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Fragile (X) X-Linked Mental Retardation I: Relationship Between Age and Intelligence and the Frequency of Expression of Fragil (X)(q28)

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Members of eight Saskatchewan families with fragile (X) X-linked mental retardation were studied in an attempt to relate frequency to age and intelligence. The mean IQ of 37 affected men was 35 (range 10–66). The mean IQ of 32 carriers was 88 (range 57–119), and the mean IQ of 13 females who remain at risk for being carriers, have no affected sons, and who failed to demonstrate the fra(X) was 100 (range 78–126). We demonstrated a significant inverse relationship between age and frequency of the fra(X) in carriers and in affected males. However, we demonstrated a more highly significant inverse relationship between frequency of the fra(X) and IQ in carriers but to a lesser extent in affected males. Of 32 carriers, only 3 (9.4%) did not demonstrate the fra(X) after addition of 5-fluoro-2′-deoxyuridine (FUdR) to the folic acid and thymidine-reduced culture medium.

From these data we would recommend that chromosome studies in individuals at risk for fra(X) X-linked mental retardation be carried out at the youngest age and that the addition of FUdR to culture medium is useful in carrier identification. It is clear that, in at least the carriers, a lower expression of the fra(X) is highly significantly correlated to higher intelligence.

Key words: X-linked mental retardation, fra(X) MR, fragile (X)(q28), intelligence, age, carrier detection, marker-X

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INTRODUCTION

The identification of the fra(X) "marker" in a form of X-linked mental retardation (fra(X)MR) has been a major insight into one of the common forms of mental handicap. The marker was first identified by Lubs [1969]; however, several years elapsed before the rediscovery of the fragile X on the X chromosome and detailed clinical delineation of the disorder by many independent investigators [Turner et al, 1975; Giraud et al, 1976; Harvey et al, 1977; Sutherland, 1977; Sutherland and Ashford, 1979; Howard-Peebles and Stoddard, 1979; Turner et al, 1980a; Jennings et al, 1980; Jacobs et al, 1980; Martin et al, 1980]. Demonstration of the fra(X) was noted to be enhanced by a number of culture medium manipulations including reduced thymidine and folic acid [Sutherland, 1977; Howard-Peebles and Howell, 1979], the necessity for methionine [Howard-Peebles et al, 1980], and addition of folic acid antagonists [Glover, 1981; Tommerup et al, 1981a]. The fra(X) has since been identified in skin fibroblasts [Jacky and Dill, 1980; Tommerup et al, 1981b; Gardner et al, 1982] leading the way to prenatal diagnosis through amniotic fluid cell cultures [Webb et al, 1981; Jenkins et al, 1981; Shapiro et al, 1982]. A more controversial aspect is the possibility of treating individuals with this condition; anecdotal evidence has been published suggesting folic acid treatment may improve the clinical management [Lejeune et al, 1981; Lejeune, 1982]. This is surprising in light of normal intracellular metabolism of folic acid in affected males [Popovich et al, 1980]. Recent reviews deal with the development of our understanding of this important and common form of MR [Turner and Opitz, 1980; Gerald, 1981].

The relationship between the fra(X) site and this X-linked recessive form of MR has not been clearly delineated. The fra(X) can be identified in all affected males and in most carriers of fra(X) MR. It is perplexing that the fra(X) cannot be identified in every cell of such individuals. Rarely, the fra(X) has been seen in normally intelligent males [Daker et al, 1981] and in normal females [Popovich et al, 1982]. In some families affected males have been only mildly retarded and, rarely, even of normal intelligence (nonpenetrance), and some have married and had offspring [Jacobs et al, 1980; Webb GC et al, 1981].

Glover [1981] has shown that the expression of fra(X) is a reflection of a disturbance in DNA synthesis due to depletion of the co-factor deoxythymidine monophosphate; however, it is yet to be confirmed that this is the mechanism for the mental retardation. In elegant work, Tommerup et al [1981a] demonstrated that the depletion of the thymidine monophosphate pool is due to inhibition of thymidylate synthetase. Thus, several biochemical aspects of fra(X) MR remain unsettled.

Several investigators have noted that many obligate fra(X) MR carriers do not demonstrate the fra(X) and that it was an impression that the fra(X) expression is lower with increasing age and higher intelligence [Jacobs et al, 1980; Turner et al, 1980a; Turner et al, 1980b; Schmidt and Passarge, 1980]. This creates uncertainty when faced with an older intelligent woman at risk of being a carrier. Further studies will be needed to elucidate the optimal age and culture conditions for fra(X) expression in these individuals.

The aim of this study was to determine what if any relationship existed between the fra(X), age, and intelligence in affected males and carriers. We have performed cytogenetic and psychological studies in a substantial number of obligate carriers, women at risk for being carriers, and affected males from eight fra(X) MR families.

We report here the apparent relationship between the expression of the fra(X), intelligence, and age in carrier and noncarrier women and affected males.

MATERIALS AND METHODS

Families

Eight unrelated fra(X) families were studied. We were able to obtain cytogenetic and psychological data on 37 affected males (mean age 40.1 years, range 6–65 years); 32 carriers (mean age ± SD: 43.3 ± 19.7 years, range 10–88 years), and 13 females at risk for being carriers who did not have affected male or carrier female children and failed to demonstrate the fra(X) (mean age ± SD: 38.4 ± 14.4 years, range 11–70). Clinical and cytogenetic data were reported previously on many individuals from six of the families (Families D, S, C, N, P, D) [Dunn et al, 1963; Jacobs et al, 1980]. Two families are new to the literature [Families E and H]. Updated pedigrees of the original six families and the two new families are found in Figure 1a–h. Although we have cytogenetic data on many other members of these families, this report includes only those individuals for whom we have both psychological and recent cytogenetic data. A consent for the study was obtained from each individual or, where appropriate, their legal guardian.

