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PHENOTYPIC VARIATION AND FMRP LEVELS IN FRAGILE X

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Data on the relationships between cognitive and physical phenotypes, and a deficit of fragile X mental retardation 1 (FMR1) gene-specific protein product, FMRP, are presented and discussed in context with earlier findings. The previously unpublished results obtained, using standard procedures of regression and correlations, showed highly significant associations in males between FMRP levels and the Wechsler summary and subtest scores and in females between these levels and the full-scale intelligence quotient (FSIQ), verbal and performance IQ, and some Wechsler subtest scores. The published results based on data from 144 extended families with fragile X, recruited from Australia and the United States within a collaborative NIH-supported project, were obtained using robust modification of maximum likelihood in pedigrees. The results indicated that processing speed, short-term memory, and the ability to control attention, especially in the context of regulating goal-directed behavior, may be primarily affected by the FMRP depletion. The effect of this depletion on physical phenotype was also demonstrated, especially on body and head height and extensibility of finger joints. It is recommended that further studies should rely on more accurate measures of FMRP levels, and use of larger samples, to overcome extensive variability in the data.
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Key Words: FMRP; IQ; anthropomorphic measures; fragile X; neurocognitive testing; physical phenotype; pedigree analysis

INTRODUCTION

Fragile X syndrome is caused by an unstable mutation in the FMR1 gene located on the X chromosome [Verkerk et al., 1991]. This mutation involves the expansion of trinucleotide (CGG) repeats in the promoter region of this gene. Small expansions, ranging from 50 to 200 CGG repeats (premutation), cause no obvious developmental delay, but they tend to expand into a "full mutation" (>200 CGG repeats) if transmitted through a female. This usually leads to an inactivation of the FMR1 gene and gross deficit of the protein gene product, FMRP [Pieretti et al., 1991]. Evidence indicates that FMRP is involved in normal brain development [Irwin et al., 2002]. A higher expression of FMRP has been documented in the human brain compared with other tissues [Hinds et al., 1993], especially in neuron-rich areas [Devys et al., 1993]. Moreover, the more recent studies using mouse brains have shown that FMRP is involved in synaptogenesis, especially in the cerebral cortex, cerebellum, and hippocampus [Willemsen et al., 1995; Irwin et al., 2002; Mineur et al., 2002] and in modifying synaptic structure in response to environmental stimulation [Weiler et al., 1997; Weiler and Greenough 1999; Beckel-Mitchener and Greenough, this volume). The assumption is therefore justified that cognitive impairment, which is a core deficit in the fragile X syndrome, is primarily caused by the deficit of FMRP.

The phenotype of individuals with the full mutation associated with depletion of FMRP is characterized, apart from a global intellectual impairment, by the presence of distinctive neurocognitive deficits that are not always proportional to the global impairment (reviewed in Bennetto and Pennington 2002). In males, these deficits concern visuospatial ability, the processing of sequential information, and attentional skills [Crowe and Hay 1990; Freund and Reiss 1991; Munir et al., 2000; Loesch et al., 2002a] and a deviant speech pattern [Sudhalter et al., 1992]. In females, specific neurocognitive impairments include attention and concentration skills [Mazzocco et al., 1993] and visuospatial abilities [Mazzocco et al., 1993; Cornish et al., 1998], especially on tasks involving a visuoconstructive component [Cornish et al., 1999]. Many of these deficits are related to higher control processes of attention such as executive functioning [Barkley 1997], which involves cognitive flexibility, planning, initiation, behavioral and attentional regulation, feedback utilization, and self-perception. There is strong evidence for executive function deficits in both males and females with the full mutation [Mazzocco et al., 1993; Sobesky et al., 1996; Munir et al., 1998; Loesch et al., 2003b]. Studies of individual constructs of attention in boys with fragile X demonstrated deficits at higher levels of attention function/executive functioning, especially in the ability to inhibit or delay responding and divide or switch attention [Munir et al., 2000; Cornish et al., 2001].

Females are usually less affected than males because of the presence of a second unaffected X chromosome. Between 50% and 71% of females demonstrate a significant cognitive deficit

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[de Vries et al., 1996], as the unaffected X chromosome still produces normal levels of FMRP. It was demonstrated that, in full-mutation females, the spectrum of cognitive impairments, especially in Performance IQ, was related to the X-activation ratio, which represents the percentage of cells that have the normal X as the active X [Abrams et al., 1994; de Vries et al., 1996; Riddle et al., 1998; Tassone et al., 1999]. Consistently with these findings, our own data from a large sample of full-mutation females showed that this ratio was highly correlated with the FMRP levels (Loesch, unpublished), with a Spearman correlation equal to 0.94 ($P < 0.0001$).

The more direct evidence of the primary involvement of FMRP depletion in developmental delay is obtained by relating this depletion to cognitive deficits and physical anomalies in individuals with fragile X. Since the introduction of the FMRP diagnostic method, it has been shown, using standard regression, that the global intellectual impairment (assessed by cognitive or developmental measures) is highly correlated with a deficit of FMRP in blood lymphocytes, in both full-mutation males and females [Kaufmann et al., 1999; Tassone et al., 1999; Bailey et al., 2001b; Dyer-Friedman et al., 2002] and in hair follicles in females [Willemsen et al., 2003]. Functional MRI (fMRI) studies have also demonstrated positive correlations between FMRP and brain metabolic activity in females with the full mutation during a visuospatial working memory task [Kwon et al., 2001; Menon et al., 2002] and during arithmetic processing [Rivera et al., 2002]. On the other hand, FMRP has not correlated with behavior problems in boys, including the degree of autism [Bailey et al., 2001a; Hessl et al., 2001]; however, this could be related to the limited range of FMRP in these studies. FMRP has correlated with internalizing problems in girls, including withdrawn and anxious/depressed behavior [Hessl et al., 2001].

Most of the FMRP studies have been done on small numbers of patients without a detailed look at the relationship between FMRP and broader neuropsychologic measures. This gap has been filled by the 2000–2002 US–Australian collaborative genotype–phenotype relationship series of studies supported by NICHD, where the effects of FMRP deficits on the wide range of specific cognitive and executive functioning deficits, as well as on global intellectual impairment, have been investigated [Loesch et al., 2002a, 2003a, 2003b]. The relationships between FMRP levels and physical measures have also been reported [Loesch et al., 2003c]. In all these studies, pedigree models were fitted to the data collected from fragile X subjects and their relatives, which allowed us to account for the effect of the background heritable variation, thereby explaining some of the variability of the traits of interest and increasing the power of the tests. Some of these studies' results are presented in the section titled Applications of Pedigree Analysis in FMRP—Phenotype Relationship Studies through that titled Heritabilities**,** and our unpublished data on the relationship between FMRP and cognitive deficits based on standard regression and correlations is outlined under Correlations between FMRP Levels and Cognitive (Wechsler) Scores.

The data for our series of studies were collected from a combined sample of 144 families recruited from Australia and United States. The Australian participants diagnosed with fragile X syndrome, and their non-fragile X relatives, were ascertained through the registry of patients attending Genetic Health Services at the Royal Children's Hospital in Melbourne. The American participants were recruited through the Child Development Unit at the Children's Hospital in Denver, Colorado. There were 287 individuals from 66 extended families for whom the data on FSIQ were available in the Australian sample and 216 individuals from 78 families in the American sample. However, sample sizes were smaller for data on specific cognitive or executive functioning scores, physical measures, or FMRP levels. The ages of the participants ranged from 4 to 76 years.

Fragile X status in participants from both investigative sites was established using a specific DNA test [Taylor et al., 1994] carried out at Kimball Genetics (Denver, CO). The DNA results were used as a diagnostic tool to classify individuals into premutation, full-mutation, and non-fragile X status categories. The sample considered in this presentation consisted of 87 male and 58 female full-mutation subjects, 32 male and 142 female premutation subjects, and 114 male and 57 female non-fragile X relatives, although the numbers may be less for individual traits. Because of low numbers, two gray-zone individuals (40 to 49 CGG repeats) have been included in the normal category. Premutation/full-mutation mosaics, as well as unmethylated full mutations (approximately 20% of the affected males), have been included in the full-mutation category. The data on age distribution among different fragile X status categories and the two investigative sites were given in Loesch et al. [2003b], where the method used to adjust for ascertainment bias was also discussed.

