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Mental Status of Females with an FMR1 Gene Full Mutation

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Summary

The cloning of the FMR1 gene enables molecular diagnosis in patients and in carriers (male and female) of this X-linked mental retardation disorder. Unlike most X-linked disorders, a considerable proportion of the female carriers of a full mutation in the FMR1 gene is affected. In this study, the intelligence quotients (IQs) were ascertained by the Wechsler Adult Intelligence Scale in 33 adult females with a full mutation, with 28 first-degree adult female relatives (mainly sisters) without a full mutation as controls. Seventy-one percent of the females with a full mutation had IQ scores below 85. In paired analysis, no significant correlation could be detected between the IQs of the females with a full mutation and those of their first-degree female relatives, reflecting a dominant effect of the FMR1 gene full mutation in the mental development of females. Considering females with a full mutation only, we observed a significant relation between the proportion of normal FMR1 alleles on the active X chromosome and IQ. We present a model to explain this relationship.

Introduction

The fragile X syndrome is the most common single cause of inherited mental retardation, affecting (according to earlier estimates) $\pm 1/1,250$ males and $\pm 1/2,000$ females (Webb et al. 1986). Recently, a lower incidence was reported of $\pm 1/4,000$ for males (Turner et al., in press). A fraction of the X-chromosomes in affected males show, on cytogenetic testing, a fragile site at Xq27 when

cells are cultured in a folic acid-deficient medium (Lubs 1969; Sutherland 1977).

The cloning of the FMR1 gene enabled direct DNA diagnosis in both male and female fragile X patients (Fu et al. 1991; Oberlé et al. 1991; Verkerk et al. 1991; Yu et al. 1991). The FMR1 gene contains a variably sized CGG trinucleotide repeat at the 5' end, which has a length of between 6 and 53 repeats in normal individuals. Patients have >200 CGG repeats, the so-called full mutation, which is associated with methylation of a CpG island upstream of the FMR1 gene and with absence of gene transcription (Oberlé et al. 1991; Pieretti et al. 1991). Normal carrier males and females, with an exception for normal females with a full mutation, have an intermediate repeat number of between 43 and 200, the so-called premutation, with an unmethylated CpG island in front of the FMR1 gene and normal transcription (Pieretti et al. 1991).

The fragile X syndrome is transmitted as an X-linked semidominant disorder, since males and females may be affected. In earlier studies, the cognitive profile in females with the fragile X syndrome has been related to fragile X chromosome expression. Some groups found a significantly negative relationship between the percentage of fragile X expression and intelligence quotient (IQ) (Chudley et al. 1983; Wilhelm et al. 1988). Others found no such relation between IQ and fragility (taking $\geq 2\%$ of fragile X cells as evidence for the carrier status) (Cronister et al. 1991).

Before the cloning of the gene, the reported incidence of intellectual deficits (IQ < 85) among heterozygous females ranged from 35% to 53% (Sherman et al. 1985; Hagerman et al. 1992). Besides the limitations of cytogenetic methods for the diagnosis of heterozygosity in females, most of those earlier studies also lacked an adequate control group. Recent studies based on DNA mutation analysis show mental impairment in 52%–82% of the females with a full mutation (Rousseau et al. 1991a, 1991b; Smits et al. 1994; Taylor et al. 1994), whereas Reiss et al. (1993) showed that the premutation in females did not affect their intellectual development.

Molecular analysis of the FMR1 gene in carrier fe-

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males enabled a reliable determination of X-inactivation. A relation between the ratio of active normal X chromosomes to normal inactive X chromosomes and full-scale IQ was reported (Rousseau et al. 1991b; Abrams et al. 1994). Abrams et al. (1994) reported a relation between the size of the FMR1 gene mutation and IQ, which had also been observed in a multicenter study by Rousseau et al. (1994). However, another study could not confirm this observation (Taylor et al. 1994).

We report the IQs and profiles in females with the full FMR1 mutation in comparison with their first-degree female relatives without a full mutation. Second, the size of the full mutation and the proportion of normal active X chromosomes in leukocytes were studied in relation to IQ and age of the females with a full mutation.

Subjects, Material, and Methods

Subjects and Controls

Thirty-three females with a full mutation in the FMR1 gene, aged 20–70 years (mean 39.7 ± 14.4 years), from 23 fragile X families were included. All families were ascertained through a male index patient (usually a first-degree relative) and were studied after informed consent was obtained. The study was approved by the Medical Ethical Committee of the University Hospital, Rotterdam. One female did not want to participate (not counted). Twenty-one females were paired to a sister with a normal FMR1 genotype (aged 22–72 years, mean 41.3 ± 14.5 years). In case more female control sibs were available, the female with the best age match was included in the study. In the absence of a control sister, the mother with a premutation was included as a control ($n = 7$; aged 48–68 years, mean 54.7 ± 7.1 years). For five females with a full mutation from large sibships, a unique control was not available, because every control had only been used once. Those five females were excluded from paired analysis (leaving 28 pairs) but were included in the molecular association studies.

Determination of IQ Levels

In all 61 females, the IQ and profile were ascertained by using the Dutch version of the Wechsler Adult Intelligence Scale. (Stinissen et al. 1970). IQ testing was performed at the home of females by one examiner (A.W.) who was not informed about the genetic status of the women.