Family E deserves further comment. Affected male EIV-4 was ascertained after his wife delivered a stillborn female with multiple congenital anomalies (hemifacial atrophy, ear anomalies, tracheoesophageal fistula, and truncus arteriosus). We were unable to culture the skin for cytogenetic studies of the stillborn but the parents were studied. EIV-4 was noted to be mildly mentally retarded and to have macro-orchidism. Chromosome studies showed the fra(X) in a few cells (confirmed on several repeat cultures in our laboratory as well as in another laboratory in Hawaii by Dr. Patricia Jacobs). EIV-4's wife had a 47,XXX chromosome constitution. Of even greater interest is the fact that two of EIV-4's sibs (EIV-2, EIV-5) were noted to have severe mental retardation and facial dysmorphic features quite different from EIV-4's stillborn daughter. These individuals did not show the fra(X). They are likely to have a previously undescribed autosomal recessive condition; this mode of inheritance is supported by the fact that the parents are first cousins. EIV-4's mother, EIII-2, and her normal daughter were studied and both failed to demonstrate the fra(X) even in the culture medium containing FUdR. The mother has nine sisters, all of whom have healthy children. She has four apparently normally intelligent brothers, one of whom has a child who is severely mentally retarded and has phenotype similar to EIV-2 and EIV-5. We suspect that EIV-4 is a new mutation of fra(X) MR.

Psychological Tests

Intellectual functioning was assessed using the Revised Wechsler Adult Intelligence Scale (WAIS-R), the Raven Progressive Matrices, and, where mental age fell below the lower limit of these methods, the Revised Stanford Binet (RSB) test was administered. To ensure comparability of scores between the WAIS-R and the RSB (in the males) and thus the accuracy of the relationship between the IQ scores and number of fra(X) manifesting cells, the RSB deviation IQ scores were converted to Z-scores with a mean of 0 and a standard deviation of 1. These Z-score values were then multiplied by 15, and 100 was added to the result. Such RSB scores have a mean

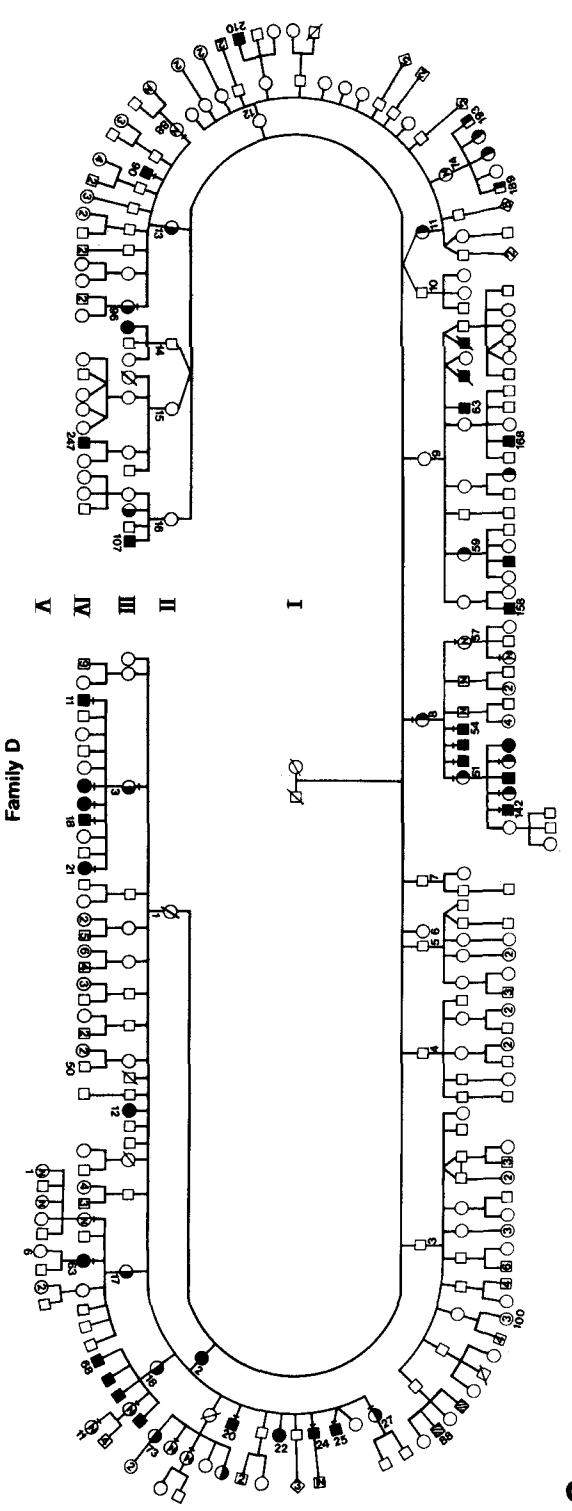


Fig. 1. a–h) Pedigrees of fra(X) MR families. a) Family D, b) Family S, c) Family C, d) Family N, e) Family P, f) Family B, g) Family E, h) Family H. Horizontal bar over symbol identifies subjects on whom we have cytogenetic and psychological data. Left half of symbol black: IQ < 80; Right half of symbol black: fra(X) (q28) identified; "N" in right hand of symbol: chromosomes normal; crosshatched symbol: PKU with mental retardation; asterisk: Trisomy 21; line in circle: 45,X Ullrich-Turner syndrome; g) EIV-2 and EIV-5: severe mental retardation, no fra(X).