The cognitive testing used the standard Wechsler scales (as in [Loesch et al., 2002a]). Executive function tests applied were as described in Loesch et al. [2003b] and included the Wisconsin Card Sorting Test (WCST) [Heaton et al., 1993] to assess working memory, planning, and flexibility in changing situations; the Rey Complex Figure Test (RCFT) [Meyers and Meyers, 1995] to assess visuospatial memory and visuospatial constructional ability; and the Behavioural Dyscontrol Scale (BDS), which consists of nine items designed to test for motor planning, inhibition, and working memory [Grigsby and Kaye, 1996]. Physical (anthropometric) traits included standard measures of the head, face, trunk and limbs as previously described [Loesch et al., 1988, 2003c].

FMRP VARIATION

FMRP assays were conducted on blood smears, which were made within 24 h of blood draw as previously described in Willemsen et al. [1995]; Tassone et al. [1999], and Loesch et al. [2002b]. FMRP level is expressed as the percentage of lymphocytes that are positive in staining for FMRP using immunocytochemical techniques [Tassone et al., 1999].

The composition of the samples was such that the largest number of male individuals were in the full-mutation category, with FMRP levels ~10%, and the largest number of female individuals were in the premutation category, with FMRP levels ~ 85%. Sixteen individuals with partially methylated/unmethylated full-mutation or premutation/full-mutation mosaicism provided intermediate FMRP levels in the male sample, which was important in achieving an adequate continuity of distribution of this measure.

CORRELATIONS BETWEEN FMRP LEVELS AND COGNITIVE (WECHSLER) SCORES

Simple correlation and regression methods (that ignore familial dependence) were used in exploratory analyses to examine trends in the data. Scatterplots and curvilinear (males) and linear (females) regression lines shown in Figure 1 were obtained by using the best fitting regression models to describe the relationship between FSIQ score and FMRP levels in the sample of 117 fragile X males

(a) and 173 fragile X females (b), with the premutation and full-mutation categories combined.

There are too few observations of males with FMRP levels between 20% and 60% (Fig. 1a), and there is a considerable variation around the fitted regression line in females, especially for FMRP values >40 (Fig. 1b). Outliers are apparent in both the male and female samples, but there is also some reduction in the variability for FMRP levels (<60% in males and <40% in females). The regression of FSIQ on FMRP in males fits both the linear and curvilinear models, although the latter shows a slightly better fit (see R^2 in legend for Fig 1a). This regression in females fits a linear model, where, consistently with earlier predictions [Reiss et al., 1995], FMRP changes account for only a small percentage of variance in FSIQ, because of a modifying effect of the second normal X chromosome of a pair. The sample of premutation females is sufficiently large to show that the FMRP level of ~80% marks the cessation of an association between the intellectual impairment and protein deficits. Beyond this threshold there is a wide spread of FSIQ scores, which are approximately normally distributed, as should be expected from the combined effect of background genes and smoothing environmental effects. A similar trend as shown for FSIQ was evident for both Performance and Verbal IQ. But for the subtests scores the variation around the fitted line was usually larger; Digit Span showed the smallest and Object Assembly, Picture Completion and Similarities showed the greatest variation.

The results of a simple regression/correlation in the full-mutation males (Table 1a) show that the contribution of FMRP deficit to the total variance of the respective summary and subtest scores (R^2) varies from a high of 59% (for Processing Speed and Digit Span) to a low of 19% (for Object Assembly). In the full-mutation females (Table 1b), the contribution of FMRP to the variance of cognitive scores is predictably lower, with R^2 not exceeding 16.5%. A linear regression was applied in both male and female samples as the most appropriate model to describe relationships between FMRP levels and cognitive scores. Because of extensive variability characteristic of fragile X data, we have also used Spearman correlations that, unlike simple regression, are resistant to outliers. For example, in Table 1a the regression for Object Assembly in males was significant, but the Spearman correlation was not, which may reflect the relatively lowest internal consistency and reliability of this test among the WAIS-III subtests. The greater variability of the scores for Object Assembly may also account for the low R^2 value shown in the same table. But the majority of results of regression listed in Table 1a and 1b are consistent with the values of the Spearman correlations.

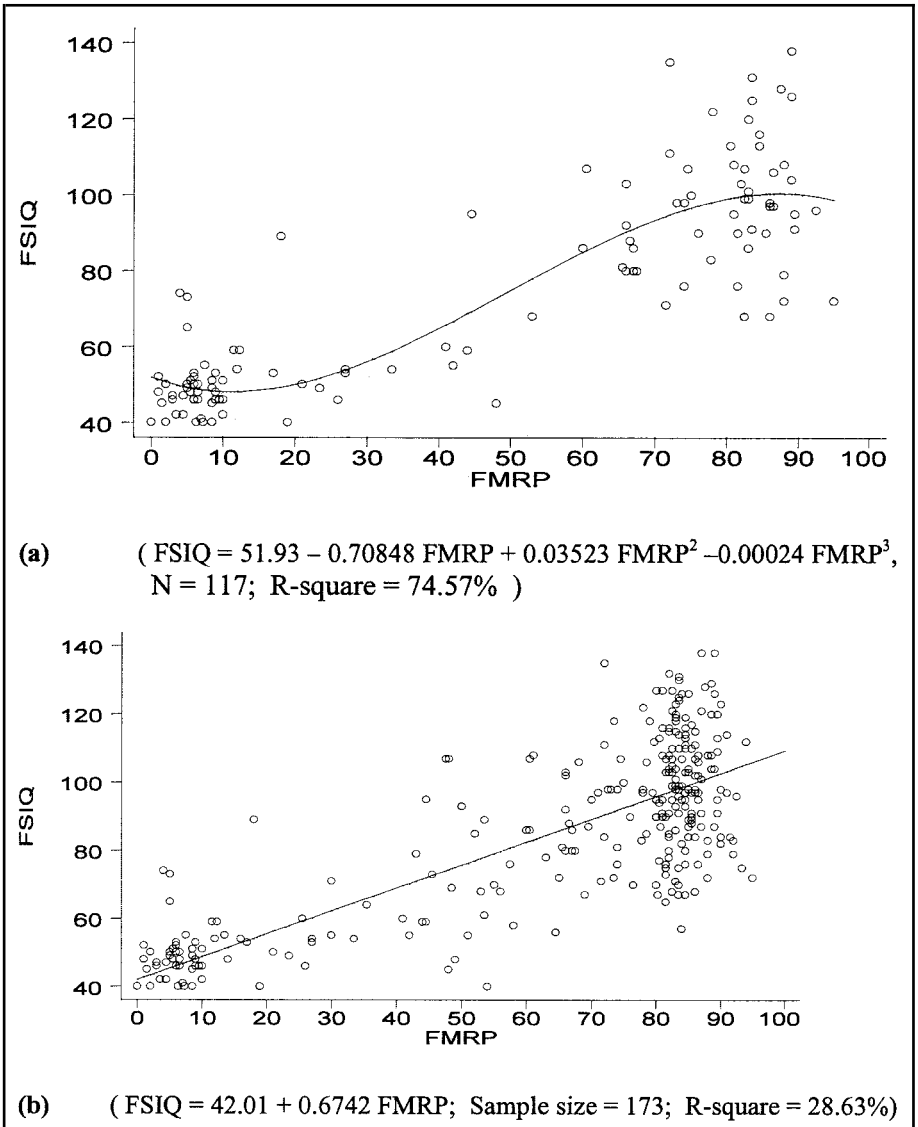

Fig. 1. Scatterplots of FSIQ on FMRP levels for fragile X premutation and full-mutation males (a) and females (b). For females, both regression parameters were highly significant ($P < 0.001$ and $R^2 = 28.8\%$). For males, both parameters were highly significant in linear regression ($P < 0.001$), and in polynomial regression, all except the second parameter in the regression model above were significant ($P < 0.01$), but R^2 was slightly higher (73.0% for linear compared with 74.6%, for polynomial model).

It was of interest to determine whether the effect of FMRP on the various summary and subtest of scores was due to factors beyond the decline in FSIQ. After we adjusted the individual summary and subtest scores in males for FSIQ, as well as for FMRP, in simple regressions, the FMRP levels were not significantly related to any of these scores, except Digit Span and Symbol Search (results not shown).

APPLICATION OF PEDIGREE ANALYSIS IN FMRP–PHENOTYPE RELATIONSHIP STUDIES

Why Use Pedigree Analysis?

