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Segregation of FRAXE in a Large Family: Clinical, Psychometric, Cytogenetic, and Molecular Data

Ben C. J. Hamel,[1] Arie P. T. Smits,[1] Esther de Graaff,[3] Dominique F. C. M. Smeets,[1] Frans Schoute,[1] Bert H. J. Eussen,[3] Samantha J. L. Knight,[4] Kay E. Davies,[4] Claire F. C. H. Assman-Hulsmans,[2] and Ben A. Oostra[3]

[1]Department of Human Genetics and [2]Department of Child Neurology, University Hospital, Nijmegen; [3]Department of Clinical Genetics, Erasmus University, Rotterdam; and [4]Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford

Summary

During an ongoing study on X-linked mental retardation, we ascertained a large family in which mild mental retardation was cosegregating with a fragile site at Xq27-28. Clinical, psychometric, cytogenetic, and molecular studies were performed. Apart from mild mental retardation, affected males and females did not show a specific clinical phenotype. Psychometric assessment of four representative affected individuals revealed low academic achievements, with verbal and performance IQs of 61–75 and 70–82, respectively. Cytogenetically the fragile site was always present in affected males and was not always present in affected females. With FISH the fragile site was located within the FRAXE region. The expanded GCC repeat of FRAXE was seen in affected males and females either as a discrete band or as a broad smear. No expansion was seen in unaffected males, whereas three unaffected females did have an enlarged GCC repeat. Maternal transmission of FRAXE may lead to expansion or contraction of the GCC repeat length, whereas in all cases of paternal transmission contraction was seen. In striking contrast to the situation in fragile X syndrome, affected males may have affected daughters. In addition, there appears to be no premutation of the FRAXE GCC repeat, since in the family studied here all males lacking the normal allele were found to be affected.

Introduction

The fragile X syndrome is the most common form of inherited mental retardation (Frijns 1989). It is associated with a fragile site at Xq27.3, and at the molecular level it is characterized by an unstable CGG repeat at the 5′ end of the FMR1 gene (Fu et al. 1991; Oberlé et al. 1991; Verkerk et al. 1991; Yu et al. 1991; for review, see Oostra et al. 1993*b*). The mechanism of mutation is expansion of the CGG repeat in patients and subsequent hypermethylation of the adjacent CpG island, resulting in silencing of the FMR1 gene (Bell et al. 1991; Pieretti et al. 1991; Vincent et al. 1991). Diagnosis of the fragile X syndrome is now based on the determination of the number of CGG repeats: normal alleles have a repeat length <50, premutation alleles have 50–200 copies, and in affected individuals full mutation alleles have >200 repeats (Fu et al. 1991). In the majority of individuals with both a cytogenetic expression of a fragile site at Xq27.3 and mental retardation, the fragile X syndrome is confirmed by identifying an increased CGG repeat in the FMR1 gene.

However, some families have been ascertained with fragile X expression but without CGG amplification. Refined cytogenetic methods using FISH have allowed differentiation of two other fragile sites, called "FRAXE" (Sutherland and Baker 1992; Flynn et al. 1993) and "FRAXF" (Hirst et al. 1993). Recently, the fragile site FRAXE was cloned, and in individuals with cytogenetic FRAXE expression amplification of a GCC repeat was found (Knight et al. 1993). In normal individuals 6–25 copies of the GCC repeat were present, with an average of 15 copies. In patients expressing FRAXE, >200 copies of the GCC repeat were found. In these patients a CpG island proximal to the GCC repeat was methylated, suggesting that methylation plays a role in the inactivation of a gene in the FRAXE region. This CpG island is located 600 kb distal to the CpG island proximal to the FMR1 gene.

Very little is known about the clinical phenotype of FRAXE-positive individuals. In the first paper describing FRAXE (Sutherland and Baker 1992), fragile-site expression was reported in mentally normal individuals. In the families described by Knight et al. (1993), almost all males who did express the fragile-site FRAXE were mildly mentally retarded. Carrier females were mentally normal. The expanded GCC repeat of FRAXE was seen in affected males as well as in carrier females and was unstable when passed through both the male and female lines. A contraction of the expanded GCC repeat was found when it was passed from an affected father to his daughter, whereas expansion was mostly found when it was passed from a

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Address for correspondence and reprints: Dr. Ben C. J. Hamel, Department of Human Genetics, University Hospital, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