DNA Analysis

The intragenic DNA probe pP2 was used for DNA analysis of the FMR1 gene (Oostra et al. 1993). Genomic DNA was isolated from blood leukocytes (Miller et al. 1988). DNA (8 μ g) was digested to completion with the restriction enzymes *Hind*III and *Eag*I according to

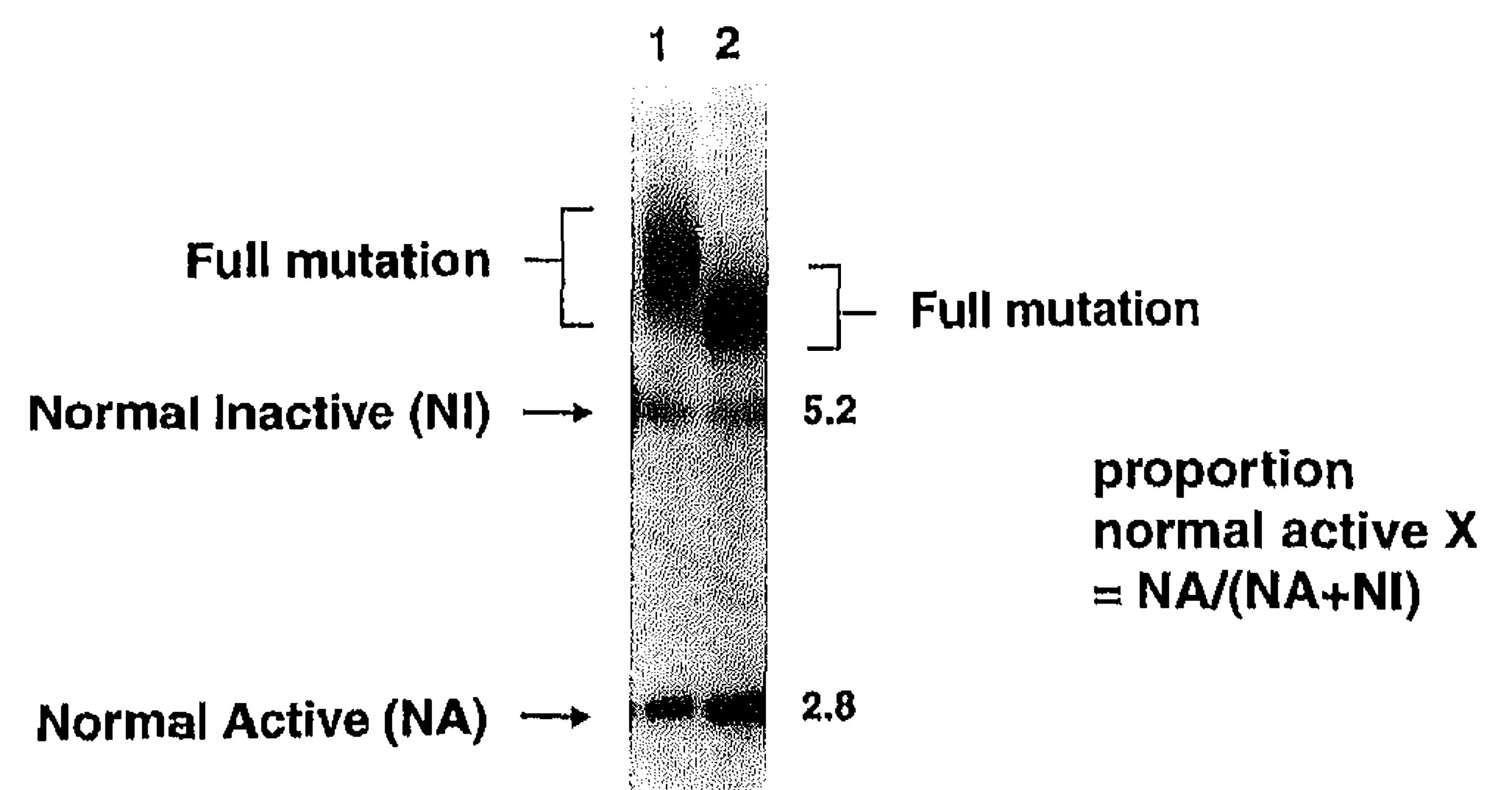


Figure 1 Analysis with probe pP2 of *Hind*III- and *Eag*I-digested DNA of two females with a full mutation, reflecting the proportion of normal FMR1 alleles on the active X chromosomes (NA) and normal FMR1 alleles on the inactive X chromosomes (NI).

the manufacturer's instructions, separated by gel electrophoresis, and subjected to Southern blot analysis according to standard procedures (Sambrook et al. 1989). The probe was labeled by the random oligonucleotide priming method (Feinberg and Vogelstein 1983). After prehybridization and hybridization, the filters were washed to $0.1 \times$ SSC at 65°C prior to autoradiography (Sambrook et al. 1989). Several autoradiograms (2–3) with different exposure times were made.

The size of the full mutation was estimated by averaging the smallest and largest detectable expansion. Sizes were estimated by determining the beginning and the end of the smear on Southern blot in carriers of the full mutation (fig. 1).

The percentages of normal FMR1 alleles on the active X chromosomes (NA) and normal FMR1 alleles on the inactive X chromosomes (NI) were ascertained by densitometry using a scanner (HP, scanjet IICX). The proportion of normal FMR1 alleles on the active X chromosome (NA) versus normal FMR1 alleles on the inactive X chromosome (NI) was calculated using the equation: $\text{NA}/(\text{NA} + \text{NI})$ (fig. 1) (Rousseau et al. 1991b).

In females with a mosaic pattern of the FMR1 gene mutation (full mutation with an additional premutation), estimation of the size of the expansion and the proportion of normal active X will be hampered by the additional premutation. Therefore, females with a premutation in addition to the full mutation ($n = 6$) were excluded from the comparison of the size of the full mutation and the X-inactivation ratio with IQ scores.

Data Analysis

A paired *t*-test for continuous data was applied to test for significant differences between the female carriers of the full mutation and their controls. The bivariate relationship between variables was estimated by means of Pearson's product-moment correlation coefficient (*r*).