The variability of both phenotypic expression and FMRP levels between fragile X individuals is caused by the unstable nature of the mutation in the FMR1 gene and the background heritable variation of the phenotypic traits. However, common statistical procedures based on unrelated samples do not account for this latter source of variation. A more efficient maximum-likelihood analysis in pedigrees, where the means and covariance structure are simultaneously estimated from pedigree data,

Table 1. Results of Simple Linear Regression of the Wechsler Summary and Subtest Scores on FMRP Levels, and Non-parametric Spearman Correlation Coefficients between These Scores and FMRP in the Combined Sample of Full Mutation Males and Females

	Males				
Wechsler variables	n	Intercept (*P*-value)	Gradient (*P*-value)	R^2	Spearman (*P*-value)
Full Scale IQ	68	44.99 (<0.001)	0.528 (<0.001)	0.522	0.509 (<0.001)
Perceptual Organization	61	53.39 (<0.001)	0.450 (<0.001)	0.414	0.476 (<0.001)
Processing Speed	56	50.71 (<0.001)	0.487 (<0.001)	0.587	0.606 (<0.001)
Verbal Comprehension	61	53.42 (<0.001)	0.376 (<0.001)	0.264	0.396 (0.002)
Performance IQ	68	49.52 (<0.001)	0.477 (<0.001)	0.499	0.474 (<0.001)
Block Design	67	1.444 (<0.001)	0.091 (<0.001)	0.385	0.322 (0.008)
Coding	55	0.772 (0.001)	0.067 (<0.001)	0.561	0.596 (<0.001)
Object Assembly	61	2.507 (<0.001)	0.072 (<0.001)	0.189	0.244 (0.058)
Picture Arrangement	61	1.468 (<0.001)	0.076 (<0.001)	0.433	0.408 (0.001)
Picture Completion	67	2.120 (<0.001)	0.075 (<0.001)	0.244	0.307 (0.012)
Symbol Search	56	0.777 (0.007)	0.078 (<0.001)	0.531	0.586 (<0.001)
Verbal IQ	69	48.85 (<0.001)	0.501 (<0.001)	0.511	0.480 (<0.001)
Arithmetic	67	0.578 (0.019)	0.074 (<0.001)	0.494	0.440 (<0.001)
Comprehension	67	1.441 (<0.001)	0.092 (<0.001)	0.395	0.466 (<0.001)
Digit Span	60	0.836 (0.001)	0.082 (<0.001)	0.586	0.589 (<0.001)
Information	67	1.945 (<0.001)	0.083 (<0.001)	0.315	0.318 (0.009)
Letter Number Sequencing	31	0.338 (0.464)	0.090 (<0.001)	0.571	0.692 (<0.001)
Matrix Reasoning	33	2.017 (<0.001)	0.087 (<0.001)	0.497	0.444 (0.010)
Similarities	67	1.371 (<0.001)	0.063 (<0.001)	0.236	0.408 (0.001)
Vocabulary	64	1.594 (<0.001)	0.073 (<0.001)	0.389	0.523 (<0.001)

	Females				
Wechsler variables	n	Intercept (*P*-value)	Gradient (*P*-value)	R^2	Spearman (*P*-value)
Full Scale IQ	56	56.92 (<0.001)	0.319 (0.008)	0.123	0.311 (0.020)
Perceptual Organization	49	57.00 (<0.001)	0.339 (0.004)	0.160	0.462 (0.001)
Processing Speed	47	68.86 (<0.001)	0.162 (0.221)	0.033	0.171 (0.251)
Verbal Comprehension	49	61.63 (<0.001)	0.286 (0.031)	0.095	0.266 (0.064)
Performance IQ	56	61.86 (<0.001)	0.270 (0.014)	0.107	0.349 (0.008)
Block Design	54	2.740 (0.041)	0.057 (0.007)	0.132	0.319 (0.019)
Coding	43	4.770 (0.005)	0.028 (0.264)	0.030	0.166 (0.289)
Object Assembly	50	3.831 (0.004)	0.044 (0.027)	0.098	0.282 (0.047)
Picture Arrangement	50	1.958 (0.161)	0.064 (0.003)	0.165	0.423 (0.002)
Picture Completion	54	3.881 (0.015)	0.058 (0.017)	0.105	0.273 (0.045)
Symbol Search	47	3.058 (0.100)	0.038 (0.162)	0.043	0.162 (0.277)
Verbal IQ	56	58.90 (<0.001)	0.311 (0.012)	0.112	0.258 (0.055)
Arithmetic	54	1.605 (0.286)	0.052 (0.028)	0.090	0.297 (0.029)
Comprehension	54	2.377 (0.163)	0.072 (0.007)	0.130	0.269 (0.049)
Digit Span	50	2.984 (0.061)	0.055 (0.025)	0.101	0.272 (0.056)
Information	54	3.661 (0.010)	0.053 (0.015)	0.109	0.253 (0.065)
Letter Number Sequencing	32	5.823 (0.187)	0.016 (0.794)	0.002	0.042 (0.820)
Matrix Reasoning	33	7.518 (0.023)	−0.014 (0.756)	0.003	0.053 (0.770)
Similarities	54	3.725 (0.033)	0.035 (0.187)	0.033	0.132 (0.340)
Vocabulary	51	2.433 (0.155)	0.061 (0.021)	0.104	0.257 (0.068)

was used in our first study of genotype–phenotype relationships in fragile X [Loesch et al., 1993]. This approach, originally developed by Lange, Westlake, and Spence [Lange et al., 1976], allows one to control for the normal heritable variation in any phenotypic measure and thus to reduce the unexplained trait variability.

Another advantage of this approach is that it addresses the problem of ascertainment bias. There were differences between the American and Australian samples in the ratio of the premutation to full-mutation subjects in both sexes, with a greater number of premutation to full mutation subjects and smaller family size in the former compared with the latter sample. This reflects differences in the follow-up of ascertained families between the two samples, with more extensive cascade testing conducted in Australia. However, the probands were selected for this study through clinical admissions based on their overall neurocognitive impairment (FSIQ), so that the other traits under investigation were not directly relevant to ascertainment. To adjust for the ascertainment bias we conditioned on the FSIQ score by including FSIQ as an explanatory variable in the pedigree models [Thompson, 1993]. Moreover, each individual's IQ was conditioned on this measure in this individual's relatives. These procedures served as an adjustment not only for ascertainment bias but also for the differences in ascertainment between the Australian and American samples.

However, an important drawback of the maximum-likelihood approach was that it did not fully address the problem of extreme (outlying) observations occurring because of the highly variable

expression of the fragile X disorder and measurement errors. To overcome this problem, a (robust) modification of the multivariate normal maximum-likelihood approach was developed [Huggins 1993], which allowed the objective down-weighting of outliers and thus concentrated on modeling the central symmetrical portion of the data. This modification was applied in all recently published studies concerning the FMRP—phenotype relationships in fragile X based on pedigree data [Loesch et al., 2002a, 2002b, 2003a, 2003b, 2003c], and the results of these studies are discussed in the following sections of this article.

EFFECTS OF FMRP DEFICITS ON PSYCHOLOGICAL PHENOTYPE ASSESSED BY PEDIGREE ANALYSIS

Wechsler Scale

The results presented in this section were obtained in our earlier studies [Loesch et al., 2002a, 2002b], where the models for the means and covariance structure were defined, and the methods of testing for significance of parameters estimated from pedigree data that represent effects of FMRP deficit on individual traits (*P* values) have been described. The *P* values for these parameters for FSIQ, as well as for unadjusted summary and subtest scores, are shown in Table 2. These data are comparable with the *P* values for the regression slope based on standard regression presented in Table 1, because simple linear regression was considered in the mean model in pedigree analysis, where within- and between-family variation was also accounted for in the concurrent models for the covariance structure. The results for males (Table 2a) show a very close similarity to the corresponding *P* values in Table 1a in that the effect of FMRP on all summary and subtest Wechsler scores is highly significant. The results for females confirm the significance of FMRP effects as shown by standard regression in Table 1b. However, the *P* values for the parameters for the FMRP effect on individual scores in females obtained from pedigree analysis are higher, and, in contrast with the data in Table 1b, this effect is significant for Processing Speed as well. This example illustrates a greater power of pedigree analysis to detect the relationships between the measures due to reduced variability in the data.

After adjustment for participants' own FSIQ in pedigree analysis, the effect was highly significant in males only for Digit Span and Symbol Search and in females only for Picture Arrangement [Loesch et al., 2002a]. This effect on Object Assembly and Similarities was also significant in both sexes, and on Information in males only, but after a conservative Bonferroni-type correction for multiple comparisons, the *P* values were >0.05 (ibid).