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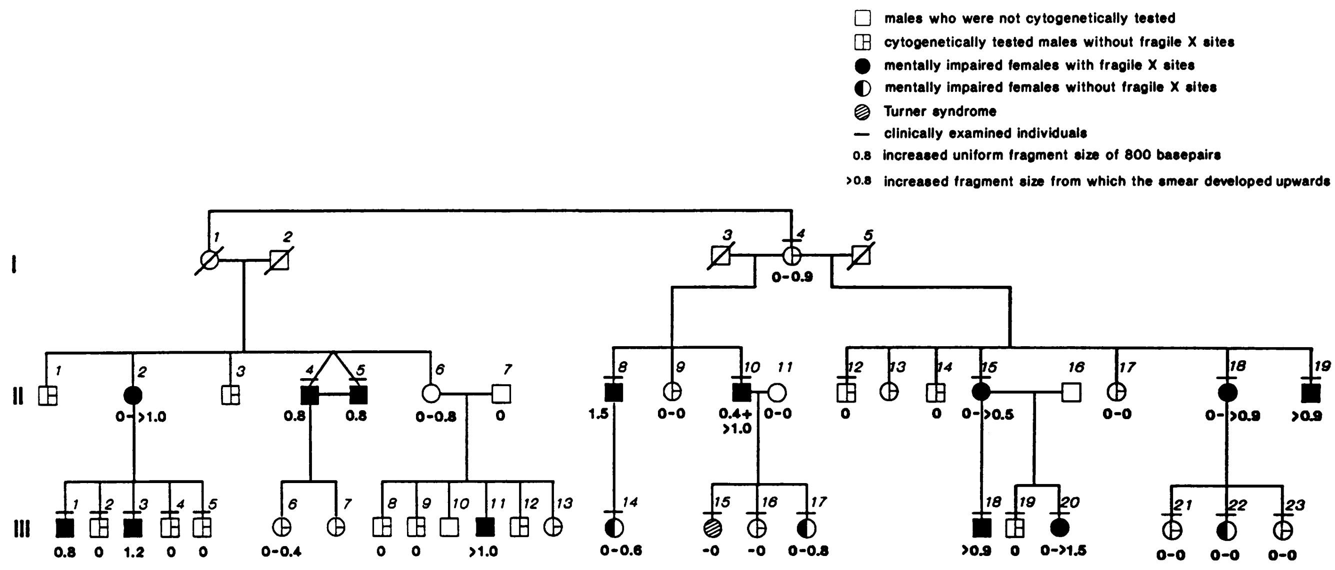

Figure 1 Pedigree

carrier mother to her affected son (Knight et al. 1993). In this paper we describe a large FRAXE family in which FRAXE is cosegregating with nonspecific mild mental retardation.

Subjects, Material, and Methods

The family (fig. 1) was ascertained from >80 families with fragile X expression. The index patient II-5 was admitted for lower-back pain at the neurology department, and a fragile X screening was requested because of familial mild mental retardation. Eight affected males, five unaffected males, seven affected females, and four unaffected females were examined by two of us (B.C.J.H. and A.P.T.S.). Clinical photographs were taken, and blood sampling was performed. Affected family members were those who attended a special school for children with learning difficulties, while the unaffected individuals received regular education.

Psychometry

Four patients (II-18, II-19, III-18, and III-22) were psychometrically assessed by using highly standardized tests. These patients were thought to be representative in terms of schooling and intellectual and social functioning. For the intelligence test the WAIS-R (Stinissen et al. 1970) was used for adults, and the WISC-R (Van Der Steene et al. 1986) was used for children. Attention was scored with the test for sustained attention (Bourdon and Vos 1988). The Bender Gestalt Test (Koppitz 1964) and Visual Motor Integration Test (Beery 1989) were used to assess the visual/motor skills. The academic achievements, including the prerequisites for reading and writing, were scored with aspects of the Groninger School Onderzoek (Kema and Kema-van Leggelo 1987) and with tests that are specifically designed to assess the reading (Wiegersma 1971; Van Den Berg and Te Lintelo 1977; Brus and Voeten 1979), writing (Struiksma et al. 1986), and arithmetic skills (Heesen et al. 1974; Ojeman 1977).

Cytogenetics

For cytogenetic analysis, peripheral lymphocytes were cultured for 92 h in medium TC 199, supplemented with 5% FCS. Chromosome slides were made according to routine procedures. One hundred metaphases of each individual were examined for the presence of a fragile X chromosome after solid Giemsa staining. Potential fragile X chromosomes were photographed, destained, and subsequently GTG-banded for evaluation.

