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A Study of FRAXE in Mentally Retarded Individuals Referred for Fragile X Syndrome (FRAXA) Testing in the United Kingdom

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Summary

The folate-sensitive fragile site FRAXE is located in proximal Xq28 of the human X chromosome and lies ~600 kb distal to the fragile X syndrome (FRAXA) fragile site at Xq27.3. The cytogenetic expression of FRAXE is thought to be associated with mental handicap, but this is usually mild compared to that of the more common fragile X syndrome that is associated with the expression of the FRAXA fragile site. The exact incidence of FRAXE mental retardation is uncertain. We describe here the results of a U.K. survey designed to assess the frequency of FRAXE in a population of individuals referred for fragile X syndrome testing and found to be negative for expansion events at the FRAXA locus. No FRAXE expansion events were found in 362 cytogenetically negative males studied, and one expansion event was identified in a sample of 534 males for whom cytogenetic analyses were either unrecorded or not performed. Further FRAXE expansion events were detected in two related females known to be cytogenetically positive for a fragile site in Xq27.3-28. To gain insight into the FRAXE phenotype, the clinical details of the identified FRAXE male plus three other FRAXE individuals identified through previous referrals for fragile X syndrome testing are presented. For the population studied, we conclude that FRAXE mental retardation is a relatively rare but significant form of mental retardation for which genetic diagnosis would be appropriate.

Introduction

The folate-sensitive fragile site FRAXE lies in proximal Xq28 of the human X chromosome and is associated with a mild form of mental retardation (Knight et al. 1993, 1994; Hamel et al. 1994; Mulley et al. 1995). By use of conventional cytogenetic techniques, FRAXE cannot be distinguished from either the FRAXA or FRAXF fragile sites, which are also located in the Xq27.3-q28 region of the human X chromosome (Sutherland and Baker 1992; Flynn et al. 1993; Hirst et al. 1993; Mulley et al. 1995). The cytogenetic expression of FRAXA at Xq27.3 is associated with the fragile X syndrome, which has an estimated incidence of ~1/1,250 live born males and is thus the most prevalent form of inherited mental retardation (Brown 1990). FRAXE and FRAXF were identified through screening programs designed to detect the fragile X syndrome mutation; a number of individuals cytogenetically positive for a fragile site in Xq27.3-Xq28 were found to be negative for the CGG expansions associated with FRAXA expression and did not exhibit hypermethylation or transcriptional silencing of FMR1, the gene responsible for the fragile X phenotype (Dennis et al. 1992.; Sutherland and Baker 1992; Flynn et al. 1993; Hirst et al. 1993). A proportion of these individuals were subsequently shown to express either the FRAXE fragile site ~600 kb distal to FRAXA or the FRAXF site further distal in Xq28 (Knight et al. 1993, 1994; Hamel et al. 1994; Mulley et al. 1995; Parrish et al. 1994; Ritchie et al. 1994).

Both FRAXE and FRAXF have been cloned and the fragility shown to be due to expansions of GCC trinucleotide repeats adjacent to hypermethylated CpG islands in Xq28 (Knight et al. 1993; Parrish et al. 1994; Ritchie et al. 1994). Like FRAXA, the cytogenetic expression of FRAXE appears to be associated with a form of mental retardation, but this is generally milder than that of the fragile X syndrome. Whether the cytogenetic expression of FRAXE is truly associated with a phenotype or whether the observed mental handicap in FRAXE individuals can be attributed to ascertainment bias has been the subject of some debate. A recent study by Allingham-Hawkins and Ray (1995) led these authors to hypothesize that FRAXE may be a benign fragile site unrelated to a clinical phenotype. However, there

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are a number of studies reported in the literature in which clinical similarities between mentally retarded FRAXE individuals support the existence of an associated phenotype (Knight et al. 1994; Hamel et al. 1994; Mulley et al. 1995). The exact criteria by which this phenotype may be defined are not clear, but accumulated data suggest that affected FRAXE individuals have no consistent dysmorphology, and the main clinical features appear to be learning difficulties, in particular, speech delay and reading and writing problems; there may also be behavioral concerns.