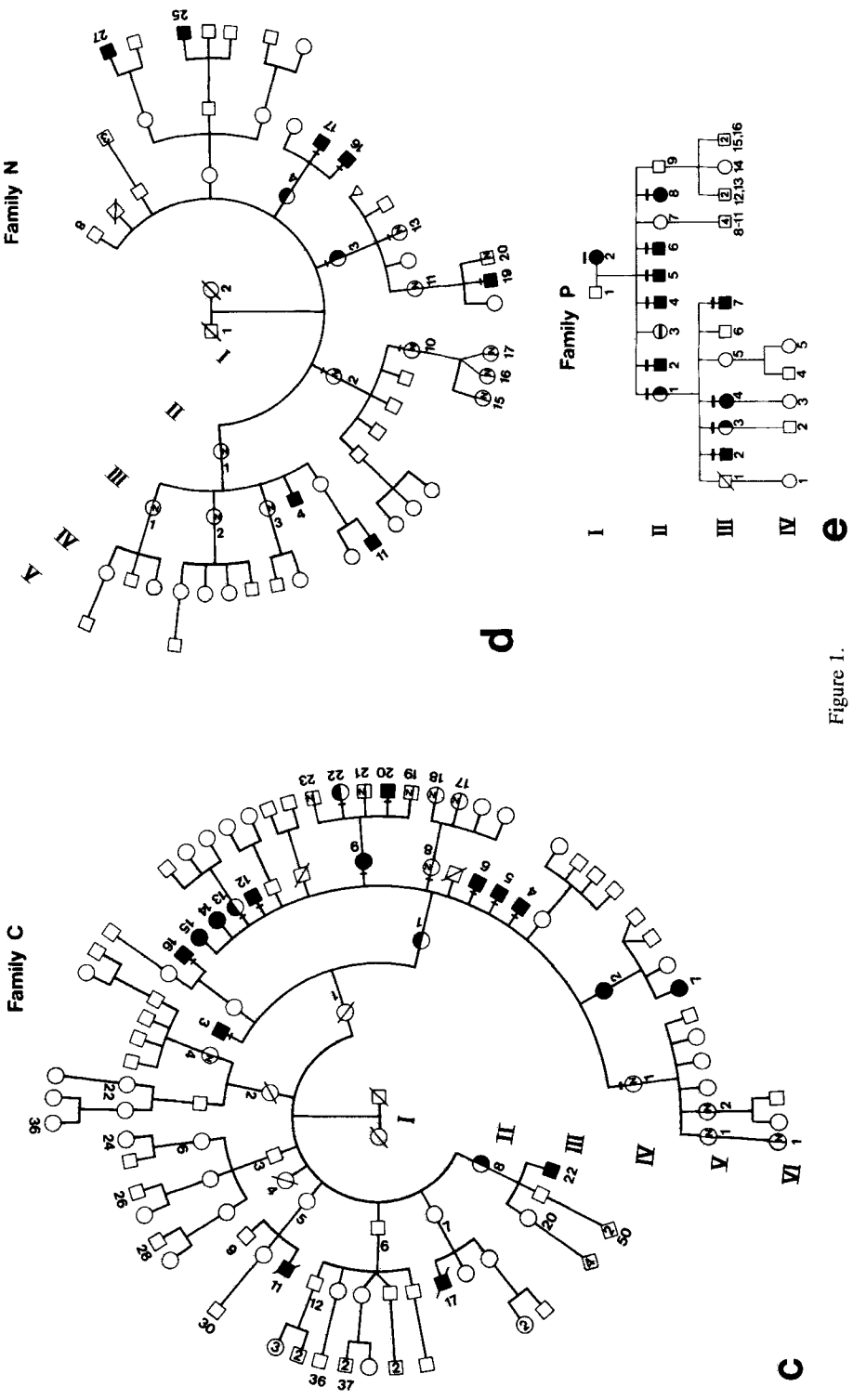
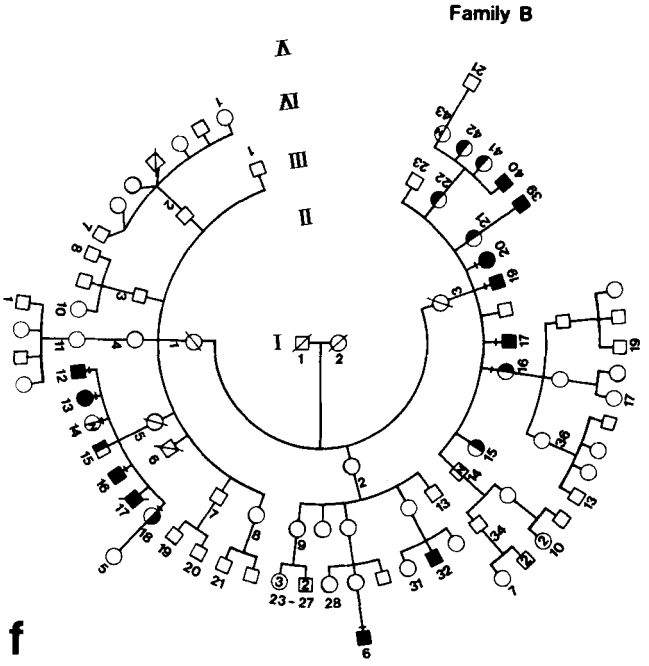


Figure 1.