In situ hybridization was performed according to the procedure of Kievits et al. (1990) and Verkerk et al. (1992). Whole-cosmid DNA, c4.1 (Verkerk et al. 1991), C1/C10 (containing marker Do33), and VK21 (Oostra et al. 1993b) were labeled with the Bio-Nickkit (BRL). Biotinylated DNA specific for the X centromere pBamX5 (Willard et al. 1983) was cohybridized for X chromosome identification. Each hybridization mix contained 2–4 ng cosmid probe/μl, 0.1 ng pBamX5/μl, and a 50-fold excess of competitor Cot-1 human DNA (BRL). This mix was denatured and preannealed for 1 h at 37°C, followed by an overnight hybridization at 37°C. After the slides were washed in 1 × SSC at 65°C the probes were detected by alternate layers of fluorescein-conjugated avidin (DCS Vector) and biotinylatd anti-avidin antibody (Vector), both diluted to 5 μg/ml in 4 × SSC with 0.5% blocking milk (Boehringer).

Slides were washed in 4 × SSC with 0.05% Tween 20. Slides were rinsed in PBS and were mounted in antifading

solution (2% DABCO/glycerol; Sigma) containing 0.03 μg propidium iodide/ml and 0.6 μg DAPI/ml. Microscopic analysis was performed with a Leica Aritoplan microscope. For the slides stained with C1/C10, a Kodak Ektachrome 400 ASA daylight film was used, while the slides stained with VK21 were captured by a cooled CCD camera in combination with Macprobe software (Probemaster unit; PSI).

DNA Analysis

Genomic DNA was isolated from leukocytes as described elsewhere (Miller et al. 1988), and 8 μg was digested to completion with either *Eco*RI (FRAXA) or *Hin*dIII (FRAXE). The samples were separated on a 0.7% agarose gel and were subjected to Southern analysis using the probe pP2 (Oostra et al. 1993*a*) and OxE20 (Knight et al. 1993) for characterizing the FRAXA and FRAXE region, respectively. The probes were labeled by the random oligonucleotide-priming method (Feinberg and Vogelstein 1983). Before hybridization the labeled probe OxE20 was incubated with 100 μg total human DNA for 2 h at 65°C. After 2 h prehybridization and overnight hybridization, the filters were washed in 0.1 × SSC, 1% SDS at 65°C, prior to exposure to X-ray film. Amplification of the GCC repeat was performed as described elsewhere (Knight et al. 1993). PCR analysis of the FRAXA CGG repeat was performed according to the procedure described by Fu et al. (1991). In order to study DXS1691, a (CA)n repeat located 2.5–5.3 kb distal to the FRAXE CpG island, 60 ng genomic DNA was amplified in a total volume of 10 μl consisting of 1 mM $MgCl_2$, 0.2 mM each of dCTP, dTTP and dGTP, 0.025 mM dATP, 10 mM Tris-Cl pH 8.3, 15 mM KCl, 0.01 % gelatin, 4 μCi ^{32}P-dATP, 2.5 U *Taq* polymerase (BRL), 0.25 μM primers F322 and F010, as described elsewhere (S. J. L. Knight, unpublished results). The reactions were initially denatured for 4 min at 95°C, followed by 33 cycles of 1 min at 95°C, 1 min at 65°C, and 1 min at 72°C. A final, 4-min extension was performed at 72°C. The amplifications were performed on a 9600 thermocycler (Perkin-Elmer). The primer sequences were F322, 5′-GCAATGATAATGTTGAGTTCTACC; and F010, 5′-CTCAAGACCAAACTTGAAGAAACC.

Results

Phenotype

Though some affected males had mild craniofacial anomalies, there was no consistent clinical phenotype present in the affected individuals. Clinical information on the affected family members is given in tables 1 and 2.

Patient III-1 (fig. 2) is a representative example of this family (fig. 1) and will be described in more detail. He was born at term, after an uneventful pregnancy and delivery. Motor milestones were reached within normal limits; speech development, however, was retarded. He attended a special school for children with severe learning difficulties. Presently, he reads and writes with difficulty but is unable to complete forms (e.g., insurance). He works in a sheltered environment. He is healthy, is married, and has three healthy sons. At the age of 34 years his height is 195 cm (97th centile), arm span 196 cm, weight 105 kg (>97th centile), occipito-frontal circumference (OFC) 60.0 cm (>97th centile), ears 72 mm (97th centile), and testicular volume 25 ml (50th–90th centile). He has a long and narrow face, mild midfacial hypoplasia, long and narrow ears, and a high-arched palate. His neck is long. No other abnormalities were found, in particular no macroorchidism or hyperlaxity. The male patients II-4, II-5, II-19, III-1, III-3, and III-18 show some resemblance to each other. However, patient III-1 also resembles his normal brothers (III-2, III-4, and III-5). For comparison, clinical information on some unaffected males is given in table 3, while in figure 2 are seen the affected males II-5, III-1, and III-18, the affected female II-18, and, for comparison, the unaffected males III-2 and III-19 and the unaffected female III-21. Patient III-15 showed features of Turner syndrome (see below). The only living member of generation I (I-4) is mentally normal.