The direction of the effects of FMRP levels is best demonstrated graphically by using the parameters estimated from pedigree models. To demonstrate the magnitude and direction of the effects of FMRP deficits on phenotypic measures adjusted for FSIQ or another explanatory variable, these effects were standardized for various levels of FMRP to be the percentage deviation from the estimated mean for a normal sample, as described in Loesch et al. [2002a], and the FMRP levels considered were 10% to 90%, in increments of 40%. These deviations are illustrated for FSIQ-adjusted summary scores in Figure 2 for males (a) and females (b) and for the subtest scores in Figure 3 for males (a) and females (b). It is remarkable that, among the summary scores, the greatest effect of progressive FMRP deficit in both sexes is on Processing Speed (Fig. 3). Among the subtest scores, the greatest effect is in Symbol Search in both sexes, particularly in females, in Digit Span in males, and in Picture Arrangement in females.

Table 2. Significance of the Effects of FMRP Deficits in Wechsler Summary and Subtest Scores, in Full Mutation Males and Females, Estimated from Pedigree Analysis, with Simple Linear Regression Considered in the Mean Model

Wechsler variables	Males	Females
Full Scale IQ	<0.0001	0.0009
Perceptual Organization	<0.0001	<0.0001
Processing Speed	<0.0001	0.0499
Verbal Comprehension	<0.0001	0.0056
Performance IQ	<0.0001	<0.0001
Block Design	0.0001	0.0002
Coding	<0.0001	0.1435
Object Assembly	<0.0001	0.0375
Picture Arrangement	0.0001	<0.0001
Picture Completion	<0.0001	0.0190
Symbol Search	<0.0001	0.1147
Verbal IQ	<0.0001	0.0185
Arithmetic	<0.0001	0.0216
Comprehension	0.0003	0.0093
Digit Span	<0.0001	0.0058
Information	<0.0001	0.0480
Letter Number Sequencing	0.0019	0.3827
Matrix Reasoning	0.0006	0.8217
Similarities	0.0012	0.1234
Vocabulary	<0.0001	0.0037

P-values were determined using the Wald test to simultaneously test hypotheses about these effects for males or females separately, as explained in Loesch et al. (2003b). The Wald test adjusts for the covariance between the estimates, and a chi-square distribution is used to determine the *P*-values.

EXECUTIVE FUNCTIONING

The scores from the three major tests for executive functioning (WCST, RCFT, and BDS) were all significantly affected by the levels of FMRP [Loesch et al., 2003b]. However, for the FSIQ-adjusted measures, the effect of FMRP depletion was significant only in lowering the total BDS score. When nine individual BDS items' scores adjusted for FSIQ (and age if appropriate) were examined using standard logistic regression (pedigree analysis was not applicable to individual BDS items because of categorical scoring), the effect of FMRP deficit was significant in lowering performance on items 2–7. These items are involved in working memory, attentional control, and motor control and are represented by tapping (2 and 4) and Go-No go (3) tasks and the tests for motor procedural learning (5 and 6), and echopraxia (7).

The discrepancy between the results from three executive function tests might be attributed to the finding, in the same study [Loesch et al., 2003b], that the BDS score was relatively independent of FSIQ, whereas the scores from the remaining two tests (WCST, RCFT) were found to be closely correlated with the level of the overall cognitive impairment. Another reason may be that only a limited number of individuals in our sample could be tested on WCST and RCFT, because of low IQ (<50), or young age, whereas at least 90% of fragile

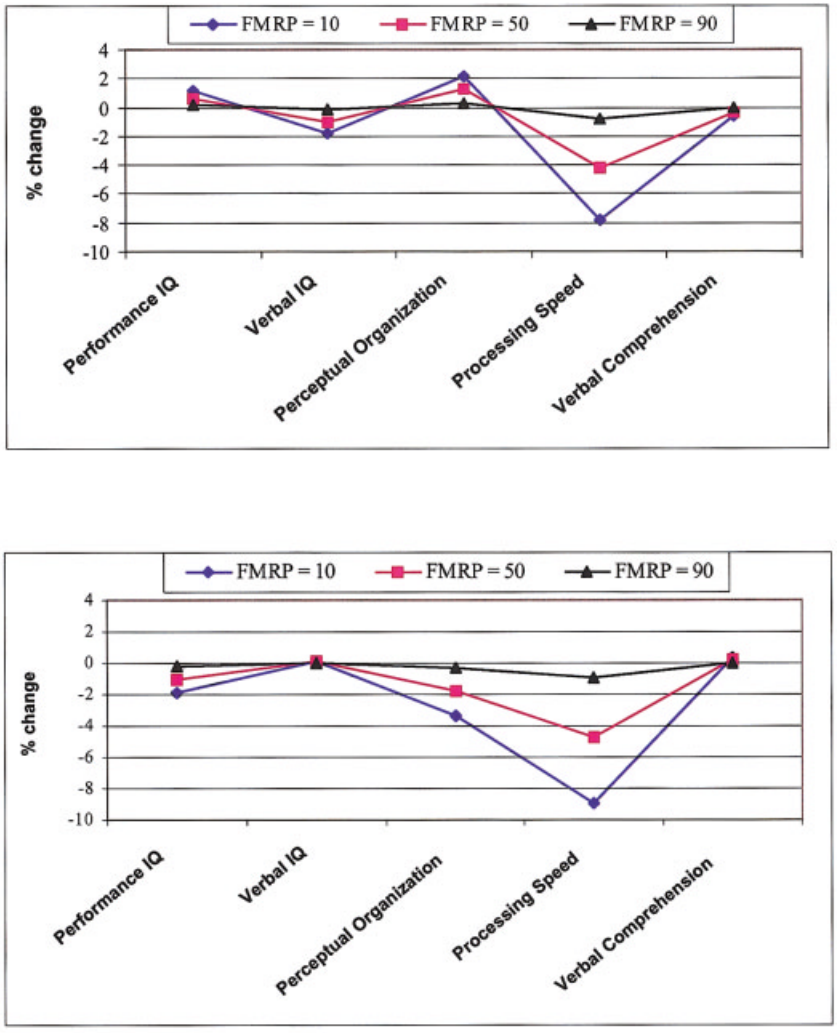

Fig. 2. Standardized percentage change in the mean values of the FSIQ-adjusted Wechsler summary scores due to FMRP deficit for various FMRP levels (10%, 50%, and 90%) in fragile X (full-mutation) male (a) and female (b) samples. The percentage change was standardized at the estimated FSIQ mean for the nonfragile relatives of fragile X subjects in our sample (from Loesch et al. J Dev Behav Pediat 2002, 23 p. 420, Fig. 1). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

X subjects were successfully tested on the BDS [Loesch et al., 2003b].

EFFECTS OF FMRP DEFICITS ON PHYSICAL PHENOTYPE ASSESSED BY PEDIGREE ANALYSIS

The earliest study of the effect of FMRP levels on physical phenotype assessed using the robust modification of the maximum likelihood approach based on pedigree data concerned two dermatoglyphic measures, ridge count and ridge breadth [Loesch et al., 2002b]. In the more recent study, a series of head and facial measurements, weight, height, 2 trunk measures and 3 limb measures were related to the FMRP deficits [Loesch et al., 2003c]. The relationship of a gradual decrement of FMRP with these measures encountered in the above study is illustrated in Fig. 4, where the effects of this decrement were standardized for various FMRP levels to become percentage deviation from the estimated means for a normal sample, as described in [Loesch et al., 2003c]. The parameters estimated from pedigree models, representing effect of FMRP deficits, were significant for the majority of the measures included for the two samples combined, with more variables affected by these deficits in males than in females. The most evident effect in both sexes was toward decreased body height and increased head height (Figs. 4a–4d) and increased extensibility of the middle finger (data shown in Loesch et al., 2003c). Moreover, in males, a significant effect of FMRP deficit was toward decreased facial widths (represented by bizygomatic and bigonial measures) and increased ear size (height and width) and weight, whereas in females, a unique effect was toward increased ear prominence and jaw length. Although the magnitude of the effect of FMRP was greater in males than in females, the trend illustrated in Figure 4 is very similar in both sexes and across age groups.

HERITABILITIES

The heritability (*H*) is estimated as the proportion of the genetic variance to the total variance in a trait, and the results were given in our earlier publications for cognitive [Loesch et al., 2002a, 2003a, 2003b] and physical [Loesch et al., 2002b, 2003c] measures.

