periods ranging from a few months to three years. A further sample was obtained from eight such females from six of the families. The results from the second blood sample were almost identical to those from the first and the identification of a low level carrier was not really improved by repeating the analysis, although it may have been tightened up a little (table 1).

SEGREGATION RATIOS WITHIN FAMILIES

Two of the families (family 5 and family 11) had a pair of brothers as independent probands. These four therefore had to be considered both as 'brothers' and as probands. In addition there was one normal brother in family 5 and two normal brothers in family 11. These three had to be considered as brothers to both the probands in their families and so had to be counted twice. In order to calculate segregation ratios there were then 17 brothers, of whom 16 were studied cytogenetically, 12 were of normal intelligence, and five were mentally retarded. All of the five retarded brothers could be shown to carry the marker but none of those with normal IQ did.

There were 14 living sisters of the probands, all of whom were studied. For reasons explained above, the two sisters from family 11 were considered twice, giving 16 sisters in all. Both of the two who were classed as retarded showed the presence of the marker. Of those 14 who attended normal schools and were considered to be of normal intelligence, eight showed the marker. Thus, in the probands' generation five of 16 (31%) brothers and 10 of 16 (62%) sisters were found to carry the fragile X(q27.3).

In order to study the inheritance of the fragile X syndrome in other family members, the risks must be calculated by considering that the disease can be transmitted through a normal male.

The fragile X was not shown in any family member other than the proband in three of the 12 families. These three could have been the consequence of a new mutation (families 4, 6, and 8).

When the generation before the proband was considered alone it was found that of 24 maternal aunts, six (25%) were mentally retarded and carried the marker X, nine (38%) carried the fragile site but were of normal IQ, while only eight (33%) had neither the fragile site nor a low IQ. There was one missed obligate heterozygote.

In all, 16 maternal uncles were studied and, of

TABLE 1 Comparison between results of fragile X estimation in low level females on two separate occasions.

Subject	First attempt	Second attempt
	Total over all media (%)	Total over all media (%)
SB	4.1	3.8
JB	3.5	1.1
PL	2.6	0
SC	3.4	4.0
TL	2.9	4.0
MW	1.0	0
FM	3.0	4.0
TB	1.6	1.0

FIGURE Pedigrees of the 12 families.

Twelve families with fragile X(q27)

these, one was mentally retarded and carried the fragile X. Even if the two sons of the male transmitter from family 10 are removed, then the incidence of affected uncles becomes only one in 14. Of the remaining 13, one was mentally retarded, but despite investigation of two separate blood samples could not be shown to have the marker (II.4 in family 11) and another was a normal male transmitter (III.1 in family 7). This still gives the ratio of three of 14 maternal uncles who have possibly inherited the gene, with only two of 14 expressing it.

To try to determine whether these disturbed segregation ratios are due wholly or in part to the presence of normal male transmitters who do not show the fragility of the X chromosome, it is necessary to consider only those family members who have 50% risk of inheriting the syndrome. Closer scrutiny of the pedigrees, however, revealed that transmission from the maternal grandfather or the mother of the proband having a new mutation could not be ruled out in families 1, 2, 3, 5, or 9.

This left only four pedigrees (families 7, 10, 11, and 12) in which the fragile X had been definitely inherited from the maternal grandmother or, in the case of family 10, maternal great grandmother. If all the family members at a 50% risk of inheriting the gene are considered for these four families, then 18 of 32 (56%) males and 33 of 45 (73%) females have done so. As the 18 males include both the 'normal transmitter' from family 7 and the chromosomally non-manifesting but retarded uncle from family 11, it was decided that a more unbiased result might be obtained by considering only the children of maternal aunts from all 12 families who have themselves been shown to carry the fragile X. The ratios then become four in 10 (40%) for males and 12 in 19 (63%) for females. There is thus a disturbed segregation ratio for those subjects at 50% risk of inheriting the syndrome.

Discussion

As a consequence of the unbiased ascertainment of 14 male subjects with the fragile X syndrome in a defined population area, 12 families were studied. Each family was studied by the same cytogeneticist using exactly the same culture conditions, in order to minimise between subject or laboratory differences.

It was found that the segregation ratios expected for an X linked recessive condition did not hold for the extended families. There were too many female heterozygotes and too few affected males. When the generation before the proband was considered alone, this disproportion became even more striking as there were too many female carriers in the generation before the severely affected male proband, 16 of 24

(67%) maternal aunts carrying the gene. A proportion of this imbalance could be accounted for by normal male transmission. In order to test this, only the four families in which the gene was shown to be female transmitted were considered, and only the children of maternal aunts from all 12 families who were actually shown to carry the fragile X(q27.3) themselves were considered. The segregation ratios were still disturbed, particularly for females. In case this was due to errors in the over detection of fragile X in these families, two other sets of families were considered: those families which had been referred by clinical geneticists as being likely carriers of the fragile X syndrome, where sufficient members had been followed up to give useful comparisons, and, secondly, published reports of families which again were large enough and had sufficient members studied cytogenetically. Both of these types of families showed the same trend, in that there were too many female heterozygotes in the generation before the proband. In the referred families, six of seven maternal aunts carried the gene, shown either by the presence of the fragile X or because they were obligate heterozygotes. However, transmission from the maternal grandfather was a possibility in one of them.

