Original articles

FRAXE and mental retardation

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Abstract

Mental impairment and instability of the CCG repeat at FRAXE is described in six kindreds. Cosegregation of FRAXA and FRAXE was found within one of these kindreds. Cytogenetic expression of FRAXE was shown to skip a generation when associated with a reduction in size of the CCG expansion when transmitted through a male; however, in general, transmission occurred through females and copy number increased from one generation to the next. In these respects the behaviour of FRAXE paralleled that of FRAXA. A relationship between FRAXE and non-specific mental impairment is strongly suggested by the occurrence in these families of more mentally impaired male and female carriers, after removal of index cases, than could reasonably be expected by chance.

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Cytogenetic detection of the fragile X (FRAXA) has been confused by the discovery of two nearby rare folate sensitive fragile sites. These were designated FRAXE^{1,2} and FRAXF.³ FRAXE sequences have been isolated approximately 600 kb distal to FRAXA⁴ and FRAXF sequences have been isolated further distal.⁵ These three fragile sites cannot be differentiated by conventional cytogenetic analysis but were initially delineated by fluorescence in situ hybridisation (FISH) using DNA probes which separate them. Direct molecular analysis of specific restriction fragments now enables definitive diagnosis by Southern analysis for all three fragile sites.

The fragile sites FRAXE and FRAXF have recently been shown to have cytogenetic and molecular features in common with FRAXA.¹⁻⁶ The mutations responsible for the cytogenetic expression of FRAXA, FRAXE, and FRAXF are amplifications of (CCG)_n trinucleotide repeats; in the case of FRAXA, the CCG repeat is adjacent to the CpG island at the 5' end of the FMR1 gene.⁷ Amplification of CCG to a critical size at FRAXA and FRAXE has been shown to be associated with hypermethylation at nearby CpG islands. The

FMR1 gene is usually silenced (presumably by hypermethylation of the CpG island) when the trinucleotide copy number exceeds approximately 230 copies,⁸ causing fragile X syndrome. The findings of Knight *et al*⁹ suggest that a similar mechanism may inactivate a gene near the CpG island associated with FRAXE and thereby cause mental retardation.

To investigate the relationship between FRAXE and mental retardation we describe the phenotypic, cytogenetic, and molecular features in six FRAXE families collected from four centres. One of these families was ascertained for FRAXE in an unbiased fashion as FRAXE was found accidentally during family follow up after diagnosis of fragile X (FRAXA) syndrome in the proband. The mode of ascertainment for the remaining FRAXE families was by cytogenetic detection and molecular confirmation of FRAXE in a mentally impaired family member. Detailed phenotypic investigations and molecular delineation of carriers of FRAXA and FRAXE was carried out in the family segregating with both fragile sites, FRAXE and FRAXA.

Methods and clinical data

DELINEATION OF FRAGILE SITES BY FISH

The fragile sites in the first four families were delineated as FRAXE by FISH before the molecular characterisation of FRAXE. This was achieved using methods as in Sutherland and Baker.¹

LOCALISATION OF DXS1123 BY FISH

The marker DXS1123 is closely linked (1.2 cM) and distal to FRAXA¹⁰ and must therefore be closely linked to FRAXE. FISH was carried out using the lambda clone DNA of DXS1123 using methods previously described¹ in order to orientate DXS1123 physically in relation to FRAXE. FISH established the physical location of the AC dinucleotide repeat marker DXS1123 to be distal to FRAXE. Thus, the closest flanking markers for indirectly tracking the segregation of FRAXE through families are the AC dinucleotide repeat markers FRAXAC1 and FRAXAC2 within the FMR1 locus proximally and DXS1123 distally.

Families studied showing percentage of cytogenetic expression of fragile sites (two values indicate repeated testing) above the Δ in kb for FRAXE. The estimated degree of methylation as a percentage, for the FRAXE associated CpG island only, is given as a superscript to the FRAXE Δ value. The fragile site expression in family 1 relates to FRAXE for I.1 and III.6, FRAXE or FRAXA for II.3 and FRAXA for III.3, III.4, and III.5. The Δ values for FRAXA for the relevant family members are given separately in the text. Families 2 to 6 have only FRAXE.