Table 1

Summary of the IQ Data of the Subjects and Controls

	SUBJECTS				PAIRED CONTROL FIRST-DEGREE RELATIVES			
	FM ^a Only (<i>n</i> = 27)		PM ^b + FM ^a (<i>n</i> = 6)		N ^c (<i>n</i> = 21)		PM ^b (<i>n</i> = 7)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	39.1	13.7	42.3	18.1	41.3	14.5	52.3	7.1
FSIQ	74.1	18.4	84.2	26.8	116.0	18.4	111.6	22.5
PIQ	81.9	19.7	90.0	28.9	119.1	18.8	111.4	23.7
VIQ	72.3	16.3	82.2	22.1	111.2	18.0	110.3	19.2
Proportion normal active X	.71	.17

^a Full-mutation FMR1 gene.^b Premutation FMR1 gene.^c Normal FMR1 gene.

This correlation coefficient was considered to be an indicator of relative importance, while the corresponding *P* value represents the level of significance statistically. Adjusting for age, the partial correlation coefficient was the indicator of relative importance.

Results

IQ and Profile in Females with the Full Mutation versus Controls

The mean full-scale IQ (FSIQ), performance IQ (PIQ), and verbal IQ (VIQ) of the different groups studied are shown in table 1. The 28 female carriers of a full mutation had significantly lower mean FSIQ, PIQ, and VIQ scores than their paired controls with a normal FMR1 gene or with a premutation (for all means $P < .001$, paired-sample *t*-test).

There was no significant difference of FSIQ between the females with a full mutation only ($n = 27$) and the females with a full mutation and an additional premutation, the so-called mosaics ($n = 6$) ($r = .22$; $P = .21$; table 1). Also, no significant difference of FSIQ, PIQ, and VIQ was observed between the females with a normal FMR1 genotype and the females with a premutation ($r = -.1$, $P = .61$; $r = -.17$, $P = .39$, respectively, $r = -.02$, $P = .9$).

Paired Analysis: Females with a Full Mutation Compared with the Controls

In figure 2, each pair (female with a full mutation and control) and each female's IQ are shown. In the group of 28 pairs, 20 (71%) of 28 carriers of the full mutation had an IQ < 85 points. When the more strict criteria for mental retardation (IQ < 70 points) was used, 14 (50%) of 28 carriers of the full mutation were mentally retarded, whereas none of the control females had an IQ < 70 points.

Sixteen (57%) of 28 carriers of the full mutation scored >30 IQ points (2 SD) lower than their control. This ratio remained nearly unchanged when only the 21 sister-sister pairs are considered (62%). Remarkably, two females with the full mutation had a higher IQ than did their controls (fig. 2). No significant correlation could be detected between the FSIQ of the females with the full mutation and the FSIQ of the controls ($r = .22$; $P = .27$). Likewise, there was no significant correlation for the VIQ of the females with a full mutation and the VIQ of the controls ($r = .07$; $P = .74$), but the PIQ correlation was significant ($r = .39$, $P = .04$).

The Size of the FMR1 Gene Mutation and Mental Status in Females with the Full Mutation Only

For females with a full mutation, only the FSIQ, PIQ, and VIQ scores were negatively related with age (r