Psychometry

The overall intelligence of the tested patients was below average (total IQ <85). Verbal intelligence was not significantly lower than performance intelligence. Three patients (II-18, II-19, and III-18) could be classified as severely impaired, on all tested psychological functions as well as on the academic achievements (reading, writing, and arithmetic). The fourth patient (III-22) performed, in comparison with the other patients, relatively well on the psychological functions and reading, whereas writing and arithmetic skill were severely impaired (table 4).

Cytogenetic Analysis

A folate-sensitive fragile site at Xq27-28 was shown in all nine affected males (range 1%–40%) and in none of the unaffected. Of the 7 affected females, 4 expressed a folate-sensitive site at Xq27-28 (range 4%–46%), and all 15 unaffected females were fragile X negative. Patient III-15 has a 45,X/46,X,r(X)(92/8) karyotype. FISH analysis of r(X) with X-specific probes showed the presence of Xp and the centromeric region (data not shown).

FISH analysis with cosmids C1/C10 located between FRAXA and FRAXE (Oostra et al. 1993*b*) gave a signal proximal to the fragile site (fig. 3*a*), whereas probe VK21, which is located distally from FRAXE, showed a signal distal from the fragile site (fig. 3*b*). From these results it can be concluded that the fragile site detected in this family is FRAXE.

DNA Analysis

The CGG repeat in the FRAXA region was within the normal range in both unaffected and affected members of

Table 1

Selected Clinical Features (Centiles) of Affected Males

	II-4	II-5	II-8	II-10	II-19	III-1	III-3	III-18
Age (years)	54	54	51	46	32	34	31	16
Height (cm)	175 (10–50)	177 (10–50)	169 (<10)	178 (10–50)	188.5 (50–90)	195 (>90)	194 (>90)	176 (10–50)
OFC (cm)	58 (97)	58.5 (97)	57 (90–97)	58 (97)	59.1 (>97)	60.0 (>97)	59.2 (>97)	54.5 (10–50)
Testes (ml)	25 (50–90)	20 (50)	25 (50–90)	25 (50–90)	20 (50)	25 (50–90)	25 (50–90)	20 (50)
Long, narrow face	+	+	−	−	+	+	+	+
Midfacial hypoplasia	−	+	+	+	+	+	+	+
High-arched palate	−	+	−	−	+	+	+	+
Long neck	−	−	−	−	+	+	+	+
Prognathism	−	−	−	−	−	+	−	−
Miscellaneous	Obese	Obese	−	Obese	Obese	Obese	−	−

this family. Southern analysis of the GCC repeat in the FRAXE region was performed by *Hin*dIII digestion and subsequent hybridization with the probe OxE20 (Knight et al. 1993). In unaffected individuals a band of 5.2 kb was detected (fig. 4). In affected individuals the expansion in the GCC repeat resulted in an enlarged *Hin*dIII fragment visible either as a discrete band or as a smear. All nine affected males had smears, with increases in size that were 800 bp (II-4 and II-5) to far >1,000 bp (III-11). One of the males (II-10) appeared mosaic; besides the smear, an additional band of 5.6 kb was visible after longer exposure. In females, both discrete bands and smears were seen (fig. 1). Interestingly, only the four females who showed a smear on the Southern blot (II-2, II-15, II-18, and III-20) had cytogenetic expression of the FRAXE site, whereas females who showed a discrete band (I-4, II-6, III-6, III-14, and III-17) did not express this fragile site. All individuals with an expanded GCC repeat were found to be mildly mentally retarded, with the exception of the normal females I-4, II-6, and III-6, who showed an increase of 900 bp, 800 bp, and 400 bp, respectively. In contrast, the affected female III-22 appeared to have a normal 5.2-kb *Hin*dIII fragment. The intensity of this fragment was equal to the band found in her two normal sisters, indicating that she had received two normal alleles. No smear was detected after longer exposure. Linkage analysis with the CGG repeat in FRAXA, marker St14, and DXS1691 located 2.5–5.5 kb distal from the FRAXE CpG island (S. J. L. Knight, unpublished results) showed that she had received the risk allele from her mother (data not shown). Analysis of the GCC repeat of FRAXE by PCR showed that she had both a normal allele consisting of 17 GCCs, derived from her father, and a second allele consisting of 25 GCCs. Thus, the enlarged repeat of ~400 copies in the mother has decreased to only 25 copies in the daughter.