CYTOGENETIC ANALYSIS

The fragile site FRAXE was induced from lymphocyte cultures as previously described.¹ The percentage of cells expressing the fragile site in distal Xq is shown in the figure.

SOUTHERN ANALYSIS

Direct molecular diagnosis of the amplifications at FRAXA and FRAXE was carried out by standard Southern analysis as previously described using the probe pfxa3 specific for FRAXA¹¹ and the probe OxE20 specific for FRAXE.⁴ The FRAXE CCG amplification (Δ) was determined from the *Hind*III fragment

(normal size 5.2 kb). Assessment of methylation status of the CpG island adjacent to FRAXE was initially attempted by *Hind*III/*Sac*II double digestion (normal fragment size 1.8 kb). However, an additional *Sac*II restriction site omitted from the original restriction map⁴ occurs immediately proximal to the OxE20 probe between the OxE20 sequence and the CCG repeat. This restriction site prevented detection of incremental fragments when unmethylated (where there were small CCG amplifications). Methylation was reassessed by *Hind*III/*Not*I double digestion (normal fragment size 2.8 kb). Methylation status of the CpG island adjacent to FRAXE for

carrier males and carrier females was estimated simply as the relative intensity of the expanded *HindIII* fragment and the expanded *HindIII/NotI* fragment. Absence of an expanded *HindIII/NotI* fragment in a FRAXE carrier indicated complete methylation. Blots of FRAXE carriers were then reprobed with pfxa3, as a control probe, to exclude incomplete digestion as an explanation for apparent methylation of the FRAXE CpG island. Presence of a single *HindIII/NotI* fragment of 2.4 kb excluded the possibility of partial digestion. Reprobing the original *HindIII/SacII* blots on FRAXE subjects with pfxa3 gave normal fragments of size 2.4 kb indicating that all FRAXE carriers were unmethylated across the FRAXA CpG island.

The FRAXA amplification in family 1 was determined from the *PstI* fragment (normal size 1.0 kb) and the methylation status of the CpG island adjacent to FRAXA was assessed by *EcoRI/EagI* double digestion (normal size of the *EcoRI* fragment 5.2 kb and normal size of the *EcoRI/EagI* fragment 2.8 kb).

PCR ANALYSIS

The trinucleotide repeat polymorphism associated with FRAXE was genotyped as copy number.⁴

TESTING FOR COGNITIVE ABILITIES IN FAMILY 1

A comprehensive battery of testing was administered to family 1 to measure specific abilities¹² but only data on Peabody Picture Vocabulary Test (PPVT), Block Design (BD), Digit Span (DS), and Similarities (Sim) are reported here. Block Design and Similarities were subtests from the Wechsler Pre-School and Primary Scale of Intelligence or the British Ability Scales, depending on the person's mental age. The Digit Span task was that in the Wechsler Intelligence Scale for Children-Revised. All scores have been scaled to a uniform mean of 100 and standard deviation of 15. PPVT and Block Design have been combined to give an overall IQ score, using the method described by Sattler¹³ for deriving short form IQs.

Results

DESCRIPTION OF FAMILIES

The families described were ascertained by referral of a mentally impaired family member for routine cytogenetic analysis, leading to the detection of a fragile site in distal Xq. Families 2 and 3 were excluded as FRAXA families by Southern analysis using the probe pfxa3, classified as FRAXE families by FISH, and subsequently confirmed as FRAXE by direct analysis when the OxE20 probe was isolated. Families 4, 5, and 6 were excluded as FRAXA and classified as FRAXE by direct Southern analysis using the probes pfxa3 and OxE20. In family 1, originally ascertained by the presence of fragile X (FRAXA) syndrome in a family member, the cosegregation of FRAXE in addition to FRAXA was inferred when I.2 rather than I.1 had an amplified fragment detectable

with the probe pfxa3 which is specific for FRAXA. I.1 only, in this generation, was the one with cytogenetic expression of a fragile site. FISH was then used to classify the fragile site in I.1 as FRAXE. The pattern of segregation of both FRAXA and FRAXE in family 1 was subsequently confirmed by direct molecular diagnosis using the probes pfxa3 for FRAXA and OxE20 for FRAXE. The figure shows the FRAXE amplification with methylation status. The pattern of transmission of instability at the FRAXE locus for the first three pedigrees, as determined by Southern analysis, was identical to that predetermined by the closely linked PCR markers flanking FRAXE (data not shown). PCR analysis of FRAXE expressed as copy number in all pedigrees (data not shown) was consistent with results determined using the probe OxE20; however, absence of a PCR product as a criterion for diagnosis must be used with caution as it could result from either expansion of the CCG repeat or the failure of a normal allele to PCR amplify for unknown technical reasons. Closely linked flanking markers such as FRAXAC1, FRAXAC2, and DXS1123, and the FRAXE polymorphism itself, represent a backup to diagnosis in the event of ambiguity in the interpretation of Southern analysis.