To date, a total of 38 FRAXE families from a number of countries have been recorded in our database. Each family has at least one developmentally delayed male who exhibits an expansion across the FRAXE site (authors' unpublished observations), and each was identified through initial referral for fragile X syndrome (FRAXA) testing. However, despite the increasing number of FRAXE families being identified, studies to determine the exact frequency of FRAXE families being discovered in this way and to present the reported phenotypes have been limited. The aims of the study presented here were twofold: (1) to determine the frequency of FRAXE individuals in a sample of U.K. patients referred for fragile X syndrome testing and found to be negative for CGG expansions at the FRAXA fragile site and (2) to report and evaluate the clinical details of any identified FRAXE individuals in addition to three unrelated FRAXE individuals previously unreported in the literature.

Material and Methods

Survey Samples

Samples that had previously been referred for fragile X syndrome testing and found to be negative for CGG expansions across the FRAXA fragile site were obtained as DNA from a number of U.K. genetic laboratories involved in FRAXA screening (see Acknowledgments). With the exception of eight cytogenetically positive females (a mother and daughter plus six unrelated females), all samples were from unrelated males and were included whether they were cytogenetically positive, negative, or had been tested at all for the expression of a fragile site in Xq27-q28. In total, 911 samples were received, and of these 15 were known to be cytogenetically positive for a fragile site in Xq27-q28, whereas 362 were known to be cytogenetically negative. For the remaining samples the cytogenetic results were either unavailable or had not been performed at the time of testing. Of the 15 samples cytogenetically positive for a fragile site in Xq27.3-q28, 8 (3 females and 5 males) had been analyzed on banded chromosomes and 7 (5 females and 2 males) on unbanded chromosomes.

PCR Amplification of the FRAXE GCC Repeat from Extracted DNA

Oligonucleotides 598 (5′-GCG AGG AAG CGG CGG CAG TGG CAC TGG G-3′) and 603 (5′-CCT GTG AGT GTG TAA GTG TGT GAT GCT GCC G-3′) derived from sequences flanking the FRAXE GCC repeat were used as primers to amplify genomic DNA using extracted DNA as a template source. The DNA was diluted to a concentration of 100 ng/μl and 0.5 μl used in a 10 μl reaction volume containing 1X Cloned *PfU* Buffer (from 10 × stock, Stratagene), 1 × dNTPs (from 100 × stock, Pharmacia) 0.25 U Cloned PfU Polymerase (Stratagene), 5% dimethylsulfoxide (Sigma), oligonucleotides 598 and 603 each at 0.5 μM, and the final volume made up using dH_20 (BDH Analar grade). Each reaction was overlayed with mineral oil and denatured at 98°C for 5 min, followed by 33 cycles of 98°C for 35 s and 70°C for 6 min 30 s. Following PCR, 10 μl of each reaction were electrophoresed at 90 V for 2–3 h through a 20-cm × 25-cm 1% agarose gel containing ethidium bromide (0.5 μg/ml). The PCR products were visualized and photographed under UV irradiation. Samples that failed to amplify were diluted and the PCR repeated. Samples failing to amplify on five separate occasions indicated possible candidates for FRAXE expansion events and were investigated further by Southern analysis.

Detection of FRAXE Expansion Events by Southern Analysis

The eight female samples plus the male samples failing to amplify by PCR on five separate occasions were subjected to Southern analysis. The DNA samples were digested with *Hin*dIII, and the digested samples were run on 20-cm × 25-cm 0.8% agarose gels and allowed to migrate at 65 V for 20 h or until the 5-kb marker reached the center of the gel (to give maximum resolution of any expanded allele). Electrophoresed samples were transferred onto Hybond-N membrane (Amersham) and hybridized with OxE20 as described by Knight et al. (1993).

Results

In order to analyze large sample numbers in a rapid, nonisotopic, and cost-effective way, a PCR-based approach was selected. Failure to PCR amplify across the FRAXE site indicated the possibility that the corresponding sample may have a GCC expansion, thereby preventing successful PCR amplification. Female samples were not included in the PCR screens, because of the difficulty in distinguishing homozygous alleles.