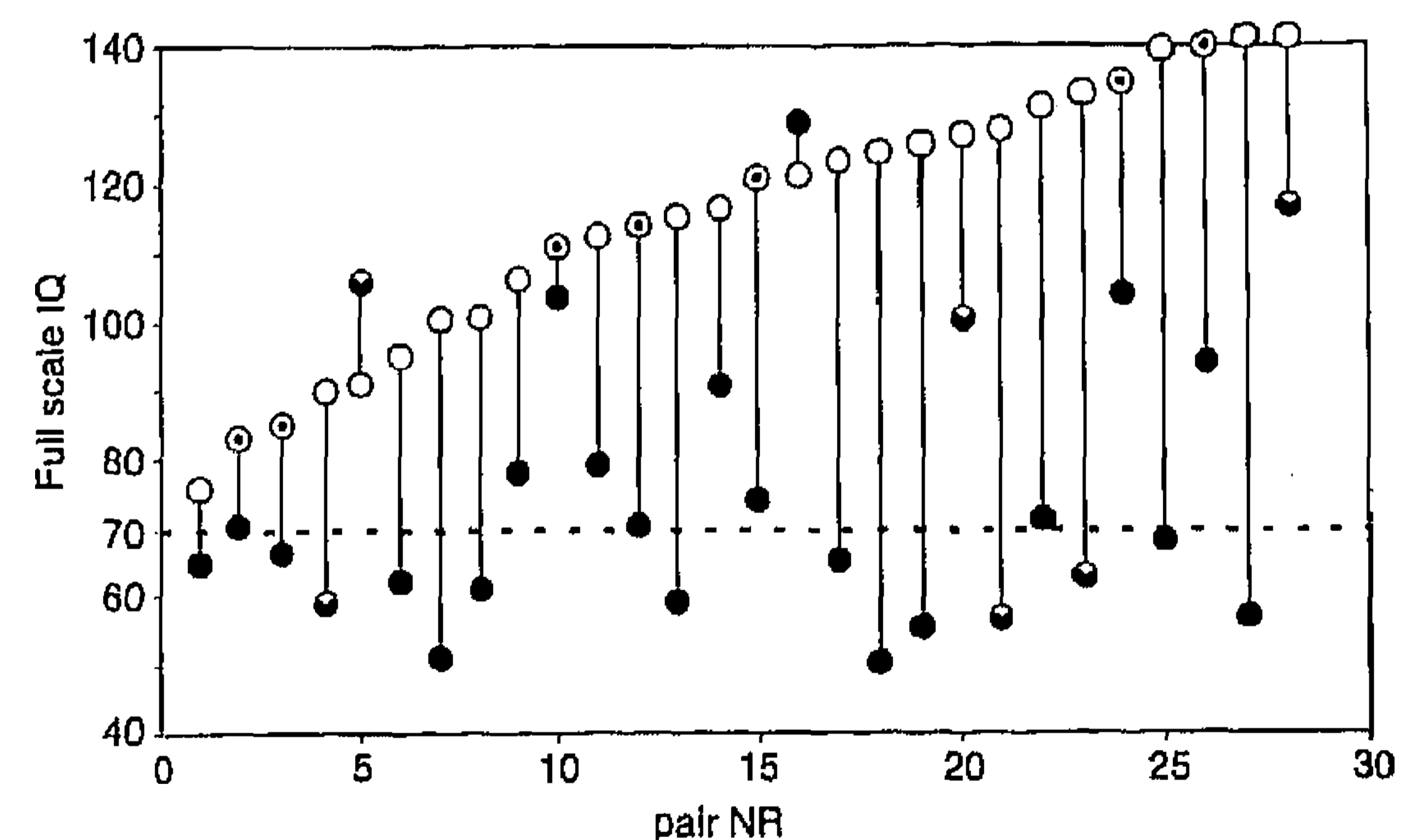


Figure 2 IQ level of the females with a full mutation (filled or 2/3-filled symbols) compared with their first-degree relatives (open symbols). Females with full mutation only are represented by filled circles, females with a premutation and a full mutation by 2/3-filled circles, sisters with normal FMR1 genes by open circles, and mothers with a premutation by open circles with a dot.

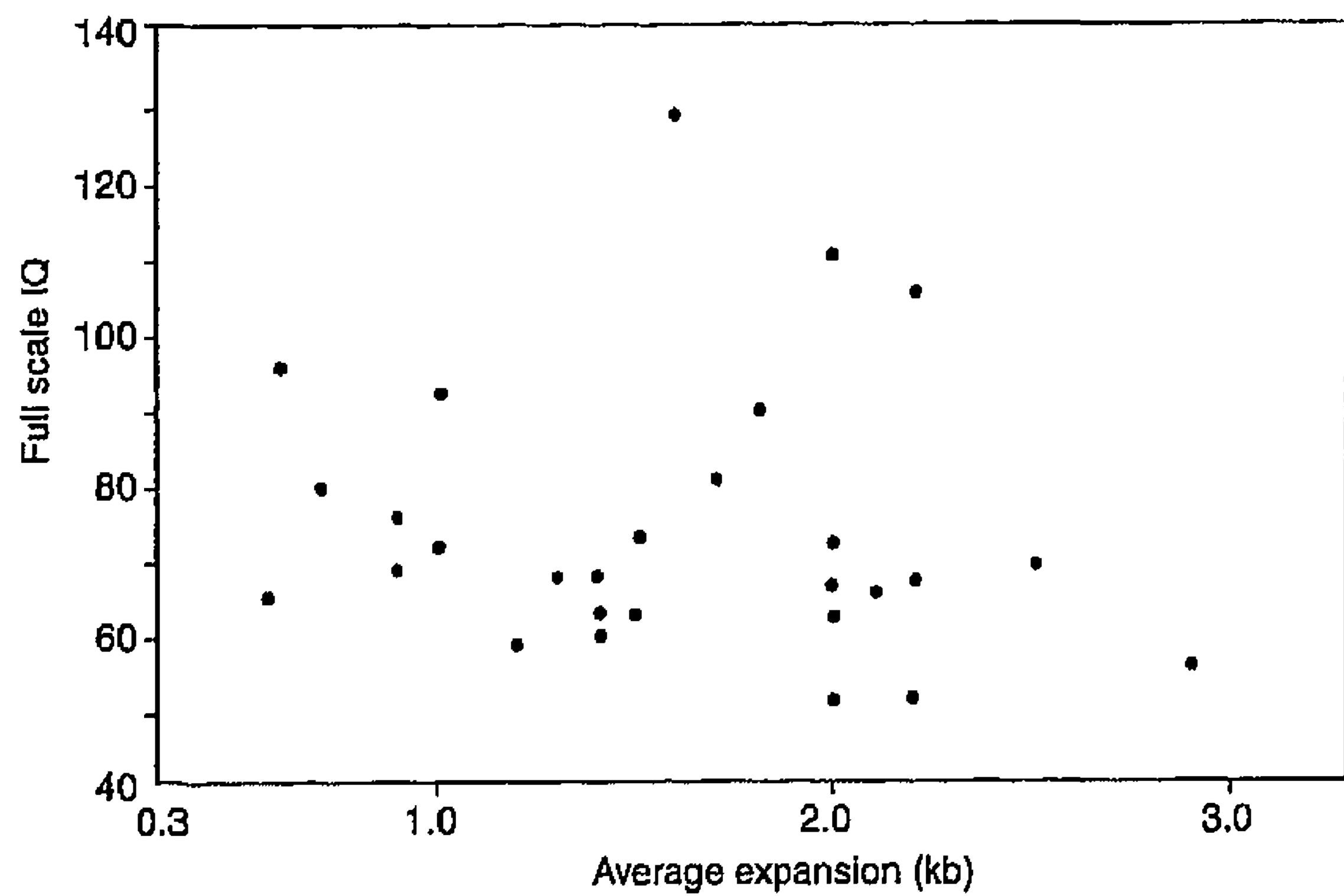


Figure 3 Average expansion size of the full mutation in the FMR1 gene related to the FSIQ in females with a full mutation only.