Family B
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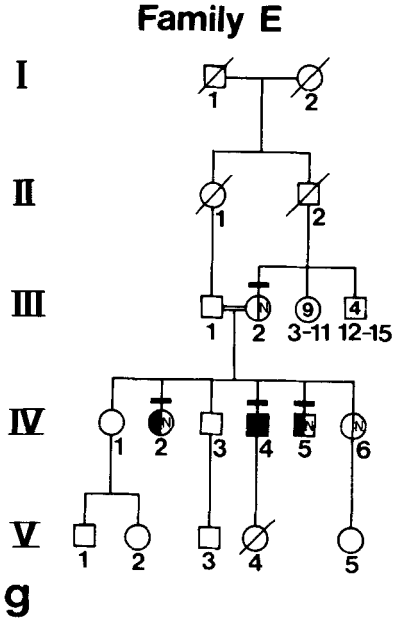
Family E
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12-15
g

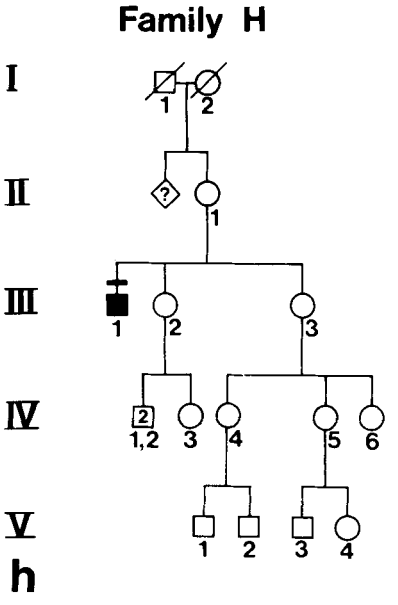
Family H
I
II
III
IV
V
1,2
h

Figure 1.

of 100 and a standard deviation of 15 and are thus comparable to the WAIS-R scores. All psychological testing was performed by one of the authors (E.M. or J.A.).

Cytogenetic Studies

Heparinized blood specimens were set up in culture within 24 hr. Medium 199 (GIBCO) or "M" medium (modified medium F-10 without folic acid, thymidine, or hypoxanthine—GIBCO) with 5% fetal calf serum was used. Most cultures on females and some males were also set up with 5-fluoro-2′-deoxyuridine (FUdR) (Sigma) at a concentration of 0.1 μmol/L 24–48 hr before harvesting.

Cultures were harvested in the usual manner at 96 hr, the slides were humid-air dried, and metaphases were examined after G-banding. Attempts were made to score at least 100 cells for the fra(X). A control blood specimen was set up at the same time as the study blood and the specimens were appropriately coded to ensure a blind study. All cultures and analyses were performed by one of us (J.K.). The consistency and reliability of J.K.'s scoring of the fra(X) was confirmed in a frequency study done on another family over a 6-month period.

Statistics

Correlation coefficients and partial correlation coefficients were determined by the Pearson product-moment method.

Multiple regression analyses were used to determine the impact of age and IQ on proportion of fra(X) expressed. Standard tests of significance were utilized.

RESULTS

Psychological Tests

The IQ scores of the 37 affected males on the RSB test ranged from 10 to 66 (mean 34.8), most falling within the severely to profoundly retarded range (Table I). The average mental age was 4.3 years (range 1.4–7.8).

The mean full scale IQ of the 32 carriers (obligate or cytogenetically proven heterozygotes) was 87.9 ± 17.3 (range 57–119). Twelve of the 32 (37.5%) carriers had IQ scores below 80 and 7 of the 32 (21.8%) had IQ scores below 70 (Table II).

The third group studied included 13 females from the families in whom we did not identify the fra(X), who did not have affected sons or carrier daughters, and whom we still consider to be at risk. This group should not be considered a true control group since we are aware that a proportion of carriers do not express the fra(X) even after the addition of FUdR and the use of a folic acid- and thymidine-free medium. The mean IQ score of this group was 99.46 ± 14.3 which was almost 12 points higher than the confirmed heterozygotes ($p < 0.05$). The ages of the two female groups were not significantly different ($p > 0.03$) (Table III).

Cytogenetics

Data on cytogenetic findings are shown in Tables I–III. In affected males, the mean frequency of the marker was 20.1% (range 1–40%). In the carrier female group the mean frequency was 9.5% (range 0–44%). Seven females in the carrier group did not demonstrate the fra(X) using a folic acid- and thymidine-reduced medium. However, after the addition of FUdR to this medium, four of these seven carriers

TABLE I. Data on Affected Males

Pedigree no.	Age (years)	IQ	fra(X)/cells	fra(X) (percent)
DIII-20	61	21	30/106	28.30
DIII-24	53	62	12/100	12.00
DIII-25	51	34	33/107	30.84
DIII-52	54	32	9/100	9.00
DIII-53	50	66	12/115	10.43
DIII-54	49	23	16/100	16.00
DIII-90	47	30	38/111	34.23
DIV-11	43	27	40/100	40.00
DIV-18	28	54	31/103	30.10
DIV-142	34	32	29/108	26.85
SIII-2	41	40	27/114	23.68
SIII-12	30	31	34/100	34.0
SIV-7	13	44	39/100	39.0
SIV-8	11	58	35/100	35.0
CIII-3	58	25	11/100	11.0
CIV-4	44	22	11/100	11.0
CIV-5	42	25	23/117	19.7
CIV-6	40	18	32/118	27.12
CIV-12	32	20	29/100	29.0
CIV-16	40	41	25/100	25.0
CV-20	14	43	21/100	21.0
NIII-16	29	38	97/556	17.45
NIII-17	25	22	120/619	19.39
NIV-19	10	63	11/100	11.0
PII-2	61	27	12/100	12.0
PII-4	58	28	23/104	22.12
PII-5	56	20	8/100	8.0
PII-6	53	23	14/107	13.08
PIII-2	37	25	44/121	36.36
PIII-7	23	32	26/108	24.07
BIII-17	65	39	14/102	13.73
BIII-19	60	10	16/103	15.53
BIV-12	48	24	24/106	22.6
BIV-16	38	35	11/100	11.0
BV-6	6	66	5/54	9.3
EIV-4[a]	27	63	1/100	1.00
HIII-1	53	23	22/108	20.37