FAMILY 1 (FIGURE)

The lower functioning subjects III.3, III.4, and III.5 carry only FRAXA and have fragile X syndrome. The somatically heterogeneous FRAXA expansion is represented by amplifications between 1.7 and 2.0 kb for III.3, of 1.1 and 2.4 kb for III.4, and 0.6 and 1.1 kb for III.5. These people do not carry FRAXE and are irrelevant for the investigation of an association between FRAXE and mental impairment. II.3 carries both FRAXA ($\Delta=0.6$) and FRAXE ($\Delta=0.2$) and, therefore, the relative contributions to mental impairment from FRAXE and FRAXA cannot be delineated. II.1 and II.3 both have small FRAXE amplifications ($\Delta=0.3$ and 0.2 respectively), but II.1 performs considerably better on cognitive tests than II.3 who has both fragile sites (table 1). The members of this family with mental impairment associated with the presence of FRAXE are I.1, III.1, and III.6. III.2 has a small FRAXE amplification ($\Delta=0.4$ kb), and does not show intellectual deficits.

Family 1 is unique since both fragile sites, FRAXA and FRAXE, are segregating. FRAXE was transmitted from I.1 who had a smear of completely methylated high molecular weight fragments ($\Delta=1.2-1.8$). The CCG expansion was reduced when transmitted to each daughter. FRAXE was cytogenetically undetectable in the daughter II.1 who had a Δ value of 0.3 kb. A fragile site was cytogenetically detectable at a low level in the other daughter, II.3, who had a contraction to a Δ value of 0.2 kb. Whether the fragile site in this daughter was FRAXA or FRAXE could not be determined by the conventional cytogenetic analysis.

There was little expansion when FRAXE was passed on from II.1, to III.1 and III.2, who

Table 1 Summary of clinical status and intellectual performance in all members of family 1

No in pedigree and sex	Age at examination	Education and occupation	Intellectual status					Mental and neurological status	Physical (minor) anomalies
			PPVT	BD	DS	Sim	IQ		
I.1 (FRAXE) Male	65	Never good at school; various unqualified jobs	63	20	65	50	53	Several hospital treated "nervous breakdowns"; takes tranquillisers because of anxiety; explosive speech with bad stutter	Large head, prominent eyebrows, narrow lower face, large sticking out ears, Dupuytren contracture bilaterally
II.1 (FRAXE) Female	35	Civil Servant after Technical College	92	76	95	103	80	Panic attack in public transport	Unusually short hallux bilaterally
III.1 (FRAXE) Female	22	Attended special school; no occupation	60	52	55	48	49	Psychiatric diagnosis of schizophrenia confirmed by SCID-R Structured Clinical Interview for DSMIII-R. ¹⁴ Bad stutter, hand mannerisms	Irregular teeth, high arched and narrow palate, moderate joint laxity
III.2 (FRAXE) Male	19	Apprenticeship for carpentry	93	116	70	103	104	NAD	Moderately high arched palate
III.6 (FRAXE) Female	10	Difficulties in learning and concentration; works as salesman's assistant	75	78	95	83	73	NAD	Hypertelorism, joint laxity, unusually short hallux bilaterally
II.3 (FRAXE & FRAXA) Female	37	Difficulties in mathematics during 8 years' schooling; occasional clerical job	90	54	110	93	68	NAD	Long chin, dysmorphic ears, shortened 5th metacarpal bone on right, unusually short hallux, enlarged thyroid gland
III.3 (FRAXA) Male	19	Special school, sheltered workshop	45	20	65	27	33*	Hand mannerisms, perseverations, dyslalia	Antimongoloid slant with hypertelorism, long narrow face, long bulbous nose, upper prognathism, short philtrum, facial asymmetry, irregular teeth, moderately high arched palate, joint laxity, clinodactyly of fingers 2 and 5
III.4 (FRAXA) Female	17	Learning difficulties, technical college (cooking) not completed; no occupation	98	49	85	65	76	Very clumsy movements, poor motor coordination; extreme short sightedness	Epicanthic folds, long bulbous nose, long chin, short philtrum, high narrow palate, joint laxity
III.5 (FRAXA) Female	14	Learning difficulties at school; works as a waitress	89	46	105	81	68	Emotionally unstable, panic attacks at night	Facial asymmetry, high and narrow palate
I.2 (FRAXA) Female	61	Occasional cleaning jobs	91	54	80	57	68	Emotionally unstable	NAD (palate and teeth not examined)
II.4 Male	38	Part time carpentry job	84	83	85	93	83	NAD	Moderately high arched palate