$r = -.43$, $P = .03$; $r = -.39$, $P = .05$; and $r = -.43$, $P = .03$, respectively). Therefore, the further data were, in addition, analyzed with adjustment for this age-dependent effect. Since the results of those analyses did not differ from the analyses without adjustment for age, we choose to present only unadjusted data.

The mean of the average sizes of the full mutation was 1.6 ± 0.6 kb ($n = 27$; females with full mutation only). No significant relation was found ($r = -.16$; $P = .42$) between average size of the full mutation and FSIQ (fig. 3), or with one of the subtests, PIQ and VIQ ($r = -.19$, $P = .34$; and $r = -.13$, $P = .52$, respectively). A similar result was observed with the smallest or largest size of the full mutation (data not shown).

The Proportion of Normal FMR1 Alleles on the Active X Chromosome and Mental Status in Females with the Full Mutation Only

The average of the proportions of the normal FMR1 alleles on the active X chromosome ($.71 \pm .17$) in females with a full mutation only, was significantly $>.5$ ($n = 27$; $P \ll .001$). As an internal control, we measured the control females with normal FMR1 genes ($n = 21$) who showed $.50 \pm .09$ X-inactivation.

In figure 4, the proportion of normal FMR1 alleles on the active X chromosome in relation to FSIQ is shown for females with a full mutation only. The (moderate) relation between the proportion of normal FMR1 alleles on the active X chromosome and FSIQ is significant ($r = .45$; $P = .02$). The relation with the proportion normal active X was significant for the PIQ ($r = .56$; $P = .003$). However, a significant relation between the proportion of normal active X and VIQ was not observed ($r = .31$; $P = .11$).

No significant relation could be detected between the proportion normal active X and age in the 27 females with a full mutation only ($r = -.14$; $P = .50$; data not shown).

Discussion

The Full Mutation in FMR1 Gene in Females Has a Dominant Effect over Other Factors Involved in Intellectual Development

In the present study, 71% (20/28) of the females with a full mutation in the FMR1 gene had IQ scores <85 , representing borderline or mild/moderate retardation. Other groups also using DNA analysis of the FMR1 gene reported lower percentages, varying from 52% to 65%, of mental impairment (IQ <85) among the females with a full mutation (Rousseau et al. 1991b, 1994; Taylor et al. 1994) (table 2), except for Smits et al. (1994) who reported 82%. However, in three of these studies, the mental development was clinically estimated without accurate IQ testing (Rousseau et al. 1991b, 1994; Smits et al. 1994).

Using strict criteria for mental retardation (i.e., IQ <70 points), 50% (14/28) of the females with a full mutation in this study were mentally retarded. This is higher than the observation of Taylor et al. (1994), who reported 22% (5/23) of the females to have an IQ <70 . Their results might be biased by the use of four different IQ tests and the low average age of the subjects they studied. Further, they could not exclude an ascertainment bias because of "unavoidable selection for motivation to come to the clinic" (p. 513), which is a major problem in several comparable studies. The present study avoided such a bias, both by selecting the families through a male index patient and by visiting the families at their homes. Although the possibility that females with a full mutation, ascertained through a male index patient, might be functioning on a different cognitive level as females with a full mutation without an affected, first-degree, male relative might be conceivable, at present no difference of this type is known.

The first-degree female relatives without a full mutation in the FMR1 gene (mainly sisters without an FMR1 gene