[a]Utilizing FUdR, 3/111 cells express fra(X).

demonstrated the fra(X). Therefore, 3 of 32 (9.4%) of our identified carriers failed to demonstrate the fra(X).

In DIV-142, 2 cells out of 150 were found with a 47,XXY constitution. It is of interest that one of the 47,XXY cells also had a fra(X). No other individual had any other structural or numerical abnormality apart from the fra(X).

Relationship Between fra(X) Expression, Age, and IQ

There appears to be an inverse relationship between IQ and expression of the fra(X) in both sexes. However, since there also appears to be an effect of age on the IQ score (independent of the standard adjustments in the IQ tests), partial correlations were used to compare age with fra(X) (where IQ is kept constant), and IQ with fra(X)

TABLE II. Data on Carriers

Pedigree no.	Carrier status	Age (years)	IQ	fra(X)/cells	fra(X) (percent)
DII-12	obligate	88	73	2/114	1.75
DII-11	obligate	74	88	3/100	3.00
DIII-3[a]	obligate	66	98	0/100	0.00
DIII-27	50% risk	49	83	10/100	10.00
DIII-51	obligate	56	91	5/100	5.00
DIII-96	50% risk	30	104	2/100	2.00
DIV-16	50% risk	34	62	42/103	40.78
DIV-17	50% risk	31	63	22/108	20.37
DIV-21	50% risk	23	77	11/105	10.48
DIV-63	50% risk	37	69	14/104	13.46
DIV-71	50% risk	39	97	0/100	0.00
DIV-143	50% risk	33	89	18/101	17.82
DIV-145	50% risk	30	103	3/100	3.00
DV-11[a]	25% risk	17	99	0/100	0.00
SII-1	obligate	61	109	0/100	0
SII-5[a]	obligate	52	89	0/100	0
SIII-4	obligate	40	111	1/107	0
SIII-13	50% risk	27	106	5/111	4.5
SIV-6	25% risk	10	105	3/100	3.0
CIV-9	obligate	38	73	29/104	27.88
CIV-13	50% risk	30	57	28/116	24.14
CV-22	50% risk	11	96	26/101	25.74
NII-3[a]	obligate	60	117	0/100	0
PI-1	obligate	82	76	3/78	3.85
PII-1	obligate	63	94	5/111	4.5
PII-8	50% risk	44	69	7/100	7.0
PIII-3	50% risk	31	97	1/100	1.0
PIII-4	50% risk	28	69	18/100	18.0
BIII-16	50% risk	66	90	2/101	1.98
BIII-20	50% risk	59	62	60/137	43.8
BIV-13	50% risk	45	77	10/100	10.0
BIV-18	50% risk	30	119	2/100	2.0
EIII-2[b]	?	59	—	0/100	0.00

[a]Subsequently shown to have fra(X) with the addition of FUdR.
[b]Not included in analysis as a carrier, does not demonstrate fra(X), unremarkable family history for X-linked MR, although son EIV-4 has fra(X).

TABLE III. Data on Females at Risk Who Do Not Demonstrate the Fragile X

Pedigree no.	Carrier status	Age (years)	IQ	fra(X)/cells
DIII-57	50% risk	41	97	0/100
DIII-88	50% risk	49	113	0/100
DIV-61	50% risk	40	109	0/100
DIV-73	25% risk	39	106	0/100
DIV-74	25% risk	35	80	0/100
DIV-155	25% risk	20	97	0/100
CIV-1	50% risk	50	100	0/100
CIV-8	50% risk	40	86	0/100
CIV-13	50% risk	31	78	0/100
NII-2	50% risk	70	85	0/100
NIII-13	50% risk	30	113	0/100
BIV-14	50% risk	43	126	0/100
SIV-5	25% risk	11	103	0/100

(where age is kept constant). The effect of age on IQ was seen in both sexes but was more obvious in the males (females: $r = -0.10$; males: $r = -0.52$). A true age effect on IQ in the males cannot be entirely discounted and it has been the experience of others that institutionalized males with the fra(X) showed decline in intelligence with time. Independent of the lack of stimulation and deterioration in performance in institutions, we wonder if there is a biological effect resulting in mental deterioration over time in affected males, as with the Down syndrome.