* Approximate assessment because of very low performance level

had Δ values of 0.5 kb and 0.4 kb respectively. Methylation was approximately 80% and 90% respectively for III.1 and III.2. Absence of a CCG PCR product for III.2 excluded copy number mosaicism in lymphocytes involving copies within the normal range. There was an expansion when FRAXE was passed on to III.6 from II.3. The Δ value increased from 0.2 kb to 0.7 kb. The CpG island was fully methylated in III.6. The female FRAXE carriers in this family show no consistent correlation between genotypic and phenotypic status. For example, both III.1 and III.6 are mentally impaired. III.6 is completely methylated at the FRAXE CpG island but III.1 has approximately the same methylation status as her unaffected brother III.2. These observations could be explained by somatic mosaicism and differential tissue expression but this is not testable. There does appear to be some correspondence between FRAXE genotype and clinical phenotype in this family for the two male carriers I.1 and III.2, since the first one with the larger Δ value and complete methylation is the mentally impaired person, whereas the second one with a much smaller Δ value is unimpaired. I.1 has a fully methylated CpG island whereas methylation of the CpG island for III.2 is incomplete.

INTELLECTUAL PERFORMANCE IN MEMBERS OF FAMILY 1

The clinical features and results of psychometric assessments of this family are sum-

marised in table 1. The most outstanding feature in some subjects who carry FRAXE, but not FRAXA, is borderline or mild intellectual deficit. This concerns verbal and non-verbal abilities, combined with psychiatric problems such as ill-defined "nervous breakdowns", requiring hospital treatment in I.1 or schizophrenia in III.1. This woman (III.1) reported auditory hallucinations starting at the age of 8. The main theme was a male voice telling her to "get rid of" or "kill" her mother. She has responded to Trifluoperazine HCl (Stelazine) but has residual symptoms including a very flat affect. Both I.1 and III.1 also expressed speech problems in the form of a stutter. Physical features included various minor limb malformations, high arched palate, and some laxity of joints. The remaining families described below did not undergo the detailed psychometric and psychiatric examinations as described for family 1.

FAMILY 2

This is an extension of family 2 of Sutherland and Baker.¹ Direct molecular diagnosis confirmed the identity of the fragile site as FRAXE. The proband IV.1 is a boy with no dysmorphic features. He had mild mental impairment and attends special school. IV.2 completed the first year of secondary schooling and after a difficult second year left school to find employment. He is described by his mother as the most intelligent of her three sons. IV.3 attends a

normal secondary school, is below average in his school work, and receives remedial teaching with reading. III.4 has normal intelligence and satisfactorily completed three years of secondary schooling. III.3 has low normal intelligence, is illiterate, and did not undertake secondary schooling. He is employed as a truck driver. Possible explanations for his sons' intellectual disability need to take into account his own intellectual difficulties. II.2 has normal intelligence and before her retirement had employment as a cleaner. III.5 appears to be of normal intelligence and has worked as a gardener for 20 years.

In family 2, FRAXE was transmitted from II.2 to III.4 and III.5, and from III.4 to her three sons. Only IV.1, the index case in this family, was clearly mentally impaired on the basis of clinical impression. His Δ value was 1.3. Partial methylation was detected only for IV.2; the remaining tested FRAXE subjects showed full methylation. Obviously, no clear correlation can be seen between FRAXE fragment size, or methylation status, with mental impairment from the male carriers III.5, IV.2, and IV.3; but it must be emphasised that this family did not undergo the detailed psychometric testing reported for family 1 and therefore existence of some correlation cannot be excluded. IV.1 is clearly mentally impaired and IV.3 receives remedial teaching for reading. III.5 is a key person who appears to be normal, but comprehensive testing is essential to determine his true intellectual status.