The expanded GCC repeat was found to be unstable when transmitted to the offspring. Transmission through a female resulted in an expansion of the repeat in 12 of 15 cases, whereas after transmission through males a decrease in length was found in all 3 cases tested.

In one branch of this family (father II-10 and his daughters III-15, III-16, and III-17) molecular findings were particularly remarkable. The affected female III-17 is the only daughter to receive an expanded GCC repeat from her mentally retarded father (II-10). Daughter III-15 had Turner syndrome; her X chromosome was found to lack the GCC-repeat expansion and was therefore likely to have derived from her mother. In DNA of the unaffected

Table 2

Selected Clinical Features (Centiles) of Affected Females

	II-15	II-18	III-14	III-15	III-17	III-20	III-22
Age (years)	37	33	16	21	17	8	9
Height (cm	166 (10–50)	165.5 (10–50)	161 (10)	153.5 (<3)	172 (50–90)	134 (50–90)	140.5 (90)
OFC (cm)	54 (10–50)	54.4 (10–50)	53.5 (10–50)	54 (10–50)	55 (50)	51.6 (50)	50.8 (10–50)
Long, narrow face	−	−	−	−	+	−	−
Midfacial hypoplasia	−	−	−	+	−	−	−
High-arched palate	−	−	−	+	−	−	−
Long neck	−	−	−	−	−	−	−
Prognathism	−	−	−	−	−	−	−
Miscellaneous	Obese	Obese	Obese	45,X/46,XrX	−	−	−

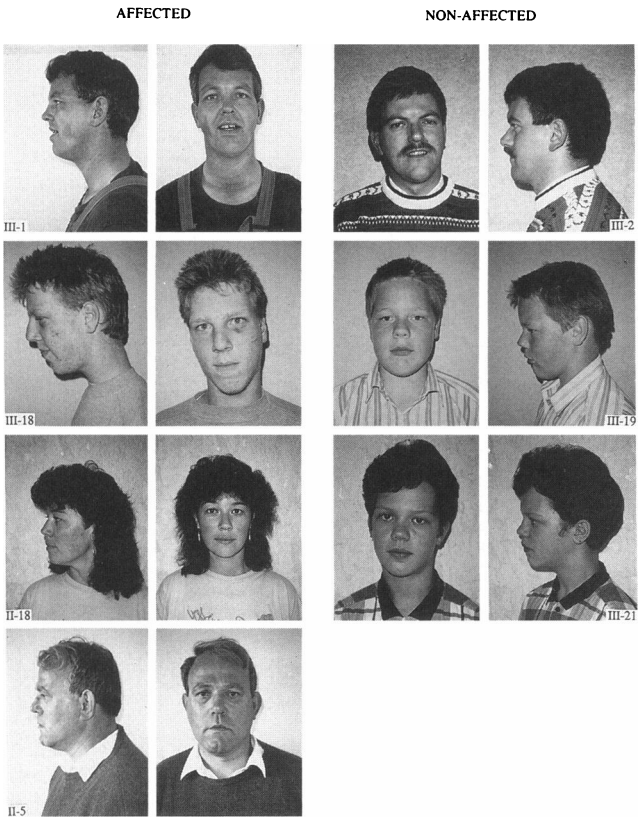

Figure 2 Patients III-1, III-18, II-18, and II-5 and unaffected family members III-2, III-19, and III-21

daughter III-16, only the normal 5.2-kb *Hin*dIII fragment was detected; no additional smears or bands were seen, even after a long exposure. The intensity of this normal band was equal to that of the band found in her sisters, both known were known to possess only one normal maternal allele. This suggested the presence of only one allele. To determine whether the daughter III-16 had received the risk allele from her father, we tested DXS1491, a (CA)n repeat 2.5–5.3 kb distal to the FRAXE GCC repeat. The risk allele, transmitted by the father to his affected daughter, was transmitted neither to his daughter with Turner syndrome nor to his normal daughter. Instead, only one allele appeared to be present in III-16, suggesting that on the paternal X chromosome the DXS1691 locus had been deleted. No abnormalities were detected in her karyogram. Amplification of the CGG repeat of the FMR1 gene 600 kb upstream of the FRAXE site revealed both a maternal allele and a paternal allele, indicating that the deletion did not extend into the CpG island of the FRAXA region. Preliminary results have thus far indicated that the size of the deletion is 7.4 kb, beginning in the region of the FRAXE HTF island and extending distally (S. J. L. Knight, unpublished results).