The males showed a decline in the expression of fra(X) with increasing age (straight correlation: $r = -0.22$, $p > 0.05$; partial correlation: $r = -0.39$, $p < 0.05$) (Fig. 2a). The partial correlation is an attempt to keep constant the effect of IQ on age and IQ on expression of the fra(X). There was a lower expression of fra(X) associated with higher intelligence (straight correlation: $r = -0.19$, $p > 0.05$; partial correlation: $r = -0.37$, $p < 0.05$) (Fig. 2b). Again the partial correlation in this

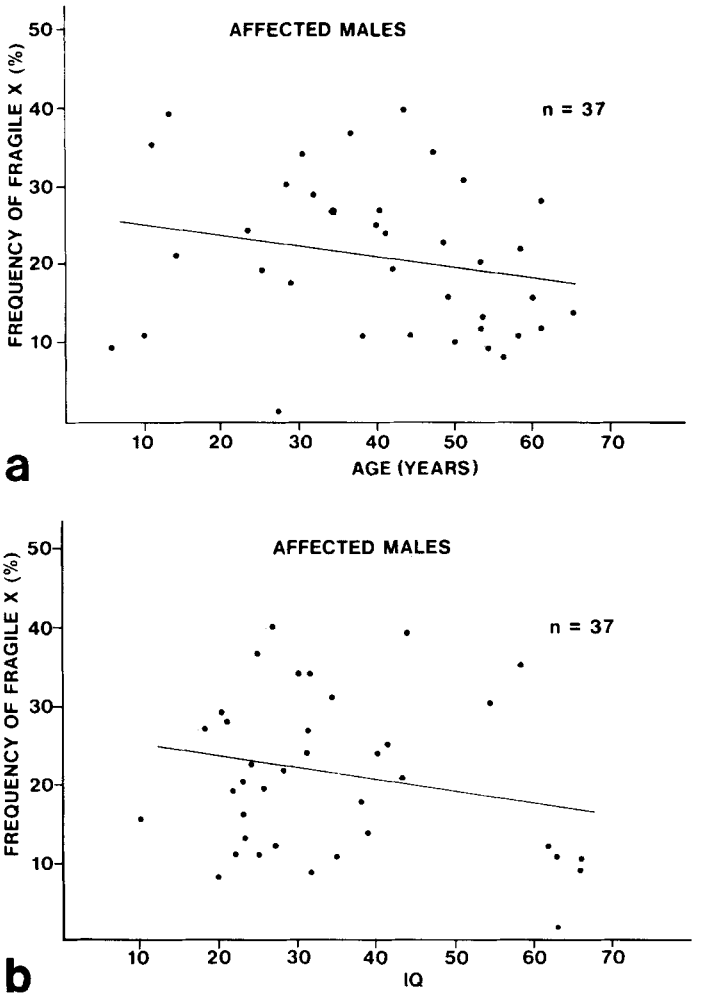

Fig. 2. a) Scatter plot with regression line in hemizygotes: fra(X) expression vs. age: $y = -0.13709x + 26.320$. Partial correlation: $r = -0.39$, $p < 0.05$; b) scatter plot with regression line in hemizygotes. fra(X) expression vs. IQ: $y = -0.12792x + 25.264$. Partial correlation: $r = -0.37$, $p < 0.05$.

instance attempts to control for the effects of age on fra(X) expression and effects of age on IQ.

Heterozygotes showed a decline in expression of fra(X) with increasing age (straight correlation: $r = -0.23$, $p > 0.05$; partial correlation: $r = -0.41$, $p < 0.05$) (Fig. 3a). However, a highly significant inverse relationship was seen between the expression of fra(X) and intelligence (straight correlation: $r = -0.68$; partial correlation: $r = -0.73$, both $p < 0.001$). The IQ accounted for 13% of the variability of expression of the fra(X) in the males and for 50% of the variability of expression of the fra(X) in the females. Age accounted for only 5% of the variability of