PCR across the FRAXE Site of Unrelated Males Referred for Fragile X Syndrome Testing

For the FRAXE study, a total of 903 male DNA samples were received from diagnostic laboratories across

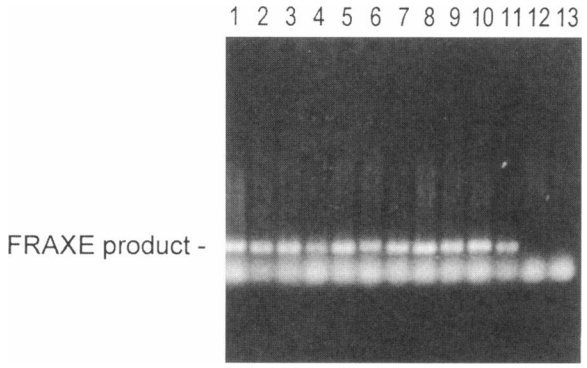

Figure 1 Example of PCR analysis across the FRAXE site, using oligonucleotides 598 and 603. Lanes 1–11 show PCR products in the normal size range of ~291–366 bp from 11 unrelated male samples. Lanes 12 and 13 show samples from two unrelated male samples that failed to PCR amplify.

the United Kingdom, and all had previously tested negative for expansions across the FMR-1 (FRAXA) CGG repeat. Of these samples, 887 gave a normal sized fragment on PCR amplification across the FRAXE GCC repeat, and 16 samples failed to amplify on five separate occasions. An example of a typical FRAXE PCR analysis is shown in figure 1. Lanes 1–11 show PCR products amplified from DNA samples of 11 unrelated males. Each of the observed products was considered to be in the normal size range for the FRAXE GCC repeat (291–366 bp, corresponding to alleles ranging from 1 to 26 GCC copies). Lanes 12 and 13 of figure 1 represent examples of PCR reactions that failed to amplify across the FRAXE site. Control reactions containing water (BDH Analar grade) in the place of DNA also failed to amplify (data not shown).

Southern Analysis of the FRAXE Region

The 8 female samples, plus each of the 16 male samples that failed to PCR amplify, were studied further by Southern analysis using OxE20 as a hybridization probe. At this level of resolution, 15 of the male samples gave the normal 5.2-kb *Hin*dIII fragment and 1 sample, "FRAXE case 1," showed an expansion event at FRAXE. Of the samples from the cytogenetically positive females, two gave expanded fragments in addition to the expected 5.2-kb *Hin*dIII fragment. These samples were from the mother and grandmother of two cytogenetically positive males for whom DNA samples were not available for study. An example of the Southern analysis is shown in figure 2. Figure 2*a* shows the expected 5.2-kb *Hin*dIII fragment in a normal female and a normal male (lanes 1 and 2, respectively) and a very faint expanded fragment of $\Delta = \sim 1$ kb in the male sample, FRAXE case 1 (lane 3). In figure 2*b*, lanes 1 and 4 are samples from a normal female and a normal male and show the expected 5.2-kb *Hin*dIII fragment. Lane 2 represents the cytogenetically positive (36%) grandmother with a FRAXE expansion of $\Delta = \sim 2.3$ kb, whereas lane 3 represents the mother (18% cytogenetic expression) with an expansion of $\Delta = \sim 2$ kb. Each has the normal 5.2-kb fragment. In order to investigate the possibility of mosaicism, Southern analyses were also

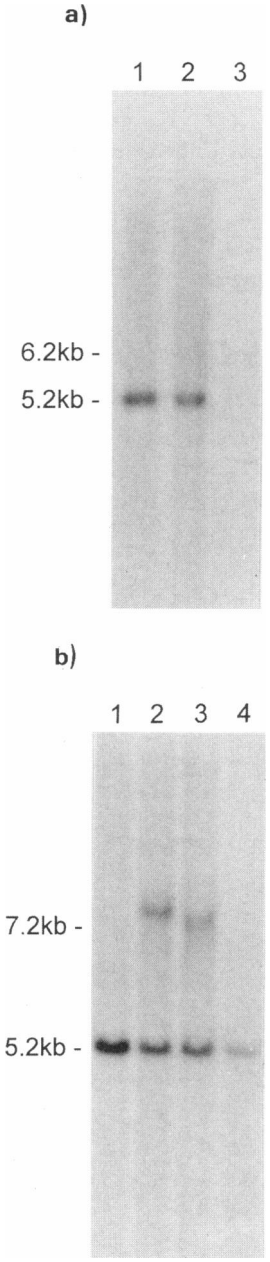

Figure 2 Southern analysis of *Hin*dIII-digested genomic DNAs hybridized with the OxE20 probe. *a*, Hybridization patterns of a normal female (lane 1), a normal male (lane 2), and a FRAXE male identified during these studies (lane 3). *b*, Hybridization patterns of normal female (lane 1), a normal male (lane 4), and the cytogenetically positive FRAXE grandmother (lane 2) and mother (lane 3) identified during these studies.