FAMILY 3

FISH and direct molecular diagnosis showed that the fragile site in this family was FRAXE. The 18 year old proband II.1 is mildly intellectually handicapped with some definite behavioural difficulties. He could read and write in a limited way with very good eye-hand coordination but little understanding of some of the material. Physically he was of average height and strong build with a square face and smallish ears, prominent upper incisors, and high narrow palate. His eyes had an antimongoloid slant with hypotelorism and epicanthic folds. The nipples were low placed and the hands and feet relatively small. Head circumference was 62 cm. Speech abnormality was his most characteristic finding with constant verbal repetitive utterances. He also had a number of hand mannerisms with posturing and patting; if these were stopped, he became confused and aggressive. Speech abnormality and hand mannerisms were similar to those described for some affected members of family 1 in table 1. His behaviour in the hostel has been described as sexually overactive, intrusive, and aggressive, but he is responding to behavioural management. Testes were above the 90th centile but not grossly enlarged. There was no joint laxity or skin hyperextensibility, the skin was not unusually soft, and there was no gynaecomastia. The sister II.2 who is positive cytogenetically appears by clinical impression to be normal in all respects, as is the obligate carrier mother, I.2.

Family 3 consists of three FRAXE subjects. FRAXE was transmitted from I.2 to her daughter II.2, with the amplification increased from 0.3 kb to 0.8 kb and fully methylated in II.2. The only obviously mentally impaired person was the index case II.1 with a value of 1.0 kb and fully methylated CpG island. The value for II.1 (affected) in family 3 was similar to that for IV.2 (unaffected) in family 2. However, the latter was only 50% methylated whereas the former was fully methylated.

FAMILY 4

Direct molecular diagnosis excluded FRAXA but indicated the presence of FRAXE in this family. The index case III.1 was an 8 year old intellectually disabled boy with multiple spider angiomas. FRAXE was transmitted to him by his mother II.2 who received it from her mother I.2. Both the mother and the grandmother appeared intellectually normal. During transmission the amplification steadily increased in size consistent with its behaviour during transmission through the other families.

FAMILY 5

This kindred has two mentally retarded males, the proband III.7 and his cousin III.11. The proband has moderate mental retardation but the degree of mental retardation for III.11, who has not been personally examined, remains unspecified. The amplifications in both boys are almost fully methylated. II.2, II.4, II.6, III.2, and III.6 are confirmed female carriers with the amplification fully methylated in III.2 and III.6. The amplification has expanded when passed through the females in generation II to males and females in generation III. The origin of the mutation carried by II.2, II.4, and II.6 is paternal, given that their mother (I.2) was inferred to be heterozygous for 15 and 19 copies of the FRAXE CCG repeat, and assuming that the pedigree as given is correct. The CCG copy number for I.2 could be inferred because the copy number for II.2, II.4, and II.6 was 15, 19, and 15 respectively. Hence, the maternal genotype was 15, 19 and the paternally derived allele of II.2, II.4, and II.6 failed to amplify because of the CCG expansion inherited from I.1.

FAMILY 6

Affected status was based on clinical impression. The proband III.1 is severely retarded and his brother III.5 is moderately retarded. Their mother II.2 is moderately retarded and the aunt II.3 is dull. The remaining aunts and uncles II.6, II.8, and II.10 are moderate to borderline in mental retardation status. Although the grandmother is cytogenetically FRAXE negative, she is the likely carrier given that her son II.10 is cytogenetically positive (although his fragile site was not confirmed as FRAXE by molecular testing). All of the mentally impaired family members carry a fragile site and this was confirmed by molecular analysis as FRAXE for II.2, II.3, III.1, and III.5.