Discussion

In 1981 Daker et al. reported on two mentally normal brothers with fragile-site expression at Xq27-28. Since then, several other fragile X–positive probands and families without the CGG amplification of the FMR1 gene have been reported (see table 5, which includes the here-reported family K). FISH analysis has demonstrated that the fragile site in families C, D, H, J, and K was FRAXE, and in families D, J, and K the FRAXE GCC amplification indeed was found; in families F and I the fragile site appeared to be FRAXF. Only in families J and K is FRAXE associated with mild mental retardation, whereas in family H all fragile X positives are mentally normal. In family C 5 of 10 fragile X positives were mentally retarded, whereas in family D only the proband showed mild mental retardation. The location of the fragile sites in two additional families, E and G, remains to be determined. In our family K no specific and consistent clinical phenotype was found, apart from mild mental retardation; this is in contrast to the fragile X syndrome, with its Martin-Bell phenotype.

FRAXE seems to be rare. We have found 1 FRAXE family among >80 families with fragile X expression. In general, FRAXE patients are not in need of medical care, and

Table 3

Selected Clinical Features (Centiles) of Unaffected Males

	II-12	III-2	III-4	III-5	III-19
Age (years)	44	33	29	27	10
Height (cm)	172.5 (<10)	186 (50–90)	187 (50–90)	185 (50–90)	152 (>90)
OFC (cm)	54.5 (10–50)	59 (>97)	59.5 (>97)	60 (>97)	54.5 (50)
Testes (ml)	25 (50–90)	25 (50–90)	Not done	20 (50)	3–4 (50)
Long, narrow face	–	+	–	+	–
Midfacial hypoplasia	–	+	–	–	–
High-arched palate	–	+	+	–	–
Long neck	–	–	–	–	–
Prognathism	–	+	–	+	–
Miscellaneous	–	–	–	–	–

Table 4

Psychological and Academic Achievement

Case (age)	Intelligence Classification (IQ)			Attention[a]	Visual/ Motor Skills[a]	Prerequisites[a]	Academic Achievement[a]		
	Verbal	Performance	Total				Reading	Writing	Arithmetic
III-18 (16)	66	70	64	+/−	−	+/−	−	−	−
II-19 (32)	61	76	65	−	−	−	Unable	Unable	Unable
II-18 (34).....	72	78	72	−	−	−	−	−	−
III-22 (10)	75	82	76	+/−	+/−	+/−	+/−	−	−

[a] − = Severely impaired (SD ≤ −2); +/− = mildly impaired (−2 < SD ≤ −1); and + = unimpaired (SD > −1).

so they do not come to our attention. Besides, with the present molecular-diagnostic practice, fragile X positives other than FRAXA will be missed. This all makes it, at present, impossible to estimate its frequency in the general population. On formal testing of four representative patients, there appeared to be a tendency for verbal IQ to be lower than performance IQ, whereas in the fragile X syndrome the opposite is found (Brainard et al. 1991).

The expanded GCC repeat was found to be unstable on transmission, similar to the situation in transmission of the CGG repeat in the fragile X syndrome. Reyniers et al. (1993) demonstrated that in fragile X males who have a full FRAXA mutation in their lymphocytes a premutation and not a full mutation is present in their sperm cells. By analogy, it is very likely that in FRAXE-expressing males a smaller GCC repeat is present in sperm as compared with lymphocytes. (Preliminary results indicate that, in FRAXE, affected males indeed have a smaller *Hin*dIII fragment in sperm cells, although the additional presence of a full mutation could not be excluded.) In striking contrast to the situation in the fragile X syndrome, however, FRAXE-expressing males may have affected daughters. These daughters were found to lack cytogenetic expression of the FRAXE site, indicating that their reduced repeat length did not allow expression of the fragile site.

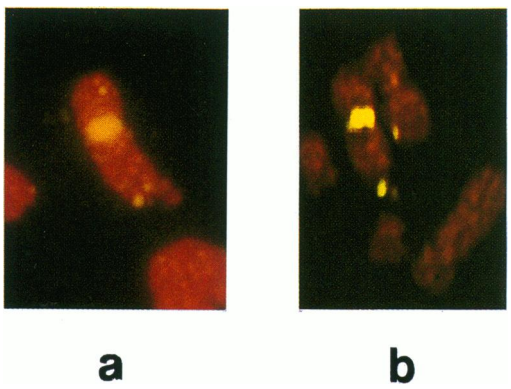

Figure 3 FISH analysis. In situ hybridization of cosmid C1/C10 (*a*) and cosmid Vk21 (*b*) to chromosome preparations of an affected member of the family. The X chromosome–specific centromere probe pBAMX5 was used for X chromosome identification. Slides were either recorded on film (*a*) or digitized (*b*).