Table 1

Summary of FRAXE PCR Survey

No. Tested	Sex	Cytogenetic Result	FRAXE	FRAXF
534	Male	Not known/ not tested	1	Not tested
362	Male	−	0	Not tested
2	Male	+ (unbanded)	0	0
5	Female	+ (unbanded)	0	0
5	Male	+ (banded)	0	2
3	Female	+ (banded)	2	1

NOTE.—A plus sign (+) indicates fragile site in Xq27.3-q28; a minus sign (−) indicates no fragile site in Xq27.3-q28.

performed for all male samples yielding normal sized PCR products and yet reported to be cytogenetically positive. All of these gave a normal 5.2-kb fragment with OxE20, and there was no obvious indication of mosaicism. The samples of the other cytogenetically positive females also showed only the normal 5.2-kb fragment (data not shown).

The FRAXE expansions detected in the mother and grandmother of two cytogenetically positive males account for the fragility in two of eight samples cytogenetically positive on banded chromosomes for a fragile site in Xq27-q28. The remaining six samples were from unrelated individuals. Southern analysis using the probe OxF14, described by Ritchie et al. (1994), has revealed that three of these six samples, one female and two males, have expansions across the FRAXF GCC repeat (data not shown), and a repeat study of a further sample this time proved to be cytogenetically negative (G. Cross, unpublished observations). The remaining two individuals were negative for expansions across the FRAXF site. Consent to obtain fresh samples from these two individuals and to retest them at both a cytogenetic and molecular level is pending.

An overall summary of these findings is given in table 1. Of the two unrelated FRAXE GCC expansion events detected, one was identified in a mother and daughter previously shown to be cytogenetically positive for a fragile site in Xq27-q28 (on banded chromosomes) and the other in a male for whom cytogenetic results were unknown at the time of testing. Subsequently, the clinical notes for this male were obtained and revealed a cytogenetic report of 2% fragile site expression. Of the 362 samples known to be cytogenetically negative and the 7 samples reported to be cytogenetically positive on unbanded chromosomes, no expansion events were detected.

Clinical Phenotypes and Case Histories

The clinical information regarding the FRAXE male, FRAXE case 1, identified during this survey is presented below. The details from three previously unreported FRAXE males, FRAXE cases 2–4, identified through fragile X syndrome testing are also presented. These additional cases were selected from the 38 database samples because they had been directly referred to our laboratory for FRAXE testing, and thus the clinical details were readily accessible. The remaining cases in the database were documented mainly through feedback from collaborating groups and include a number that have been reported previously in the literature.

FRAXE case 1.—This boy was born at 35 wk by Cesarean section after intrauterine growth retardation and fetal distress were noted. His birth weight was 1.44 kg, and he subsequently showed developmental delay and microcephaly. Educational psychological assessment at the chronological age of 33 mo on the Griffiths Scales Mark showed him to be at the 20-mo level on the personal-social scales and the 18-mo level for eye-hand and performance. Speech therapy assessment at the chronological age of 38 mo showed comprehension equivalent to 24 mo of age and expressive language at 18 mo of age. The developmental delay prompted chromosomal studies, which revealed fragile X expression in 2% of cells, and the fragile X syndrome was the favored diagnosis. He initially attended a normal primary school but was transferred to a special school at the age of 5 years. Following reevaluation at the age of 9 years 10 mo, the diagnosis of fragile X syndrome was considered unlikely, and this was later confirmed by DNA studies. A recent physical examination at the age of 10 years 5 mo revealed his height and weight to be below the third percentile. Mildly dysmorphic features consisting of thick lips with a long upper lip and misplaced teeth were also noted, in addition to a hoarse voice and stellate irides. These features combined with an "engaging" personality were considered to be consistent with William syndrome, but follow-up studies did not reveal any cardiac abnormalities. A review at the age of 10 years summarized the diagnosis as learning difficulties, mild dysmorphic features, and short stature. At this stage, the patient did not have obvious features of William syndrome. Subsequently, FISH studies of the elastin gene on chromosome 7 were shown to be normal (individuals with William syndrome have been noted to have deletions resulting in hemizygosity at the elastin locus [Ewart et al. 1993]). At the age of 11 years, educational psychological assessment on the British Ability Scales showed word recognition at 6.4 years (<1st percentile) and base number skills at 6.1 years (<1st percentile). At present, this boy attends a special school and is said to be making good progress with no particular management problems.