Table 2 Contraction of CCG repeat size during paternal transmission

	Father Δ	Daughter Δ		Reference
FRAXA	0.8–1.1 kb	0.2–0.3 kb	(2 daughters)	11
	Not stated	Not stated		20
	1.0–1.5 kb	0.2–0.3 kb	(3 daughters)	21
FRAXE	0.8 kb	0.4 kb	(1 daughter)	4
	0.65 kb	0.35–0.4 kb	(2 daughters)	4
	1.2–1.8 kb	0.2–0.3 kb	(2 daughters)	Present study

Discussion

Detailed characterisation by in situ hybridisation using probes in Xq28 initially established the existence of FRAXE in the first three families, including the one described by Sutherland and Baker¹ (family 2) and the one originally reported by Loesch *et al*¹⁵ (family 1). This diagnosis was confirmed by detection of unstable sequences using the OxE20 probe. It is worth noting that the fragile site described by Romain and Chapman⁶ and reported by Sutherland and Baker¹ in their family 1 was excluded as FRAXE using the OxE20 probe. A localisation distal to FRAXE was shown for the fragile site in this family by FISH. This family was then confirmed to have FRAXF by direct molecular analysis.⁵ Both FRAXE and FRAXA were found to be segregating in family 1, which could have easily confounded genotype-phenotype inferences in the absence of molecular diagnosis to distinguish these fragile sites. Webb *et al*¹⁶ reported I.1 from this family in the context of fragile X syndrome and Loesch *et al*¹⁵ carried out detailed psychological assessment (in their family A). Cosegregation of instability at the FRAXA locus with instability at the FRAXE or FRAXF loci is a possibility to consider when characterising families for fragile sites in distal Xq.

Reduction in the size of the FRAXE CCG expansion was documented in family 1 during transmission from the affected male I.1 to his two daughters. This behaviour of trinucleotide repeats from the upper end of the observed size distribution of expansions may be sex specific since this observation parallels the findings in FRAXA¹⁷ and another FRAXE family (table 2) and for myotonic dystrophy.^{18,19} The general lack of increase in copy number during the transmission of the FRAXA CCG repeat through males, including regression of the larger copy numbers, is consistent with the observation that FRAXA is not usually cytogenetically penetrant in daughters of transmitting males. The same might be predicted to apply to FRAXE given the cytogenetic and molecular similarities between the two fragile sites. Although there are limited data for the transmission of FRAXA or FRAXE through males with large Δ values, the available information (table 2) is suggestive of regression of large Δ values for both FRAXA and FRAXE during transmission through a male. This mechanism can explain why the cytogenetic expression of FRAXA and FRAXE can skip generations and in particular why the daughters of FRAXA and FRAXE males do not have cytogenetic expression of these fragile sites. This may prolong the presence of the unstable FRAXA mutation in the population by pro-

tecting it from natural selection, supplementing the input into the population from recurrent mutation of chromosomes with trinucleotide repeats at the upper end of the normal unexpanded range in copy number.²² The process may be similar for FRAXE.

Certain similarities, as well as differences, also appear between clinical symptomatology of FRAXA and FRAXE. Psychiatric problems, and speech defect in the form of dyslalia or stutter such as encountered in FRAXE subjects in families 1 and 3, may also occur in FRAXA subjects, but rarely in such a degree and form as in I.1 and III.1 in family 1. However, none of the four male carriers of FRAXE in family 2 had any of the discernible speech, behavioural, or psychiatric problems observed in some members of the other families. At least two of the four male carriers did have learning difficulties and this may reflect phenotypic variability (as observed for fragile X syndrome) and, as such, family 2 may fit into a different portion of the clinical spectrum.

Data on intellectual scores for family 1 in FRAXE and FRAXA people listed in table 1 show that intellectual performance is, on average, lower in FRAXA than in FRAXE members and, consistent with what has been reported earlier, it concerns specific deficits such as on Block Design and Similarities scores.^{12,23} For example, FRAXA members usually have reasonable vocabulary as measured by the PPVT but are much poorer on the verbal concept skills assessed in the Similarities subtest where similarities between two and three words have to be identified. In FRAXE this discrepancy appears to be much less and all FRAXE subjects in family 1 are characterised by somewhat lower performance across all the abilities tested. Although this small number of subjects does not allow for any formal statistical comparisons, these data may become important if similar trends can be shown in other FRAXE families where the results of psychometric testing, rather than descriptive or general IQ data, are available. Among physical features, minor limb malformation encountered in the FRAXE members of family 1 are not uncommon in FRAXA.²⁴ However, none of the FRAXE carriers in this family had facial features suggestive of the fragile X syndrome.