Because of the size of this family, we could determine the transmission of the GCC repeat by one individual to several children. We found that transmission through the same person can result in both an increase and a decrease in repeat length. The passage of the GCC repeat by the FRAXE-expressing female II-2 (330 copies) resulted in an increase to 400 copies in one affected son (III-3) and in a decrease to 265 GCC copies in another affected son (III-1).

Knight et al. (1993) suggest that the mechanism of silencing in the FRAXE region is the same as that in FRAXA: as soon as repeat number reaches a critical level, methylation occurs, resulting in lack of mRNA and thereby causing the clinical phenotype. In the family that we studied, we found that, similar to the FRAXA mutation, all GCC repeats with a length >130 copies were methylated (data not shown). In the fragile X syndrome a premutation can be transmitted through normal transmitting males. In striking contrast to the fragile X syndrome, however, there appears to be no premutation of the FRAXE GCC repeat, since, in the family that we studied, all males lacking the normal allele were found to be affected.

We identified a mosaic male (II-10) possessing both a small expansion of 120 GCCs and a large expansion of >760 copies. In contrast to the clinically unaffected mosaic male with a small amplification of 133 copies and a large amplification of 866 copies, reported by Knight et al. (1993), this male was affected. A likely explanation for the observed difference is the finding that both expanded repeats in the affected male are methylated (data not shown), whereas the small fragment in the mosaic described by Knight et al. (1993) is unmethylated.

There were two peculiar phenomena in this family. First, in the mentally impaired female III-22 a fragment of 25 GCCs was present. Its length is at the upper end of the normal range of 6–25 GCCs. The methylation pattern in this female appeared to be normal and could therefore not

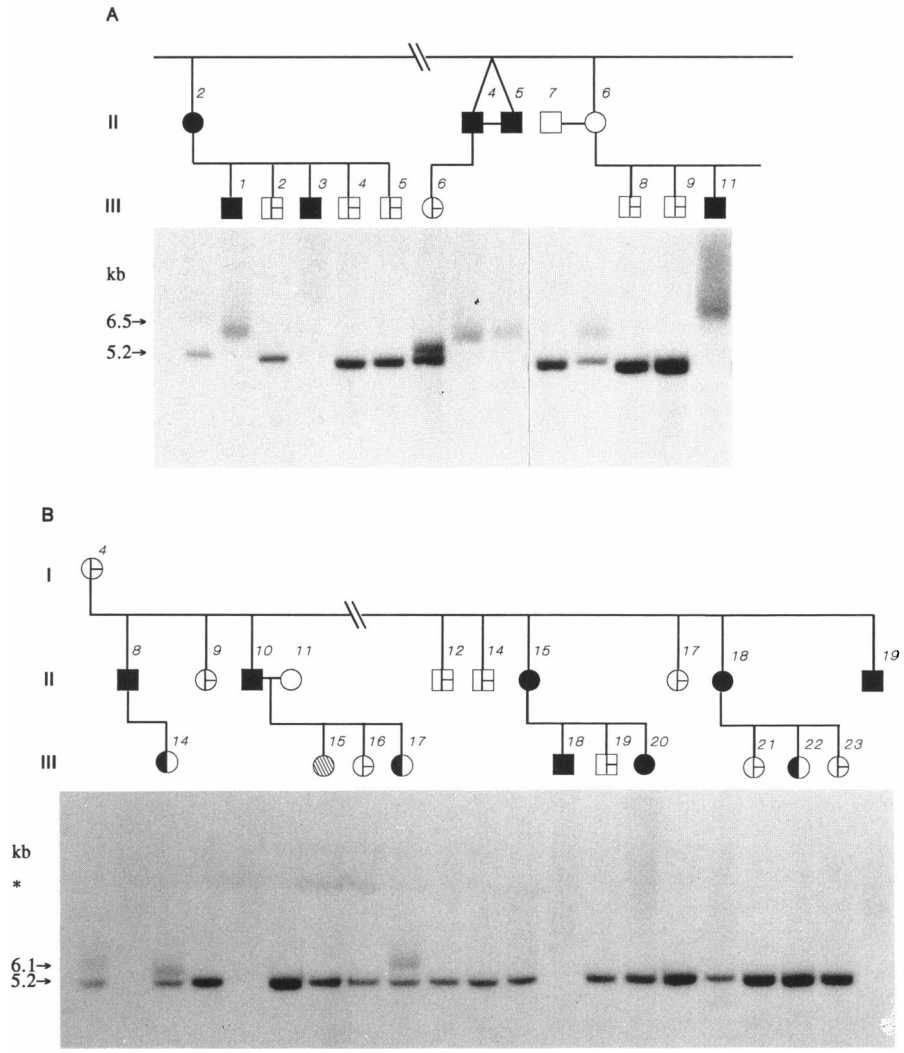

Figure 4 Southern blot analysis of two branches of the FRAXE family. DNA was digested with *Hin*dIII, and, after electrophoresis and subsequent blotting, the filters were hybridized with the probe OxE20. The asterisk indicates a constant, aspecific band, visible in all lanes after longer exposure.