FRAXE case 2.—This boy was born after a normal pregnancy. For the first few months he was considered a placid baby, but he rapidly developed very demanding

behavior. At the age of 2 years 6 mo he had only one word. A developmental assessment at nearly 5 years of age showed particularly poor coordination skills and speech delay. He spoke in short sentences and could count to 10. His general health was good, but he was very clumsy and had some mannerisms, for example, flapping his hands when excited and stroking and brushing his face. A physical examination showed normal growth, with a 52-cm head circumference (between the 50th and 75th percentile) and a height of 114 cm (on the 90th percentile). A café au lait lesion was noted on his right arm, and he had 5th finger clinodactyly. He was not considered to have any significant dysmorphic features. At the age of 7 years, his developmental level is considered to be that of a 4 year old. He can recognize only six words and is able to write his first name. He is currently described as having both behavioral and learning difficulties and was transferred to a school for children with learning difficulties because he was unable to manage in mainstream education. Cytogenetic analyses revealed 28%–40% fragile site expression, but molecular studies of the FMR1 locus were normal. Subsequent analyses of the FRAXE locus revealed an expansion across this site.

FRAXE case 3.—This boy was referred at the age of 6 years 11 mo with learning difficulties. He was the first child of healthy, unrelated parents who also have three normal daughters. He was born at 37 wk and weighed 2.5 kg. There were no neonatal problems, although during pregnancy there had been some concern regarding intrauterine growth retardation. The father attended a normal school but was poor at reading and writing. The mother has two sisters who each have a son with significant language difficulties. His parents were concerned about poor language development from 18 mo of age, and speech therapy was started at 2.5 years. Normal hearing was confirmed on several occasions. At the age of 6 years 5 mo he was referred to the local child development center because he was found to be very "backward" at school and posed a problem in the normal education stream. Initial assessment showed adequate gross and fine motor function and skills but significant learning difficulties. On the Wechsler Intelligence Scale for Children his full-scale IQ was 66 with a verbal IQ of 65 and performance IQ of 73. Physical examination showed a head circumference of 53.5 cm (>75th percentile) and a height of 115 cm (on the 25th percentile). Apart from two white patches of hair, no abnormal features were noted, and the rest of the examination was normal. Chromosome studies revealed 9%–16% expression of a fragile site in Xq27.3-q28, but molecular analyses for fragile X syndrome were normal. This individual was subsequently shown to have expansion events at the FRAXE locus.

FRAXE case 4.—This boy was referred at the age of 6 years because of concerns about his difficult behavior. However, his delay was first noted when he started a formal school program at the age of 3.5 years. At the age of 3 years 11 mo he was evaluated by use of the "Portage Developmental Checklist" and was found to have deficits in the areas of social interaction, sensory awareness, intellectual concepts, and language. At the age of 6 years he was considered to have a developmental age of 4–5 years. Behavioral concerns included temper tantrums, aggressiveness toward his sisters, a destructive nature, and sometimes a rocking motion. A physical exam at 6 years 3 mo showed a height of 130.5 cm (>97th percentile) and a head circumference of 55 cm (>98th percentile). His father's head circumference was >97th percentile. Developmental testing showed that Gesell figures were copied at the 5-year level, and his digit span was three digits forward; digits could not be reversed. Sentence recall was at the 4-year level, and he was unable to read the first of the Durrell paragraphs. He had a number concept of ≥ 10 but could not add or subtract mentally within a set of five blocks. The overall impression from these evaluations was that he has a developmental disability predominantly affecting language development with a continuum of dysfunction also including other developmental areas. The largest gaps in the developmental testing were in language-related areas such as social skills, and it was recommended that he join a special education program. Although an original amniocentesis analysis had revealed a normal fetal karyotype, a repeat chromosome analysis revealed 7% fragile site expression in Xq27.3-q28. Molecular testing for the fragile X syndrome was negative, but FRAXE studies revealed an expansion of $\Delta = \sim 2.9$ kb at the FRAXE locus.