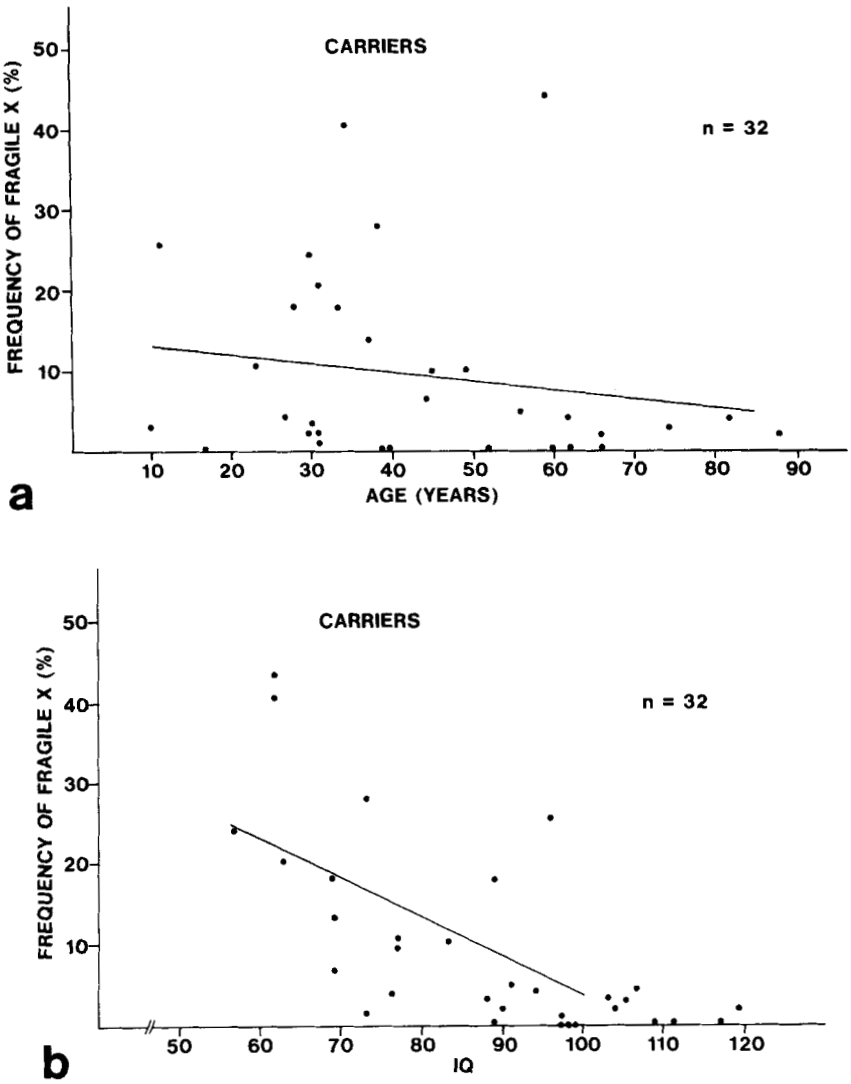

Fig. 3. a) Scatter plot with regression line in carriers: fra(X) expression vs age. $y = -0.13786x + 15.481$. Partial correlation: $r = -0.41$, $p < 0.05$; b) scatter plot with regression line in carriers: fra(X) expression vs IQ: $y = -0.47156x + 50.957$. Partial correlation: $r = -0.73$, $p < 0.001$.

expression of the fra(X) in females. It therefore appears that, at least in carrier females, intelligence has a greater relationship with expression of the fra(X) than does age.

DISCUSSION

This is the first study of a substantial sample to assess the relationship between expression of the fra(X), IQ scores, and age in hemizygotes and carriers. We have confirmed the impressions of others that there appears to be a relationship between increasing age and higher IQ and a decreasing expression of fra(X) in carriers [Jacobs et al, 1980; Turner et al, 1980a; Turner et al, 1980b]. Intelligence of carriers is more highly associated with a lower frequency of fra(X) expression than age per se, although this should be accepted with some caution since mean age of the carriers was relatively high. What came as a surprise was the apparent inverse relationship between expression of the fra(X) with age as well as with IQ in affected males. It would be important to have this finding confirmed by other investigators.

Jacobs et al [1980] proposed two hypotheses to explain the age effect of fra(X) expression. The first hypothesis states that the fragile site is more easily detectable when it is on the active X. There is a gradual selection in peripheral blood over time for cells in which the fra(X) is inactivated thereby leading to a lower frequency of fra(X) in older carriers. Although this is an attractive proposal, several studies looking at inactivation of X chromosome in fra(X) MR families have failed to provide direct supportive evidence [Lubs, 1969; Jacobs et al, 1980; Martin et al, 1980].

Jacobs' second hypothesis states that the fragile site is susceptible to breakage with subsequent loss of band (X)(q28) resulting in cell death when the site is on the active X chromosome, but not when it is on the inactive X. This hypothesis would predict a reduction in expression of the fra(X) with time in heterozygotes but not in the hemizygotes. Assuming that our data in males are real and that the relationship between age and fra(X) frequency can be confirmed, Jacobs' second hypothesis can be rejected. We would agree with Jacobs et al [1980] that high-resolution banding on extended chromosome preparations would be invaluable in looking for terminal X-chromosome deletions in affected males.

Despite a clear relationship between age, IQ, and expression of fra(X), the more intelligent males do not necessarily have the lowest expression of the fra(X); the reverse is also not necessarily true. Therefore, the fra(X) frequency cannot be used as an accurate prediction of intelligence. This is also true in females although, in general, the older and more intelligent carriers tend to have lower fra(X) frequencies. It would be important to look at the relationship between expression of fra(X), age, and IQ in skin fibroblast preparations in both males and females.

A rather high proportion of our carrier females was noted to be of borderline intelligence or to be mildly mentally retarded (37.5% had an IQ of less than 80; 22% had an IQ of less than 70). This is in keeping with the report by Turner et al [1980b], and we would presume that this is the result of random X-chromosome inactivation. We cannot eliminate a selection bias when studying our carriers since the dull carriers would be more easily accessible to us than the brighter ones who would tend to leave their families to seek better opportunities. It is also possible that we were more assiduous in finding females who are described as dull by other relatives. A major bias is the fact that absence of the fra(X) in peripheral blood cultures does not exclude the carrier state.

It is probable that dull carriers have a higher proportion of active fra(X) than the brighter carriers. Recent studies in our laboratory looking at X-chromosome inactivation in carriers from fra(X) MR families seem to bear this out. Other reasons for the wide range in IQs among the heterozygotes and hemizygotes probably reflect other modifying genetic and/or environmental factor(s) (eg, perhaps increased birth weight in hemizygotes and heterozygotes increasing the risk of birth asphyxia, variable dietary intake of folic acid, folic acid reduction with anticonvulsant therapy). The fact that IQ appears to be correlated to the proportion of fra(X) (which has been confirmed in the heterozygotes) suggests: 1) the modifying factors coincidentally affect both IQ and expression of fra(X); or, the more likely explanation, 2) the fra(X) is a direct and intimate reflection of the underlying biochemical disturbance which leads to a lowering of the IQ, and any modifying influence that enhances or reduces the expression of the fra(X) may also result in an alteration in cerebral function.

The finding of possible sex-chromosome aneuploidy in one of our affected males and our previous detection of an affected male who also had the Down syndrome (DIV-72) [Dunn et al, 1963; Jacobs et al, 1980], and another unaffected individual in one of our families with 45,X chromosome constitution (PII-3) suggests that there is an excess of nondisjunction in fra(X) MR families. Further reports will be necessary to confirm this relationship.

We have confirmed an enhanced effect of fra(X) expression in carriers through folic acid- and thymidine-reduction, and with the use of FUdR. We recommend that both males and females at risk be studied as young as possible and that enhancing factors be routinely employed in the culture media.