be used to account for the observed mental impairment. It is noteworthy that in the psychometry this patient had the highest scores; her mental impairment might as well have another cause. Second, there is a remarkable branch in this family, in which the instability in the FRAXE region is clearly shown. An affected mosaic male (II-10) has three daughters, and all three were different at the molecular level: one showed an expansion of the GCC repeat of the paternal allele; a second had Turner syndrome, lacking the paternal allele; and the third appeared to have a deletion, containing the GCC repeat derived from the paternal allele. Despite the deletion, this female was mentally normal. There might be two explanations for this peculiar phenomenon. First, it is possible that the presence of one normal allele resulted in normal development. This may also explain the three mentally normal females (I-4, II-6, and III-6) with an expanded GCC repeat. However, other females who also carry a normal allele apart from the expanded GCC repeat are mentally retarded. The mental retardation in these females with an expansion may be caused by skewed X inactivation. Methylation analysis of the DNA isolated from their blood leukocytes revealed that there was no skewed X inactivation (data not shown), but one should be aware that the methylation pattern in blood lymphocytes may not be an accurate representation of other tissues such as brain.

A second possibility is that the deletion found in this patient does not affect the promoter of the gene that is otherwise silenced by the amplification of the GCC and the subsequent methylation. Further studies will be required to determine the exact length and location of the deletion, which in turn will enable us to learn more about the mechanism by which mental retardation is caused in patients with an expanded GCC repeat.

Table 5

Published Families with Fragile Site at Xq27.3 Other than FRAXA

Family[a]	Clinical Status[b]		% Fragile Site	FISH	DNA Analysis	
	Proband	Others			FRAXA CGG	FRAXE GCC
A	No MR	No MR	6–22	Not done	Not done	Not done
B	No MR	No MR	10–75	Not done	Normal	Not done
C	Mild MR	Mild MR[c]	13–42	FRAXE	Normal	Not done
D	Mild MR	No MR	26–35	FRAXE	Normal	Amplification
E	Mild MR	No MR	14–40[d]	Not done	Normal	Not done
F	No MR	No MR	5–14	FRAXF[e]	Normal	Not done
G	Mild MR[f]	No MR	12–40	Not done	Normal	Not done
H	No MR	No MR	14–28	FRAXE	Normal	Not done
I	Moderate MR	Moderate MR[g]	2–26[h]	FRAXF	Normal	Not done
J	Mild MR	Mild MR	1–24	FRAXE	Normal	Amplification
K	Mild MR	Mild MR	1–46	FRAXE	Normal	Amplification

[a] References are as follows: A—Daker et al. (1981); B—Voelckel et al. (1989) and Oberlé et al. (1992, family 3); C—Nakahori et al. (1991, family 5c), Dennis et al. (1992, family 1), and Flynn et al. (1993); D—Nakahori et al. (1991, family 5b), Dennis et al. (1992, family 2), Flynn et al. (1993), and Knight et al. (1993, family 2); E—Oberlé et al. (1991, PC family; 1992, family 1) and Rousseau et al. (1991); F—Romain and Chapman (1992) and Sutherland and Baker (1992); G—Oberlé et al. (1992, family 2); H—Sutherland and Baker (1992); I—Hirst et al. (1993); J—Knight et al. (1993, family 1); and K—present study.

[b] MR = mental retardation.

[c] Of 10 fragile X positives, 5 had MR.

[d] Folate-insensitive fragile site.

[e] Reference: J. Mulley (personal communication).

[f] XYY karyotype.

[g] Of five fragile X positives, two had MR.

[h] Possibly a folate-insensitive fragile site.

In conclusion, we have described a family in which amplification of a GCC repeat in the FRAXE region is associated with mild mental retardation without a distinct clinical phenotype. Remarkably, affected males may have affected daughters, and the absence of normal transmitting males suggests the absence of a premutation in FRAXE. Familial mild mental retardation warrants a specific search for FRAXE.

Note added in proof:—Knight et al. (1994) reported on families B and C (table 5); both exhibit GCC repeat extension at the FRAXE locus, and the only mentally retarded patient in family B is a fragile X–negative male with a 550-bp increase in size.

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