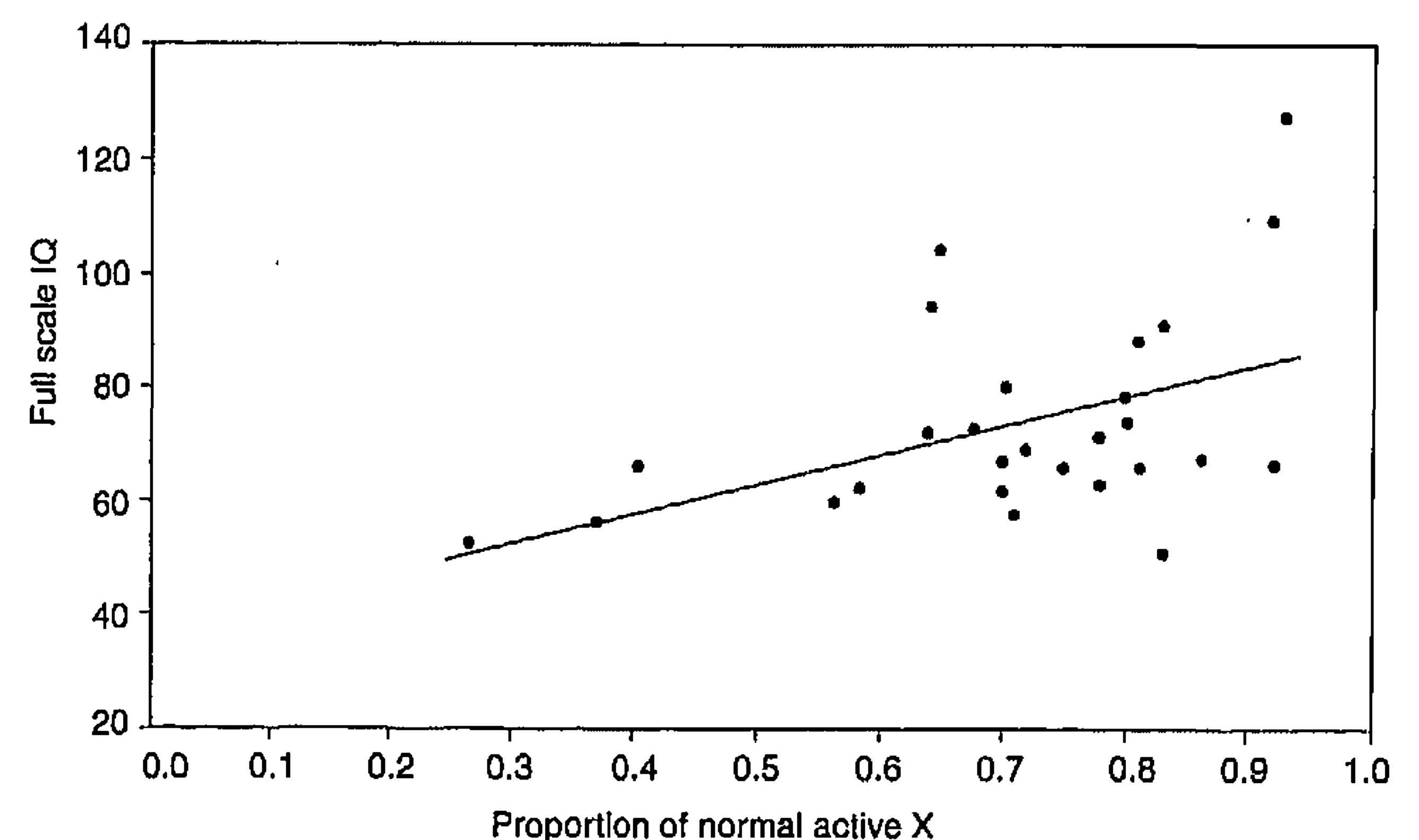


Figure 4 Proportion of normal FMR1 alleles on the active X chromosome related to FSIQ in females with a full mutation only.

Table 2**Summary of Molecular and IQ Data in Female Carriers of the Full Mutation: Overview of the Literature and the Present Study**

	N	AGE (years)	CONTROLS	IQ TEST	IQ < 85 (%)	RELATIONSHIP		
						Size of FM ^a to IQ	X inactivation ^b to IQ	X inactivation ^b to Age
Rousseau et al. (1991) (France)	27	All	Women PM ^c	Clinical estimate	52	?	Yes (+)	Yes (+)
Taylor et al. (1994) (Denver, USA)	23	3–58	Mothers	Test (4) ^d	65	No	No	Yes (+)
Abrams et al. (1994) ^e	31	4–27	Mothers (+fathers)	Test (2) ^d	?	Yes (–)	Yes (+)	No
Reiss et al. (1995) ^e (Baltimore, USA)	29	6–17	Mothers + fathers	Wechsler Intelligence Scale for Children	?	No	Yes (+)	?
Rousseau et al. (1994) (collaborative)	170	All	Women N ^f and PM ^c	Clinical estimate	59	Yes (–)	No	Yes (+)
Present study	33	20–70	Sisters (+mothers)	Weschler Adult Intelligence Scale	71	No	Yes (+)	No ^g

NOTE.—Minus sign (–) or plus sign (+) correspond to negative or positive correlation, respectively.

^a Full-mutation FMR1 gene.

^b Proportion of normal active X, as described by Rousseau et al. (1991b).

^c Premutation FMR1 gene.

^d Number of different IQ tests used.

^e Study groups partly overlap.

^f Normal FMR1 gene.

^g Adults only.

mutation) served as references in this study, allowing an intrafamilial assessment of the effect of the full mutation. The effect of the full FMR1 mutation is reflected in the IQ difference between females with the full mutation and their controls. Fifty-seven percent (16/28) of the women with a full mutation scored significantly lower (>30 points) than their control, which indicates a dominant effect of the full mutation in the FMR1 gene over shared environmental and genetic factors involved in mental development. This is supported by the observation that both FSIQ and VIQ of the females with a full mutation were uncorrelated to the controls' FSIQ and VIQ. It is remarkable that a significant relation between PIQ of females with a full mutation and their controls was found.

Molecular Characteristics of the Full Mutation and the Mental Status

The full mutation in the FMR1 gene in females can be characterized by different features, such as size of the mutation and localization on the active or inactive X chromosome. No relation between the size of the full FMR1 mutation, whether characterized by smallest, average, or largest size, and the IQ level of the females was observed in this study. These findings are inconsistent with earlier reports by Abrams et al. (1994) and

Rousseau et al. (1994), but they are in line with the reports from other groups (Rousseau et al. 1991b; Taylor et al. 1994; Reiss et al. 1995) (table 2). Because a relation between size and IQ level could not be detected in males (De Vries et al. 1993; Loesch et al. 1993), it would be unlikely to observe such relationship in females. This corresponds with the characteristics of the full mutation in the FMR1 gene. Independent of the size of a full mutation, the full mutation is associated with methylation of a CpG island in front of the FMR1 gene, leading to an absence of FMR1 protein.

As in other X-linked disorders, lyonization may influence the phenotypic expression of the normal allele in females with a full mutation in the FMR1 gene. Earlier studies using cytogenetic techniques in lymphocytes explored whether the normal X chromosome is more frequently the active (or early replicating) X chromosome in carrier females with a normal intelligence compared to carriers with an intellectual impairment. Although some studies confirmed the relation between a high percentage of normal active X chromosomes and normal intelligence (Knoll et al. 1984; Paul et al. 1984; Wilhelm et al. 1988), others did not (Nielsen et al. 1983; Fryns et al. 1984). With molecular techniques, the proportion of normal FMR1 alleles on the active X chromosome

can be estimated more accurately by using methylation assays. The cells with normal active FMR1 alleles are actually capable of producing FMR1 protein. In the 27 females with a full mutation only, the normal FMR1 alleles were significantly more frequent on the active X chromosome in intellectually higher-functioning females than in females with a mental impairment. This confirms earlier findings (Abrams et al. 1994; Rousseau et al. 1991*b*; Reiss et al. 1995) but contradicts two other studies (Rousseau et al. 1994; Taylor et al. 1994) (table 2). In the study by Rousseau et al. (1994), the intelligence level was clinically estimated without specific IQ testing, and therefore the relation might have been missed. Taylor et al. (1994) included females with a mosaic pattern (premutation and full mutation). Although we did not observe a difference of the FSIQ between females with a full mutation versus mosaics, the effects of an additional premutation on the phenotype have not been fully assessed in larger controlled studies. Therefore, including "mosaic" females might influence the results. Another reason for excluding "mosaic" females is the inability to establish the percentage of normal active X chromosomes accurately in these cases.