Discussion

The folate-sensitive fragile sites FRAXA and FRAXE are located in close proximity in Xq27.3-q28 of the human X chromosome (Sutherland and Baker 1992; Flynn et al. 1993; Hirst et al. 1993). The cytogenetic expression of FRAXA is associated with the fragile X syndrome and the cytogenetic expression of FRAXE with a milder form of mental handicap (Lubs 1969; Flynn et al. 1993; Knight et al. 1993). Although FRAXA and FRAXE are indistinguishable by use of conventional cytogenetic means, they can be delineated at the molecular level, and this provides the basis for differential diagnosis. The fragile X syndrome is known to be the most common form of inherited mental retardation, but the exact frequency of individuals with FRAXE expansions remains unknown. The studies presented here were designed to assess the frequency of FRAXE expansion events in samples referred for fragile X syndrome testing and to report the clinical phenotypes noted in identified

FRAXE individuals. In a study of 903 male samples referred for fragile X syndrome testing and found to be negative for expansions across the FRAXA GCC repeat, our studies revealed 1 individual who showed a FRAXE expansion of $\Delta = 1$ kb on Southern analysis. This corresponds to ~0.1% of the tested sample set. The frequency of FRAXA expansions detected by Wang et al. (1993) in 525 samples referred for fragile X syndrome testing was ~2.7%, whereas the frequency of FRAXE was ~14-fold less, at ~0.2%. This is consistent with our findings and the results of Allingham-Hawkins and Ray (1995) who failed to identify any FRAXE individuals in a comparatively smaller sample set of 300 males. Allingham-Hawkins and Ray (1995) concluded from their studies that FRAXE expression may be a benign characteristic that is not associated with a phenotype. They supported this by citing the documentation of two FRAXE families in which the cytogenetic expression of FRAXE did not associate with a clinical phenotype. However, one of these families, cited from Sutherland and Baker (1992), has already been shown to express not FRAXE but the FRAXF fragile site further distal in Xq28 (Mulley et al. 1995). Also, in the second family cited, the individual expressing the FRAXE site has not received formal clinical or psychometric testing, and thus his true status remains unknown (Knight et al. 1994). The importance of psychometric testing has been highlighted previously by Mulley et al. (1995). These authors described a FRAXE family in which two individuals may have been misclassified as normal on the basis of clinical impression alone. Nevertheless, there are two FRAXE families cited in the literature in which cytogenetically positive males with confirmed expansion events have been psychometrically assessed and do not have any notable mental retardation (Knight et al. 1993; Hamel et al. 1994). A simple explanation for these observations is that FRAXE genotype-phenotype relationships can be blurred by factors such as GCC copy number mosaicism, methylation mosaicism, or between-tissue mosaicism. This is already known to be the case in the normal FRAXE male with 43% cytogenetic expression described by Knight et al. (1993). This individual was shown to have an unmethylated FRAXE expansion of 400 bp and a methylated expansion of 2.6 kb, thus explaining both his cytogenetic expression of FRAXE and his normal phenotype.

Despite such observations, the possibility of ascertainment bias remains an important consideration. One way of determining whether FRAXE expansions truly cause mental handicap would be to assess whether the FRAXE expansion frequency in a large unbiased "normal" control population is significantly less than that of the mentally retarded population. To date, none of the 552 control samples reported in the literature or of the 400 control samples contributing to an ongoing study have shown expansion events across FRAXE (Knight et al. 1993; Allingham-Hawkins and Ray 1995; Nancarrow et al. 1995; Rubinsztein et al. 1995; Wang et al. 1995; R. J. Ritchie, L. Charkrabarti, S. J. L Knight, R. Harding, and K. E. Davies, unpublished observations). However, many more individuals will need to be studied before any firm conclusions can be drawn. Nevertheless, the clinical phenotypes of the 4 FRAXE individuals presented in this manuscript, together with those previously reported in the literature (Knight et al. 1993, 1994; Hamel et al. 1994; Mulley et al. 1995), and, combined with the 38 families recorded thus far in our database, all provide strong evidence that the expression of FRAXE is associated with a mental retardation phenotype. The degree of mental handicap may vary from very mild to more severe, and the consistent feature in all cases is that of a language disorder usually presenting as speech delay. Reading and writing may also be delayed, and behavioral problems can be present. Although some dysmorphic features are mentioned, these are not generally consistent between patients. The described features may not be unique to this form of mental handicap, but, nevertheless, the underlying similarities between affected individuals suggest that comprehensive clinical and psychometric assessments of additional FRAXE individuals might enable a more definitive phenotype to be determined. A further consideration is that many of the FRAXE patients identified to date have been assessed at a young age. Thus, the situation in FRAXE mental retardation may be similar to the fragile X syndrome, where one of the first nonspecific signs is developmental delay, with the clinical features being very subtle in the young and becoming more recognizable with increasing age.