Heritabilities of individual traits estimated from the models considering the regression of traits on FMRP were generally lower than those estimated from other models. Predictably, the highest values of genetic variance/heritability were for the physical measures, ranging from 80% to 90% for body height and limb length, 60% to 80% for most trunk and head measures, and some facial measures, with a low of 16% for the ear height [Loesch et al., 2003c]. Heritabilities for dermatoglyphic measures of ridge count and ridge breadth calculated from the same model were 65% and 60%, respectively [Loesch et al., 2002b]. For cognitive (Wechsler) scores, heritabilites for FSIQ, Verbal and Performance IQ, Perceptual Organization, and Processing Speed were only ~30%, but genetic variance was significant for all the above measures; for the Wechsler subtests heritabilities were <30%, and genetic variance was not significant except for Information and Vocabulary [Loesch et al., 2002a]. A similar value was obtained for a majority of executive function scores, with only two RCFT items approaching 40% [Loesch et al., 2003b]. Possible reasons for a discrepancy between these results for physical and psychological scores include greater measurement error in obtaining psychological measures compared to physical measures, which increases the value of (individual) environmental variance; the complex nature of psychological scores; and a greater contribution of nongenetic components generally. The effect of FMRP variation on covariance between relatives (which has not been investigated) may also affect heritability estimates. Unlike individual environment, common environmental component, which must considerably affect psychological scores, could not be separated from genetic variance in our analyses because of an insufficient

number of distant relatives and the relative smallness of the family sample.

SUMMARY AND IMPLICATIONS FOR FUTURE RESEARCH

The results based on standard regression have demonstrated a strong relationship of FMRP depletion in fragile X full-mutation subjects with overall cognitive deficit, as well as specific cognitive skills and executive functioning. Our data on FSIQ from the Australian–U.S. collaborative NIH study were consistent with the earlier findings of a strong relationship between cognitive testing and FMRP levels [Kaufmann et al., 1999; Tassone et al., 1999; Bailey et al., 2001a; Dyer-Friedman et al., 2002]. Moreover, our results showed that in males all the Wechsler subtests (unadjusted for FSIQ) were significantly correlated with FMRP, but in females only a few were significantly correlated. The earlier finding that arithmetic processing was significantly correlated with FMRP in full-mutation fragile X females [Rivera et al., 2002] is consistent with our data for Arithmetic. On the other hand, these data have shown that the contribution of FMRP to the total variance of this trait in females is only 9%, and it does not exceed 16.5% for the remaining subtests. Whereas in males, this contribution is 49% for Arithmetic and ranges from 19% for Object Assembly to the 59% for Processing Speed and Digit Span. A very low contribution of FMRP to the variance of Object Assembly may be largely attributed to the low reliability of this subtest in WAIS-III. But after adjustment of summary and subtest (Wechsler) scores for FSIQ, a deficit of FMRP no longer made significant contribution to the variance of the subtests in females except Picture Arrangement and only for Digit Span and Symbol Search in males.

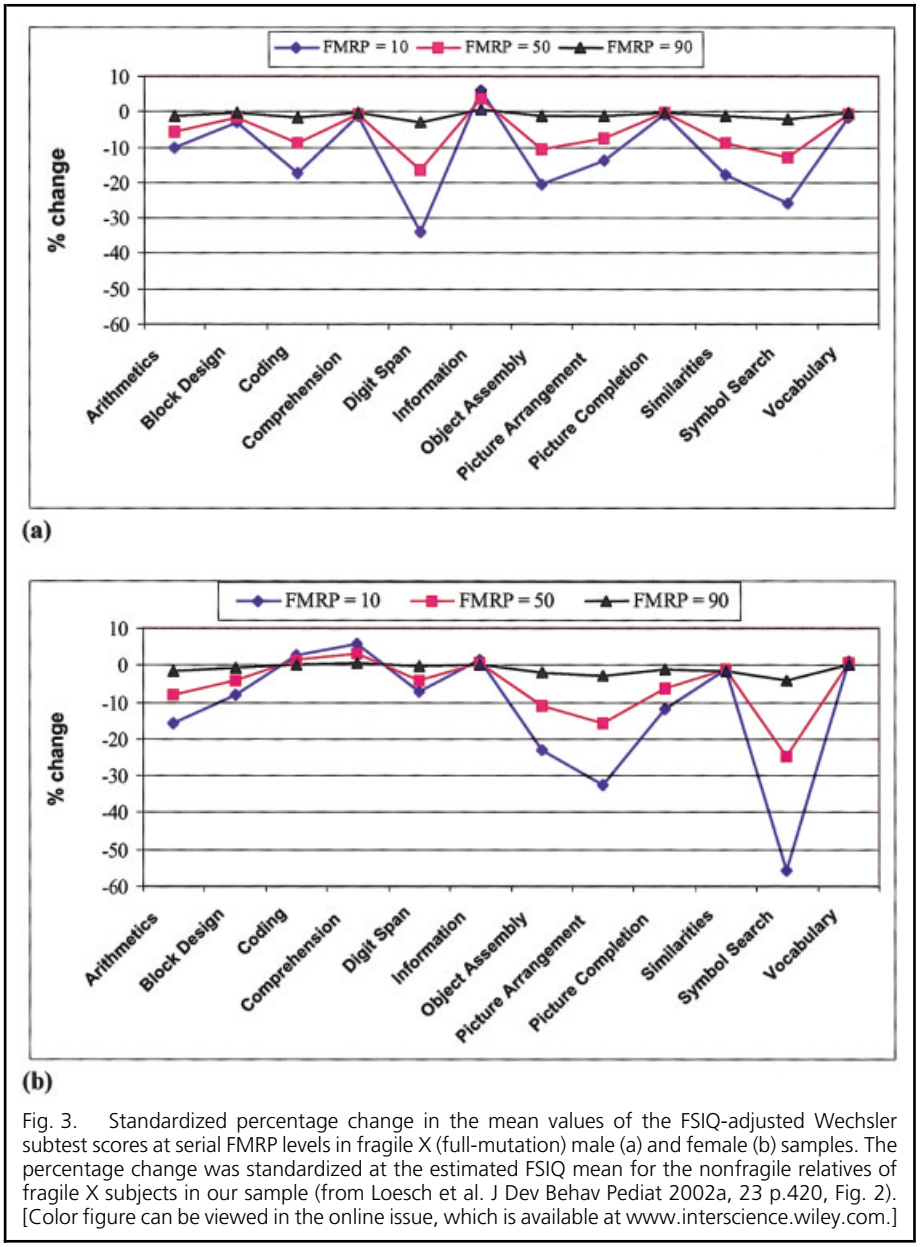

Fig. 3. Standardized percentage change in the mean values of the FSIQ-adjusted Wechsler subtest scores at serial FMRP levels in fragile X (full-mutation) male (a) and female (b) samples. The percentage change was standardized at the estimated FSIQ mean for the nonfragile relatives of fragile X subjects in our sample (from Loesch et al. J Dev Behav Pediat 2002a, 23 p.420, Fig. 2). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

However, as noted above, there are some important drawbacks in using standard statistical analysis in genotype–phenotype relationship studies. These are related to ascertainment bias caused by recruiting families through clinical admissions or combining the data collected in different investigative sites, as well as to the effects of confounding factors and of interaction between these factors, in addition to small sample sizes. Therefore, in the series of studies of the relationship between the phenotype and FMRP in fragile X [Loesch et al., 2002a, 2002b, 2003a, 2003b, 2003c], we applied the more powerful approach based on pedigree data, which overcomes some of the flaws and biases of standard correlation/regression approaches. This method allowed us to control for the effect of the background heritable variation and to test (and adjust when appropriate) for possible confounding variables and for interaction between these variables (such as between FSIQ and FMRP). Although the interaction between confounding variables is a universal problem, it is further complicated in disorders caused by dynamic mutations, where measures representing the severity of a condition may also interact with age. This results from anticipation, whereby the younger generations are usually more affected and have bigger FMRP/FSIQ deficits than the older generations. Apart from the benefits of the maximum likelihood approach in controlling for confounded variables and the interactions, a robust modification applied in our series of studies has enabled us to objectively down-weight the outliers and thus to concentrate on the analysis of the general trend in the data.