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REFERENCES

Daker MG, Chidiac P, Fear CN, Berry AC (1981): Fragile X in a normal male: A cautionary tale. Lancet i:780.

Dunn HG, Renpenning H, Gerrard JW, Miller JR, Tabata T, Federoff S (1963): Mental retardation as a sex-linked defect. Am J Ment Defic 67:827–848.

Gardner AP, Howell RT, McDermott A (1982): Fragile X chromosome, consistent demonstration of fragile site in fibroblast cultures. Lancet i:101.

Gerald PS (1981): X-linked mental retardation and the fragile X syndrome. Pediatrics 68:594–595.

Giraud F, Ayme S, Mattei JF, Mattei MG (1976): Constitutional chromosomal breakage. Hum Genet 34:125–136.

Glover TW (1981): FUdR induction of the X chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. Am J Hum Genet 33:234–242.

Harvey J, Judge C, Wiener S (1977): Familial X-linked mental retardation with an X chromosome abnormality. J Med Genet 14:46–50.

Howard-Peebles PN, Howell WM (1979): Mental retardation, marker-X chromosomes, and silver staining (NOR's). Cytogenet Cell Genet 23:277–278.

Howard-Peebles PN, Stoddard GR (1979): X-linked mental retardation with macro-orchidism and marker-X chromosomes. Hum Genet 50:247–251.

Howard-Peebles PN, Pryor JC, Stoddard GR (1980): X-linked mental retardation, the fragile site in Xq and the role of methionine. Am J Hum Genet 32:73A.

Jacky PB, Dill FJ (1980): Expression in fibroblast culture of the satellited-X chromosome associated with familial sex-linked mental retardation. Hum Genet 53:267–269.

Jacobs PA, Glover TW, Mayer M, Fox P, Gerrard JW, Dunn HG, Herbst DS (1980): X-linked mental retardation: A study of 7 families. Am J Med Genet 7:471–489.

Jenkins EC, Brown WT, Duncan CJ, Brooks J, Ben-Yishay M, Giordano FM, Nitowsky HM (1981): Feasibility of fragile X chromosome prenatal diagnosis demonstrated. Lancet ii; 1292.

Jennings M, Hall JG, Hoehn H (1980): Significance of phenotypic and chromosomal abnormalities in X-linked mental retardation (Martin-Bell or Renpenning syndrome). Am J Med Genet 7:417–432.

Lejeune J (1982): Is the fragile X syndrome amenable to treatment? Lancet i: 273–274.

Lejeune J, Maunowry C, Rethoré MO, Prieur M, Raoul O (1981): Site fragile Xq27 et métabolisme des monocarbones: Diminution significative de la frequence de la lacune chromosomique par traitment in vitro et in vivo. CR Acad Sci Paris 292 (ser 111):491–493.

Lubs HA (1969): A marker X chromosome. Am J Hum Genet 21:231–244.

Martin RH, Lin CC, Mathies BJ, Lowry RB (1980): X-linked mental retardation with macro-orchidism and marker-X chromosomes. Am J Med Genet 7:433–441.

Popovich BW, Rosenblatt DS, Vekemans M, Cooper BA (1980): Intracellular folate levels in fibroblasts from patients with X-linked mental retardation. Am J Hum Genet 32:84A.

Popovich B, Vekemans M, Rosenblatt D, Monroe P (1982): Fragile X (letter). N Engl J Med 306:1551–1552.

Schmidt A, Passarge E (1981): Cytogenetic and phenotypic manifestations in heterozygotes for an X-chromosome with a fragile site (abst). Clin Genet 20:391.

Shapiro LR, Wilmot PL, Brenholz P, Leff A, Martino M, Harris G, Mahoney MJ, Hobbins JC (1982): Prenatal diagnosis of fragile X chromosome. Lancet i:99–100.

Sutherland GR (1977): Fragile sites on human chromosomes: Demonstration of their dependence on the type of tissue culture medium. Science 197:265–266.

Sutherland GR, Ashford PLC (1979): X-linked mental retardation with macro-orchidism and the fragile site on Xq27 or 28. Hum Genet 48:117–120.

Tommerup N, Paulsen H, Brondum-Nielsen K (1981a): 5-fluoro-2′-deoxyuridine induction of the fragile site on Xq28 associated with X linked mental retardation. J Med Genet 18:374–376.

Tommerup N, Nielsen KB, Mikkelsen M (1981b): Marker X chromosome induction in fibroblasts by FUdR. Am J Med Genet 9:263–264.

Turner G, Eastman C, Casey J, McLeary A, Procopis P, Turner B (1975): X-linked mental retardation associated with macro-orchidism. J Med Genet 12:367–371.

Turner G, Daniel A, Frost M (1980a): X-linked mental retardation, macro-orchidism and the Xq27 fragile site. J Pediatr 96:837–841.

Turner G, Brookwell R, Daniel A, Selikowitz M, Zilibowitz M (1980b): Heterozygous expression of X-linked mental retardation and X-chromosome marker fra(X) (q27). N Engl J Med 303:662–664.

Turner G, Opitz JM (1980): Editorial comment: X-linked mental retardation. Am J Med Genet 7:407–415.

Webb GC: Rogers JG, Pitt DB, Holliday J, Theobald T (1981): Transmission of fragile (X) (q27) site from a male. Lancet ii:1231–1232.

Webb T, Butler D, Insley J, Weaver JB, Gree S, Rodeck C (1981): Prenatal diagnosis of Martin-Bell Syndrome associated with fragile site at Xq27–28. Lancet ii:1423.

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