When published reports of eight families were studied, in which the proband was identified and there were sufficient family members in the preceding generation, then 16 of 21 (76%) maternal aunts were reported as either carrying the fragile X or being obligate heterozygotes. These families may not have been ascertained in an unbiased manner, as our 'referred' families were not (table 2).

Both male transmission and a very high mutation rate in sperm only have been suggested as contributing to the prevalence of the syndrome.¹⁵ Two of the families in this study certainly seemed to have a male transmitter. One subject, unfortunately dead (II.4 in family 10), conformed to the reported pattern¹⁶ in that all his daughters were of normal IQ, all three showed the fragile X at a very low level (4%, 4%, and 3%, respectively), and all three had severely affected offspring. The mental retardation 'comes out' in the third generation. However, this family is unusual in that the normal male transmitter also had a severely affected mentally retarded brother with 12% of fragile X (II.2). It is not often found that a severely affected male occurs in the same generation as a normal male transmitter.¹⁶ The daughter of the male transmitter from family 7, who was detected cytogenetically, was also of normal IQ and also had a low level of fragile X (6 in 150, 4%). This family had a further male transmitter who was not studied (II.4).

TABLE 2 (a) Segregation of fragile X(q27-3) in maternal aunts: this study.

Family	Total	Missed obligates	Lowered IQ		Normal IQ	
			Fra X +ve	Fra X -ve	Fra X +ve	Fra X -ve
1	4	1			2	1
2	1					1
8	2					2
9	5		2		1	2
10	2				2	
11	5		2		2	1
12	5		2		2	1
Total	24	1	6		9	8

TABLE 2 (b) Segregation of fragile X(q27-3) in maternal aunts: referred families.

Family	Total	Missed obligates	Lowered IQ		Normal IQ	
			Fra X +ve	Fra X -ve	Fra X +ve	Fra X -ve
Gr	1					1
Cr	2				2	
D	2				2	
Ho	2				2	
Total	7				6	1

TABLE 2 (c) Segregation of fragile X(q27-3) in maternal aunts: cited from published reports.

	Total	Missed obligates	Lowered IQ		Normal IQ	
			Fra X +ve	Fra X -ve	Fra X +ve	Fra X -ve
Gardner <i>et al</i> ⁹	6	3			2	1
Froster-Iskenius <i>et al</i> ¹⁰ (1)	1				1	
Froster-Iskenius <i>et al</i> ¹⁰ (2)	2					2
Fryns and van den Berghe ⁵	2				2	
Webb <i>et al</i> ¹¹	3		1		1	1
Rhoads <i>et al</i> ¹²	1		0		1	0
Van Roy <i>et al</i> ¹³	2		1		1	
Gustavson <i>et al</i> ¹⁴	4	1			2	1
Total	21	4	2		10	5

A further five of our families may have derived from a transmitting male in generation I. If so, this would change the 'at risk' figures in these families from 50% for both sexes to 100% for females and 0 for males in generation II.

The fragile X was not found in a further 23 males at low risk, including nine of the fathers who were included as controls.

The families were also studied to see whether the level of fragile X ran true within them. It has become our experience with referred patients that there are some 'low level' families in which the fragile site is particularly difficult to detect in all members, making prenatal diagnosis even more hazardous for the heterozygotes within them. However, this was not true of the 12 families in this study as large differences in the percentage of fragile X were found to be normal for both males and females, although

retarded females were easier to detect than those heterozygotes of normal IQ. The highest level found in a retarded male (40%) and the lowest (3%) were both from the same family (table 3).

Several of the females at risk with normal IQ fell into the borderline category for the detection of fragile X(q27), making it difficult to decide whether they were carriers or not. It was not known how reproducible the results were between blood samples taken on different occasions, and a second sample could well have clarified the situation in a number of cases. When eight repeat blood samples were studied, however, the results obtained were very similar to those from the first sample and the only clarification came from the increased numbers of cells analysed, giving a greater degree of confidence in a borderline result (table 1).

It appears that in addition to the anomalies shown

TABLE 3 Level of fragile X(q27.3) within families. Cells cultured in TC199 + FCS.

Family	Males with low IQ (%)	Females with normal IQ > 20 years old (%)	Females with normal IQ < 20 years old (%)	Females with low IQ (%)
1	9	1-4	4-31	
2	32	6	32	
3	34	6	5	4-31
4	4	0		
5	16-24	6		
6	5	0		
7	9	0-6	4-5	
8	25	1		
9	16	4		10-28
10	7-20	4-10	5-22	6-23
11	0-28	0-4	12	7-29
12	3-40	4-14	4-8	6-14

Only females considered to be carrying the fragile X are included in the table, that is, those found to be cytogenetically positive and the 'missed' obligate carriers, including all mothers.

by the fragile X syndrome, in that one third of female heterozygotes are clinically affected and that the condition can be transmitted through a normal male, segregation ratios are disturbed as there is an excess of females who appear to inherit the gene and a decrease in the number of males who do so.

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Note added in proof

We have recently been successful in obtaining a blood sample from male transmitter II.4 from family 7. It was negative for the fragile X. He is 90 years old.

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