The results on the effect of FMRP deficit on global intellectual impairment (FSIQ) obtained from pedigree models are not inconsistent either with our own results (in 3) or with the earlier published data based on standard correlations [Kaufmann et al., 1999; Tassone et al., 1999; Bailey et al., 2001a; Dyer-Friedman et al., 2002]. However, pedigree analysis is more reliable in investigating phenotype–genotype relationships and flexible enough to test the range of hypotheses around the confounding effects. A highly significant effect of FMRP deficit on all the summary and subtest scores in males and on a number of these scores

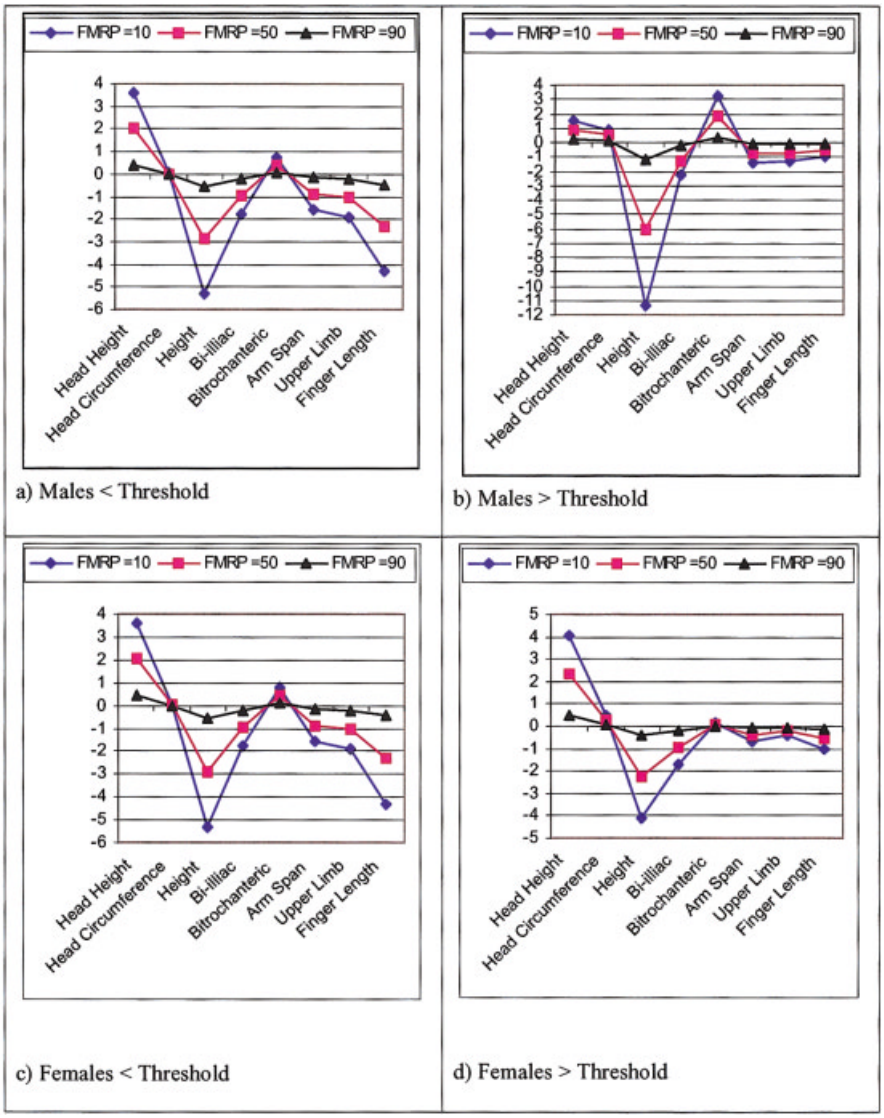

Fig. 4. Effect sizes representing the percentage change due to FMRP for various FMRP levels (10%, 50%, and 90%) for the age-adjusted physical measures (threshold model) in the fragile X sample of males (a and b) and females (c and d). These effects were estimated at the threshold age for each trait. The values are standardized and plotted separately for the "young" subjects located below the estimated individual threshold values for males (a) and females (c), and for "adult" subjects above the threshold values for males (b) and females (d). (from Loesch et al. Am J Med Genet 2003, Fig. 2). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

in females as demonstrated by pedigree analysis is not unexpected, because of strong relationships between these scores and FSIQ. But this approach is far more important in assessing this effect on the FSIQ-adjusted scores to identify specific deficits/strengths in fragile X, related to FMRP levels, but occurring independently of the level of an overall cognitive impairment.

We have identified Digit Span and Symbol Search in both sexes and Picture Arrangement in females as outstanding examples of such deficits specifically affected by FMRP depletion, and among the summary scores, Processing Speed was also significantly impaired [Loesch et al., 2002a]. These findings suggest that the brain processes, which may be primarily affected by a deficit of FMRP, especially in males, are processing speed, short-term memory, and attention. In our data Processing Speed (which is composed of Symbol Search and Coding) was affected by FMRP deficit irrespective of the overall IQ, along with Symbol Search and Coding, and short-term memory was mainly involved in Symbol Search and Digit Span tasks [Sattler 1992; Guerreiro et al., 1998]. Attention is also involved in performance on these tasks, as well as on Picture Arrangement, which was specifically affected by FMRP deficits. Decrements in processing of sequential information and attention skills have been found in previous studies of full-mutation males [Freund and Reiss 1991; Munir et al., 2000]. Our findings on the effect of FMRP on executive function tests are not inconsistent with the argument that attention may be one of the processes primarily affected by a deficit of FMRP. A progressive FMRP depletion significantly reduced the FSIQ-adjusted total BDS score, as well as a number of individual item scores [Loesch et al., 2003b], which mainly test for the ability of controlling attention, especially in the context of regulating goal-directed behavior. On the other hand, the claim that processing speed, memory, and attention are primarily affected by FMRP deficit is not incompatible with experimental evidence of the involvement of this protein in developing and shaping up brain synapses [Weiler et al., 1997; Weiler and Greenough 1999; Irwin et al., 2002].

Although the gender differences in severity of the effect of FMRP depletion on both summary and subtest IQ scores are evident from the data presented, the differences in the degree of impairment on certain subtest scores are difficult to interpret, because we have conducted no comparative analyses of cognitive profiles affected by FMRP deficit in either sex. The results so far indicate the effect of FMRP depletion is predominantly on processing speed, memory, and attention in both sexes but that visuospatial defects may be more strongly manifested in females than in the males irrespective of their overall IQ levels.

The above conclusions refer to the results from a sample of full-mutation subjects. However, in the most recent study [Loesch et al., 2003a] a significant effect of FMRP deficit on a number of Cognitive (Wechsler) scores, including FSIQ, and Performance and Verbal IQ was demonstrated in premutation males, which supports rare findings of a subtle reduction of FMRP levels in these carriers [Tassone et al., 2000a; Tassone et al., 2000b; Hagerman et al., 2001; Kenneson et al., 2001]. In addition to the impact of alleged FMRP deficit on cognitive development, there may be other mechanisms in fragile X premutation carriers contributing to some specific cognitive deficits, such as in arithmetic skills [Loesch et al., 2003a]. The elevated mRNA levels in those with the premutation may have a toxic gain-of-function effect on the brain [Tassone et al., 2000a; Hager-

man and Hagerman 2002; Jacquemont et al., 2003].

Although the impairment in overall cognitive functioning, as well as some specific skills, can be explained simply by the detrimental effect of FMRP depletion on brain development, the underlying mechanisms leading to a physical defect in fragile X full mutation is more complex and difficult to interpret in context with FMRP deficit. Demonstration of (linear) effects of progressively reduced levels of FMRP in fragile X subjects on a number of physical measurements, mainly of trunk, limbs, and head, which was in a direction consistent with the fragile X physical phenotype, has been an important step toward understanding these mechanisms. It has been suggested [Loesch et al., 2003c] that they may be relevant to hypothalamic dysfunction caused by reduced synaptic contacts because of immaturity of dendritic spines [Comery et al., 1997; Weiler and Greenough 1999]. Earlier data on the growth pattern in fragile X [Loesch et al., 1995] imply that this dysfunction may cause premature increase in the pulsatile secretion of GnRH, followed by premature secretion of high doses of estrogen, thus leading to earlier epiphyseal maturation. The FMRP-related increase in head height might reflect an increased brain size, which is most prominent in the frontal lobe and also relevant to synaptic dysmorphology [Reiss et al., 2000].

An important finding in the Loesch et al. [2003c] study concerned a significant effect of FMRP deficit in extensibility of finger joints, which may indicate a direct association of this protein with a connective tissue disorder in fragile X [Opitz et al., 1984; Riddle et al., 1998]. Because FMRP is known to play a role in mRNA transport and translation [Corbin et al., 1997], it was postulated [Loesch et al., 2003c] that the deficit or absence of this protein during development may cause a cascade alteration translation of different mRNAs, such as the ones involved in the connective tissue morphology and function.