Factors which blur a genotype-phenotype relationship for FRAXE, already an issue for fragile X syndrome, will need to be recognised and taken into account. The first of these is a lack of proper clinical and psychological assessment. If psychometric assessment had not been used with family 1 then subjects such as III.6 (IQ=73) or II.3 (IQ=69) may have been misclassified as normal on the basis of general clinical impression alone. Assessment by clinical impression alone was carried out for the members in the remaining families. The tentative classification of III.5, IV.2, and IV.3 in family 2 as normal might therefore be considered provisional in the absence of formal psychometric assessment.

The second factor affecting genotype-phenotype relationship could be copy number mo-

saicism first reported by Knight *et al.*⁴⁹ People having multiple fragments, some premutations and some full mutations (to use terminology first applied to FRAXA and fragile X syndrome), may be less severely affected. Knight *et al.*⁴⁹ found affected FRAXE males to have Δ values greater than 650 base pairs, similar to the Δ value separating premutations from full mutations in fragile X (FRAXA) syndrome. The Δ value of 0.4 kb for the male III.2 in family 1 of the present study was 90% methylated, suggesting that it is close to the FRAXE premutation-full mutation boundary. A Δ value of 1.0 for the male II.1 in family 3 was fully methylated, suggesting that it is within the FRAXE full mutation range. Degree of methylation is a less reliable indicator for females. Random inactivation of the female X chromosomes leads to an expression of 50% methylation in non-carrier females; however, random X inactivation occurring early in embryogenesis can skew the proportion of active and inactive X chromosomes. Although methylation status is given for females, no conclusions are made from these data.

The phenomenon which could also blur a genotype-phenotype relationship is methylation mosaicism.²⁵ This is the incomplete methylation of fragments with Δ values within the accepted full mutation range, which are normally methylated and associated with a clinical phenotype. For fragile X syndrome this has been observed at the lower end of the full mutation range for Δ values up to 1.2 kb. The effect on phenotype of copy number mosaicism and methylation mosaicism has been documented for the fragile X.²⁴⁻²⁶ The Δ value of 1.1 for IV.2 in family 2 of the present study was associated with 50% methylation, suggesting that this might be a methylation mosaic similar to cases documented for FRAXA. In addition, III.2 in family 1 and III.7 and III.11 in family 5 were not fully methylated, but methylation was at least 90%.

Phenotypic expression may be modulated further by between tissue mosaicism. Δ values were determined from lymphocytes, but corresponding Δ values have not been determined in neural tissue. Discrepant phenotypic expression between III.1 and III.2 in family 1, both with similar Δ and methylation values, could have such an explanation. The critical tissue for phenotypic expression of developmental delay or mental impairment is undoubtedly not blood. Tissue specific differences in amplification, between lymphocytes and muscle, have been observed in myotonic dystrophy.^{27,28}

Finally, a genotype-phenotype relationship may be confounded by the occurrence of mentally retarded subjects unrelated to the fragile site. These phenocopies have been documented in fragile X syndrome families.^{11,25} We suggest that the mental retardation of the schizophrenic FRAXE carrier III.1 in family 1 could be of this type. The early onset of her auditory hallucinations distinguishes her from any of the other intellectually disabled people in these families. She carries only a small FRAXE amplification and unless she carries extensive be-

tween tissue mosaicism she is unlikely to be affected by the FRAXE site since males with a similar size amplification in lymphocyte DNA are clearly unaffected.

The nature of the clinical relationship between FRAXE and non-specific mental retardation will be difficult to establish, in comparison with fragile X syndrome, given that the proposed phenotype is milder, manifested primarily as learning problems, and in need of formal quantification for confirmation. Nevertheless, these pedigrees (figure) show that with the exclusion of index cases there are more mentally impaired male and female carriers in these pedigrees than could reasonably be expected by chance. FRAXE and FRAXF are frequent enough among families ascertained by conventional cytogenetic analysis as having a fragile site in distal Xq such that molecular confirmation of which fragile site is involved must be carried out before genetic counselling of family members.

Note added in proof

A recent report²⁹ indicates that a FRAXE full mutation can be passed from father to daughter, so an affected male can have an affected daughter.

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