We observed an age-independent, skewed X inactivation in leukocytes favoring the X-chromosomes with normal FMR1 alleles over those with a full-mutation FMR1 allele in females both with normal and subnormal intelligence level. This suggests a positive selection for leukocytes able to produce FMR1 protein earlier in life. Some groups (Rousseau et al. 1991*b*, 1994; Taylor et al. 1994) observed an age-dependent selection in leukocytes: a higher proportion of active X chromosomes carrying the normal FMR1 gene in elderly women with a full mutation compared to younger females. Because only adults were included in the present study, we could not observe such an age-dependent selection.

In each cell, the X inactivation occurs at random in the late blastocyst stage (Nesbitt 1971; McMahan et al. 1983; Lyon 1994). The precursor cells for both brain and blood formation are the embryonic ectodermal cells, which undergo X inactivation around the 2d wk of embryonic development. The X-inactivation distribution is rather consistently equal when different tissues within one individual are compared, both in mice and men (Nesbitt 1971; Fialkow 1973, McMahan et al. 1983; Kolehmainen and Karant 1994). Because we observed females with a high proportion of normal FMR1 alleles (>.80) with either a normal or a subnormal IQ level, the currently observed relation is likely influenced by the age-dependent selection for normal FMR1 expression in leukocytes. We hypothesize that the proportion of inactivation of the normal X chromosome at the late blastocyst stage in some individuals is <.5 and in others >.5. Individuals with a lower proportion of normal ac-

tive X chromosome in the brain will have subnormal intelligence, while individuals with a higher proportion will be normal. There will be a relation between the proportion of normal FMR1 alleles on the active X chromosome in the brain and IQ. In leukocytes, however, there is selection for normal X chromosomes, leading to a change in the proportion of normal X chromosomes. This change is more likely to be higher in individuals with a low proportion of normal X chromosomes, disturbing the relationship between proportion of normal alleles and IQ. The presently observed relation between a higher proportion of normal FMR1 alleles on the active X chromosomes in leukocytes and a higher intelligence in females with a full mutation might still be a reflection of the distribution of X inactivation in brain cells. To prove this hypothesis, we propose to study the X-inactivation pattern in other tissues that might lack this selection phenomenon. Studies on protein expression in neuronal cells of either females with a full mutation or heterozygous knock-out mice might give the relevant clues.

The currently observed relation between molecular parameters and the FSIQ were also significant for the subcomponent PIQ but not for VIQ. This is in line with a recent report about girls with a full mutation, in which a stronger relation between the proportion of normal active X was suggested for PIQ than for VIQ (Reiss et al. 1995). Since the PIQ is less influenced by cultural factors than is the VIQ (Cattel 1963; Horn 1968), the PIQ seems preferable over the VIQ and FSIQ as an indicator of mental developmental capabilities in molecular-cognitive association studies.

The mental status in females with the full mutation is influenced by several factors. Besides the full mutation in the FMR1 gene, other genetic and environmental factors may play, to an unknown extent, a role in intellectual development. In this first study of comparing full mutation FMR1 female carriers with their noncarrier sisters, those shared factors were less significant, reflecting a dominant effect of the FMR1 gene full mutation on mental development in a majority of females with a full mutation. This finding will be relevant in the genetic counseling of carriers of (pre-)mutations of the fragile X syndrome. X-inactivation studies during prenatal diagnosis in female pregnancies with a full FMR1 mutation will not allow a reliable prognosis of the intellectual development of such a child. Henceforth, parents will have to decide on the general risk estimates for intellectual impairment in female pregnancies with a full mutation as observed in this study.