The cloning of the gene responsible for the developmental delay in FRAXE individuals has yet to be reported, and, in the past, the diagnosis has relied on cytogenetic studies followed by molecular analyses. In the studies presented here, none of the samples reported to be cytogenetically positive for a fragile site in Xq27-q28 using unbanded chromosomes were found to have expansion events at either FRAXA, FRAXE, or FRAXF. This may reflect the limitations of this technique in terms of assessing precise chromosomal localizations, a concern supported by the fact that all of the five FRAXE individuals presented in this study were shown to be cytogenetically positive on banded chromosomes for a fragile site in Xq27.3-28. Conversely, none of the 362 individuals known to be cytogenetically negative for a fragile site in Xq27.3-q28 showed expansion events across the FRAXE GCC repeat. Thus, cytogenetic expression of a fragile site in the Xq27.3-28 region (banded chromosomes) of FRAXA and FRAXF negative individuals can be a strong indicator of an expansion event at the FRAXE fragile site. However,

many diagnostic laboratories no longer perform routine cytogenetic analyses for fragile site expression and rely solely on molecular analyses. For diagnostic laboratories already involved in fragile X syndrome screening and considering molecular testing for FRAXE, both the issues of phenotype and of frequency of FRAXE individuals in the given referrals are important considerations. The survey presented here shows that the frequency of FRAXE in the studied population is rare, possibly 14-fold less than the estimated 1/1,250 incidence of males with the fragile X syndrome. However, this may not be an accurate reflection of the true incidence of FRAXE. One possible disadvantage of the PCR screening described here is that a normal size product may be obtained in individuals who have a degree of mosaicism across the FRAXE region. This situation has been noted previously in the FMR-1 gene (Nakahori et al. 1991; Oberlé et al. 1991) and has recently been recorded in a FRAXE family analyzed in this laboratory (authors' unpublished observations). In addition, the sample population screened in this study is biased in that all of the samples were initially referred for fragile X syndrome testing. Therefore, in light of the usually milder phenotype of FRAXE individuals relative to fragile X syndrome individuals, it is possible that the tested population does not encompass all cases of FRAXE mental retardation. As alluded to elsewhere (Knight et al. 1994), it could be that other FRAXE individuals may be found in different target populations, for example children with language delay undergoing special education.

In order to relieve the potential burden caused by FRAXE testing, a number of laboratories have devised screening rationales for the combined analysis of FRAXA and FRAXE. Wang et al. (1995) described a nonradioactive assay for the simultaneous PCR amplification of the triplet repeats at both FRAXA and FRAXE. This assay is reliable, cheap, and efficient and has particular application in laboratories that receive a large number of referrals. In this way, the vast majority of referrals can be eliminated from further study by a simple primary PCR screen, and the few remaining samples can followed up by Southern analysis.

It is interesting to note that, of the eight individuals known to be cytogenetically positive (banded chromosomes) for a fragile site in Xq27.3-28, three were found to have an expansion of the FRAXF GCC repeat. In contrast to FRAXE, a higher proportion of FRAXF patients have been described in whom the cytogenetic expression has no apparent association with mental impairment (Donnelly et al. 1994; Parrish et al. 1994; Ritchie et al. 1994). This suggests that ascertainment bias may have been involved in identification of the mentally impaired FRAXF individuals presented in this study. However, more complex scenarios involving mosaicisms of copy number, methylation, or tissue differences have yet to be ruled out.

In summary, we have conducted a study to assess the frequency of FRAXE expansion events in a population of individuals referred for fragile X syndrome testing and found to be negative for expansions across the FRAXA fragile site. We have also presented the clinical details of four FRAXE individuals previously unreported in the literature. For the population studied, we conclude that FRAXE mental retardation is a relatively rare, but significant, form of mental retardation for which genetic laboratory diagnosis would be appropriate. In this way, individuals suffering from this form of mental handicap can be identified and any special needs assessed.

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