Although using advanced analytical methods and large samples allows important information on the effects of FMRP decrement on both psychological and physical phenotype to be obtained, the analysis is most effective in full-mutation subjects, where the FMRP levels are drastically reduced, and the relationships of this deficit with the phenotype are strong. But the results of the analysis are more difficult to interpret if the FMRP deficit is small or controversial, such as in the premutation carriers [Tassone et al., 2000b; Kenneson et al., 2001]. This is partly because inferences concerning deficits of this protein in the brain have been made from the data from peripheral blood lymphocytes, irrespective of the suggested differences in FMR1 mutational patterns across different tissues (e.g., [Taylor et al., 1994; Allingham Hawkins et al., 1996; Dobkin et al., 1996; Maddalena et al., 1996]). Indeed, the recent study [Willemsen et al., 2003] that related the IQ score to FMRP levels in hair root cells (originating from the same germ layer as brain tissue) in fragile X females reported a higher correlation between these traits ($r = 0.61$) than this correlation obtained from our data ($r = 0.31$ in Table 2). However, the IQ scores were not directly comparable between these two studies, and the scatterplot presented in Willemsen et al. [2003] shows greater variability, including the presence of outliers, which may have affected their correlation results. Irrespective of the choice of biopsy tissue, the FMRP score represents the level assessed only at one time point, and thus provides no data on this level during development. But most importantly, the existing method does not constitute an assessment of the actual level of this protein in each cell, but only scores the proportion of FMRP-expressing lymphocytes among all lymphocytes counted. Therefore, this assay is not sensitive to mild deficits of FMRP and may not recognize all the isoforms of FMRP. Clearly, a test that permits the rapid, extremely sensitive, and specific measurement of FMRP levels, and that recognizes small level changes, needs to be developed; such a test and would thus be more appropriate to assess the effect of a small deficit of this protein on the phenotype of individuals carrying smaller CGG expansions.

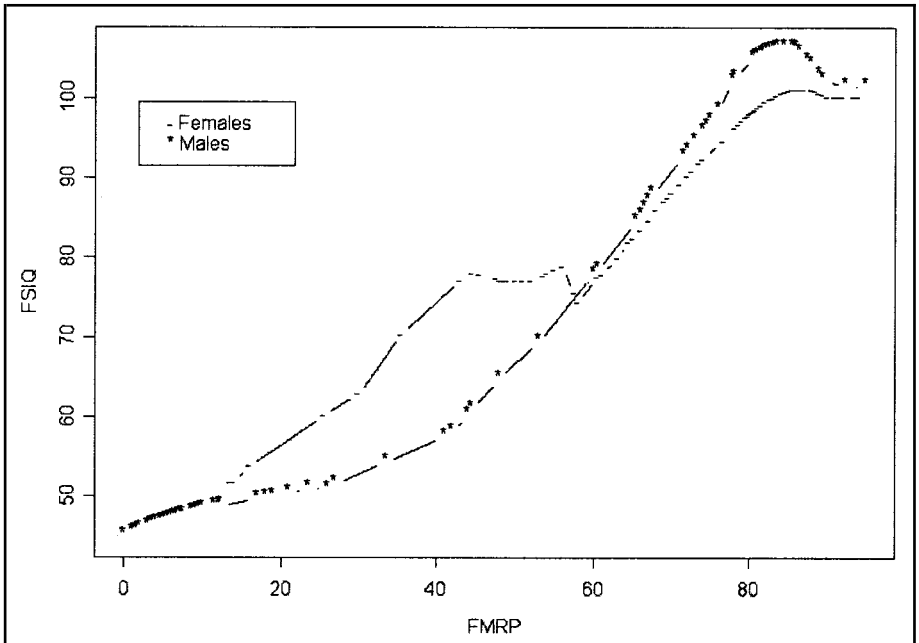

Fig. 5. Separate plots of the mean functions for males and females (full-mutation, premutation and non-fragile X relatives combined) using nonparametric estimates of the mean function.

The lack of FMRP data over the whole 0–100 range and the floor effect in the IQ scores are sources of difficulty in statistical modeling. To overcome this problem, the sample sizes should be further increased and the most seriously affected individuals should be scored using tests that are better able to discriminate among low-functioning individuals. This will enable various curvilinear and threshold models to be fitted, and a larger number of explanatory variables to be included, to assess the effect of FMRP deficits on individual traits more accurately. Nonparametric methods that allow more flexible models without the need to specify a particular parametric form are becoming common in many areas of statistics. There is clearly a need for the development of robust nonparametric approach that extend the clustered data methods of Lin and Carroll [2000] to pedigree data. For example, some preliminary results concerning the nonparametric estimation of the FMRP/FSIQ relationship in males and females are given in Figure 5. The nonparametric estimate is consistent with the curvilinear model for males shown in Figure 1, but for females the modifying effect of the second chromosome (which introduces

heterogeneity) is revealed as a disturbance in the 40–60 FMRP range. This interaction between the development of state-of-the-art statistical procedures and the new data arising in molecular genetics in general and genotype–phenotype relationships in particular, following from the previous developments of pedigree and robust methods, is an exciting and promising area of multidisciplinary research. ■

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REFERENCES

Abrams MT, Reiss AL, Freund LS, et al. 1994. Molecular–neurobehavioral associations in females with the fragile X full mutation. Am J Med Genet 51:317–327.

Allingham Hawkins DJ, Brown CA, Babul R, et al. 1996. Tissue-specific methylation differences and cognitive function in Fragile X premutation females. Am J Med Genet 64:329–333.

Bailey DB, Jr., Hatton DD, Skinner M, et al. 2001a. Autistic behavior, FMR1 protein, and developmental trajectories in young males with Fragile X syndrome. J Aut Dev Disorders 31:165–174.

Bailey DB, Jr., Hatton DD, Tassone F, et al. 2001b. Variability in FMRP and early development in males with Fragile X syndrome. Am J Ment Retard 106:16–27.

Barkley RA. 1997. Behavioral inhibition, sustained attention, and executive functions, constructing a unifying theory of Attention Deficit/Hyperactivity Disorder (ADHD). Psychol Bull 121:65–94.

Bennetto L, Pennington BF. 2002. Neuropsychology. In Hagerman RJ, Hagerman PJ, editors. Fragile X Syndrome: Diagnosis, Treatment, and Research, 3rd ed. Baltimore: Johns Hopkins University Press, pp. 206–248.

Comery TA, Harris JB, Willems PJ, et al. 1997. Abnormal dendritic spines in Fragile X knockout mice: Maturation and pruning deficits. Proc Natl Acad Sci USA 94:5401–5404.

Corbin F, Bouillon M, Fortin A, et al. 1997. The Fragile X mental retardation protein is associated with poly(A)+ mRNA in actively translating polyribosomes. Hum Mol Genet 6:1465–1472.

Cornish KM, Munir F, Cross G. 1998. The nature of the spatial deficit in young females with Fragile-X syndrome: A neuropsychological and molecular perspective. Neuropsychologia 36:1239–1246.

Cornish KM, Munir F, Cross G. 1999. Spatial cognition in males with Fragile-X syndrome: Evidence for a neuropsychological phenotype. Cortex 35:263–271.

Cornish KM, Munir F, Cross G. 2001. Differential impact of the FMR-1 full mutation on memory and attention functioning: A neuropsychological perspective. J Cogn Neurosci 13: 144–150.

Crowe SF, Hay DA. 1990. Neuropsychological dimensions of the Fragile X syndrome: support for a nondominant hemisphere dysfunction hypothesis. Neuropsychologia 28:9–16.

de Vries BB, Wiegers AM, Smits AP, et al. 1996. Mental status of females with an FMR1 gene full mutation. Am J Med Genet 58:1025–1032.

Devys D, Lutz Y, Rouyer N, et al. 1993. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a Fragile X premutation. Nat Genet 4:335–340.

Dobkin CS, Nolin SL, Cohen I, et al. 1996. Tissue differences in Fragile X mosaics: Mosaicism in blood cells may differ greatly from skin. Am J Med Genet 64:296–301.

Dyer-Friedman J, Glaser B, Hessl D, et al. 2002. Genetic and environmental influences on the cognitive outcomes of children with Fragile X syndrome. J Am Acad Child Adolesc Psychiat 41:237–244.

Freund LS, Reiss AL. 1991. Cognitive profiles associated with the fra(X) syndrome in males and females. Am J Med Genet 38:542–547.

Grigsby J, Kaye K. 1996. Behavioral Dyscontrol Scale: Manual, 2nd ed.