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References

- Abrams MT, Reiss AL, Freund LS, Baumgardner TL, Chase GA, Denckla MB (1994) Molecular-neurobehavioral associations in females with the fragile X full mutation. *Am J Med Genet* 51:317-327
- Cattell RB (1963) Theory of fluid and crystallized intelligence: a critical experiment. *J Educ Psychol* 54:1-22
- Chudley AE, Knoll J, Gerrard JW, Shepel L, McGahey E, Anderson J (1983) Fragile (X) X-linked mental retardation I: relationship between age and intelligence and the frequency of expression of fragile (X)(q28). *Am J Med Genet* 14:699-712
- Cronister A, Schreiner R, Wittenberger M, Amiri K, Harris K, Hagerman RJ (1991) Heterozygous fragile X female: historical, physical, cognitive, and cytogenetic features. *Am J Med Genet* 38:269-274
- de Vries BBA, Wiegers AM, De Graaff E, Verkerk AJMH, Van Hemel JO, Halley DJJ, Fryns JP, et al (1993) Mental status and fragile X expression in relation to FMR-1 gene mutation. *Eur J Hum Genet* 1:72-79
- Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132:6-13
- Fialkow PJ (1973) Primordial cell pool size and lineage relationships of five human celltypes. *Ann Hum Genet* 37:39-48
- Fryns JP, Kleczkowska A, Kubien E, Petit P, Van den Berghe H (1984) Inactivation pattern of the fragile X in heterozygous carriers. *Hum Genet* 65:400-401
- Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, et al (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 67:1047-1058
- Hagerman RJ, Jackson C, Amiri K, Silverman AC, O'Connor R, Sobesky W (1992) Girls with fragile X syndrome: physical and neurocognitive status and outcome. *Paediatrics* 89:395-400
- Horn JL (1968) Organization of abilities and the development of intelligence. *Psychol Rev* 75:242-259
- Knoll JH, Chudley AE, Gerrard JW (1984) Fragile (X) X-linked mental retardation. II. Frequency and replication pattern of fragile (X)(q28) in heterozygotes. *Am J Hum Genet* 36:640-645
- Kolehmainen K, Karant Y (1994) Modelling methylation and IQ scores in fragile X females and mosaic males. *Am J Med Genet* 51:328-338
- Loesch DZ, Huggins R, Hay DA, Gedeon AK, Mulley JC, Sutherland GR (1993) Genotype-phenotype relationships in fragile X syndrome: a family study. *Am J Hum Genet* 53:1064-1073
- Lubs HA (1969) A marker X chromosome. *Am J Hum Genet* 21:231-244
- Lyon MF (1994) The X inactivation centre and X chromosome imprinting. *Eur J Hum Genet* 2:255-261
- McMahon A, Fosten M, Monk M (1983) X-chromosome inactivation mosaicism in the three germ layers and the germ line of the mouse embryo. *J Embryol Exp Morphol* 74:207-220
- Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1214
- Nesbitt MN (1971) X chromosome inactivation mosaicism in the mouse. *Dev Biol* 26:252-263
- Nielsen KB, Tommerup N, Poulsen H, Jacobsen P, Beck B, Mikkelsen M (1983) Carrier detection and X-inactivation studies in the fragile X syndrome: cytogenetic studies in 63 obligate and potential carriers of the fragile X. *Hum Genet* 64:240-245
- Oberlé I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, et al (1991) Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 252:1097-1102
- Oostra BA, Jacky PB, Brown WT, Rousseau F (1993) Guidelines for the diagnosis of fragile X syndrome. *J Med Genet* 30:410-413
- Paul J, Froster-Iskenius U, Moje W, Schwinger E (1984) Heterozygous female carriers of the marker-X-chromosome: IQ estimation and replication status of fra(X)(q). *Hum Genet* 66:344-346
- Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991) Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 66:817-822
- Reiss AL, Freund L, Abrams MT, Boehm C, Kazazian H (1993) Neurobehavioral effects of the fragile X premutation in adult women: a controlled study. *Am J Hum Genet* 52:884-894
- Reiss AL, Freund LS, Baumgardner TL, Abrams MT, Denckla MB (1995) Contribution of the FMR1 gene mutation to intellectual dysfunction. *Nat Genet* 11:331-334.
- Rousseau F, Heitz D, Biancalana V, Blumenfeld S, Kretz C, Boue J, Tommerup N, et al (1991a) Direct diagnosis by DNA analysis of the fragile X syndrome of mental retardation [see comments]. *N Engl J Med* 325:1673-1681
- Rousseau F, Heitz D, Oberlé I, Mandel JL (1991b) Selection in blood cells from female carriers of the fragile X syndrome: inverse correlation between age and proportion of active X chromosomes carrying the full mutation. *J Med Genet* 28:830-836
- Rousseau F, Heitz D, Tarleton J, MacPherson J, Malmgren H, Dahl N, Barnicoat A, et al (1994) A multicenter study on genotype-phenotype correlations in the fragile X syndrome, using direct diagnosis with probe StB12.3: the first 2,253 cases. *Am J Hum Genet* 55:225-237
- Sambrook J, Fritsch EF, Maniatis T (eds) (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Sherman SL, Jacobs PA, Morton NE, Froster-Iskenius U, Howard-Peebles PN, Nielsen KB, Partington MW, et al (1985) Further segregation analysis of the fragile X syndrome with special reference to transmitting males. *Hum Genet* 69:289-299
- Smits A, Smeets D, Hamel B, Dreesen J, de Haan A, Oost B (1994) Prediction of mental status in carriers of the fragile

- X mutation using CGG repeat length. *Am J Med Genet* 51:497-500
- Stinissen J, Willems PJ, Coetsier P, Hulsman WLL (1970) Handleiding bij de Nederlandstalige bewerking van de Wechsler Adult Intelligence Scale (W.A.I.S.). Swets and Zeitlinger, Lisse
- Sutherland GR (1977) Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium. *Science* 197:265-266
- Taylor AK, Safanda JF, Fall MZ, Quince C, Lang KA, Hull CE, Carpenter I, et al (1994) Molecular predictors of cognitive involvement in female carriers of fragile X syndrome [see comments]. *JAMA* 271:507-514
- Turner G, Webb T, Wake S, Robinson H. The prevalence of the fragile X syndrome. *Am J Med Genet* (in press)
- Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, Reiner O, et al (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65:905-914
- Webb TP, Bunday S, Thake A, Todd J (1986) The frequency of the fragile X chromosome among schoolchildren in Coventry. *J Med Genet* 23:396-399
- Wilhelm D, Froster-Iskenius U, Paul J, Schwinger E (1988) Fra(X) frequency on the active X-chromosome and phenotype in heterozygous carriers of the fra(X) form of mental retardation. *Am J Med Genet* 30:407-415
- Yu S, Pritchard M, Kremer E, Lynch M, Nancarrow J, Baker E, Holman K, et al (1991) Fragile X genotype characterized by an unstable region of DNA. *Science* 252:1179-1181