Guerreiro MM, Camargo EE, Kato M, et al. 1998. Fragile X Syndrome: Clinical, electroencephalographic, and neuroimaging characteristics. Ar Qneuropsiquiatr 56:18–23.

Hagerman RJ, Hagerman PJ. 2002. The Fragile X premutation: Into the phenotypic fold. Curr Opin Genet Dev 12:278–283.

Hagerman RJ, Leehey M, Heinrichs W, et al. 2001. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of Fragile X. Neurology 57:127–130.

Heaton RK, Chelune GJ, Talley JL, et al. 1993. Wisconsin Card Sorting Test Manual, Revised and Expanded. Psychological Assessment Services, Inc.

Hessl D, Dyer-Friedman J, Glaser B, et al. 2001. The influence of environmental and genetic factors on behavior problems and autistic symptoms in boys and girls with Fragile X syndrome. Pediatrics 108:electronic e88.

Hinds HL, Ashley CT, Sutcliffe JS, et al. 1993. Tissue specific expression of FMR-1 provides evidence for a functional role in Fragile X syndrome [see comments] [published erratum appears in Nat Genet 1993 Nov,5(3):312]. Nat Genet 3:36–43.

Huggins RM. 1993. On the robust analysis of pedigree data. Austr J Statistics 35:43–57.

Irwin SA, Galvez R, Weiler IJ, et al. 2002. Brain Structure and functions of FMR1 protein. In Hagerman RJ, Hagerman PJ, editors. Fragile X Syndrome: Diagnosis, Treatment and Research, 3rd ed. Baltimore: The Johns Hopkins University Press, pp.191–205

Jacquemont S, Hagerman RJ, Leehey M, et al. 2003. Fragile X premutation tremor/ataxia syndrome: Molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72:869–878.

Kaufmann WE, Abrams MT, Chen W, et al. 1999. Genotype, molecular phenotype, and cognitive phenotype: correlations in Fragile X syndrome. Am J Med Genet 83:286–295.

Kenneson A, Zhang F, Hagedorn CH, et al. 2001. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. Hum Mol Genet 10:1449–1454.

Kwon H, Menon V, Eliez S, et al. 2001. Functional neuroanatomy of visuospatial working memory in Fragile X syndrome: Relation to behavioral and molecular measures. Am J Psychiat 158:1040–1051.

Lange K, Westlake J, Spence MA. 1976. Extensions to pedigree analysis. III. Variance components by the scoring method. Ann Hum Genet 50:385–398.

Lin X, Carroll RJ. 2000. Nonparametric function estimation for clustered data when the predictor is measured with/without error. J Am Stat Assoc 95:520–534.

Loesch D, Huggins R, Bui QM, et al. 2003a. Effect of the Fragile X status categories and FMRP deficits on cognitive profiles estimated by robust pedigree analysis. Am J Med Gen 22:13–23.

Loesch DZ, Bui QM, Grigsby J, et al. 2003b. Effect of the Fragile X status categories and the FMRP levels on executive functioning in Fragile X males and females. Neuropsychology 17(4):646–657.

Loesch DZ, Huggins R, Hay DA, et al. 1993. Genotype-phenotype relationships in Fragile X syndrome: a family study. Am J Hum Genet 53:1064–1073.

Loesch DZ, Huggins RM, Bui QM, et al. 2002a. Effect of the deficits of Fragile x mental retardation protein on cognitive status of Fragile x males and females assessed by robust pedigree analysis. J Dev Behav Pediatr 23:416–423.

Loesch DZ, Huggins RM, Bui QM, et al. 2003c. Relationship of deficits of FMR1 gene specific protein with physical phenotype of Fragile X males and females in pedigrees: A new perspective. Am J Med Genet 118A:127–134.

Loesch DZ, Huggins RM, Hoang NH. 1995. Growth in stature in Fragile X families: a mixed longitudinal study. Am J Med Genet 58:249–256.

Loesch DZ, Huggins RM, Taylor AK. 2002b. Application of robust pedigree analysis in studies of complex genotype-phenotype relationships in Fragile X syndrome. Am J Med Genet 107:136–42.

Loesch DZ, Lafranchi M, Scott D. 1988. Anthropometry in Martin-Bell syndrome. Am J Med Genet 30:149–164.

Maddalena A, Yadvish KN, Spence WC, et al. 1996. A Fragile X mosaic male with a cryptic full mutation detected in epithelium but not in blood. Am J Med Genet 64:309–312.

Mazzocco MM, Pennington BF, Hagerman RJ. 1993. The neurocognitive phenotype of female carriers of Fragile X: additional evidence for specificity. Journal of Developmental and Behavioral Pediatrics 14:328–335.

Menon V, Mackenzie K, Rivera SM, et al. 2002. Prefrontal cortex involvement in processing incorrect arithmetic equations: evidence from event-related fMRI. Hum Brain Mapp 16: 119–130.

Meyers JE, Meyers KR. 1995. Rey Complex Figure Test and Recognition Trial Manual. Odessa, Fl: Psychological Assessment Resources, Inc.

Mineur YS, Sluyter F, de Wit S, et al. 2002. Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse. Hippocampus 12:39–46.

Munir F, Cornish KM, Wilding J. 1998. Patterns of attention deficit in Fragile X syndrome: a neuropsychological perspective. Paper presented at Sixth International Fragile X Syndrome Conference. Ashville, USA

Munir F, Cornish KM, Wilding J. 2000. A neuropsychological profile of attention deficits in young males with Fragile X syndrome. Neuropsychologia 38:1261–1270.

Opitz JM, Westphal JM, Daniel A. 1984. Discovery of a connective tissue dysplasia in the Martin-Bell syndrome. Am J Med Genet 17:101–109.
Pieretti M, Zhang FP, Fu YH, et al. 1991. Absence of expression of the FMR-1 gene in Fragile X syndrome. Cell 66:817–822.
Reiss AL, Eliez S, Smith JE, et al. 2000. Brain imaging in neurogenetic conditions: Realizing the potential of behavioral neurogenetics research. Ment Retard and Developmental Disabilities Research Reviews 6:186–197.
Riddle JE, Cheema A, Sobesky WE, et al. 1998. Phenotypic involvement in females with the FMR1 gene mutation. American Journal on Ment Retard 102:590–601.
Rivera SM, Menon V, White CD, et al. 2002. Functional brain activation during arithmetic processing in females with fragile X syndrome is related to FMR1 protein expression. Human Brain Mapping 16:206–218.
Sattler JM. 1992. The Assessment of Children: Revised and Updated, 3rd ed. San Diego: Jerome M. Sattler, Inc.
Sobesky WE, Taylor AK, Pennington BF, et al. 1996. Molecular-clinical correlations in females with Fragile X. Am J Med Genet 64: 340–345.
Sudhalter V, Maranion M, Brooks P. 1992. Expressive semantic deficit in the productive language of males with Fragile X syndrome. Am J Med Genet 43:65–71.
Tassone F, Hagerman RJ, Iklé DN, et al. 1999. FMRP expression as a potential prognostic indicator in Fragile X syndrome. Am J Med Genet 84:250–261.
Tassone F, Hagerman RJ, Taylor AK, et al. 2000a. Elevated levels of *FMR1* mRNA in carrier males: a new mechanism of involvement in Fragile X syndrome. Am J Hum Genet 66:6–15.
Tassone F, Hagerman RJ, Taylor AK, et al. 2000b. Clinical involvement and protein expression in individuals with the *FMR1* premutation. Am J Med Genet 91:144–152.
Taylor AK, Safanda JF, Fall MZ, et al. 1994. Molecular predictors of cognitive involvement in female carriers of Fragile X syndrome. J Am Med Assoc 271:507–514.
Thompson EA. 1993. Sampling and Ascertainment in Genetic Epidemiology: A Tutorial Review. Department of Statistics, University of Washington.
Verkerk AJ, Pieretti M, Sutcliffe JS, 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.
Weiler IJ, Greenough WT. 1999. Synaptic synthesis of the Fragile X protein: possible involvement in synapse maturation and elimination. Am J Med Genet 83:248–252.
Weiler IJ, Irwin SA, Klintsova AY, et al. 1997. Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. Proc Natl Acad Sci USA 94:5395–5400.
Willemsen R, Mohkamsing S, de Vries B, et al. 1995. Rapid antibody test for Fragile X syndrome. Lancet 345:1147–1148.
Willemsen R, Smits A, Severijnen L-A, et al. 2003. Predictive testing for cognitive functioning in female carriers of the Fragile X syndrome using hair root analysis. J Med Genet 40:377–379.