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Longitudinal Changes in Intellectual Development in Children with Fragile X Syndrome

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Abstract

Structural equation modeling (SEM) was used to examine the development of intellectual functioning in 145 school-age pairs of siblings. Each pair included one child with Fragile X syndrome (FXS) and one unaffected sibling. All pairs of children were evaluated on the Wechsler Intelligence Scale for Children—Third Edition (WISC-III) at time 1 and 80 pairs of children received a second evaluation at time 2 approximately 4 years later. Compared to their unaffected siblings, children with FXS obtained significantly lower percentage correct scores on all subtests of the WISC at both time points. During the time between the first and second assessments, the annual rate of intellectual development was approximately 2.2 times faster in the unaffected children compared to the children with FXS. Levels of the fragile X mental retardation protein (FMRP) were highly associated with intellectual ability scores of the children with FXS at both time points ($r=0.55$ and 0.64 respectively). However, when gender, age, and the time between assessments were included as covariates in the structural equation model, FMRP accounted for only 5% of the variance in intellectual ability scores at time 1 and 13% of the variance at time 2. The results of this study suggest that slower learning contributes to the low and declining standardized IQ scores observed in children with FXS.

Keywords

Fragile X syndrome; Structural equation modeling; Intellectual functioning; IQ; FMRP

Fragile X syndrome occurs in approximately 1 in every 4,000 live births and is the most common inherited form of cognitive and behavioral disability. In 1991, Verkerk and colleagues reported that a single gene on the X chromosome (locus Xq27.3), *FMR1*, was associated with the symptoms of FXS (Verkerk et al. 1991). Subsequent research revealed that persons with FXS had increased numbers of triplet (CGG) repeats within *FMR1*. In normal alleles, the CGG repeats vary from 6 to 50, whereas expansions of ~50–200 repeats are associated with the “premutation” form of the gene seen in carrier females and males. The probability of having a child with the full mutation increases as the length of the mothers’ CGG repeat length increases—a phenomenon known as genetic anticipation. Larger expansions (200 to thousands) of CGG repeats are considered “full mutations” and are typically associated with excessive methylation of cytosines in the *FMR1* promoter. This

modification extinguishes transcription of the *FMR1* gene into mRNA, thus stopping translation of the fragile X mental retardation protein (FMRP). FMRP plays an important role in the development of synaptic function, maturation, and plasticity during development, and possibly, on an ongoing basis throughout adult life as well (Reiss et al. 1995).

Investigators have reported that individuals with FXS experience weaknesses in executive function, visual memory, visual-spatial relationships, and arithmetic, with relatively less severe impairments in verbal skills (Bennetto et al. 2001; Cornish et al. 2004; Fisch 2006; Freund and Reiss 1991; Kemper et al. 1986; Mazzocco et al. 1992; Miezieski et al. 1986; Munir et al. 2000). While this profile appears similar for both genders, females with FXS experience significantly and substantially less severe deficits than males, presumably because of their status as heterozygotes for the *FMR1* mutation, with resulting higher FMRP levels.

To date, our understanding of intellectual development in school-age children or older individuals with FXS is based primarily on cross-sectional studies (Dykens et al. 1988, 1989a; Wieggers et al. 1993) or longitudinal investigations that have been limited by small sample sizes, restricted age ranges, widely varying time intervals between assessments, or retrospective designs (Dykens et al. 1989a, b; Fisch et al. 1999a, b; Wright-Talamante et al. 1996). Despite these limitations, these studies have suggested that intellectual functioning in individuals with FXS develops more slowly than same-aged peers, a phenomenon thought to contribute to the observation of declining standardized IQs (Skinner et al. 2005).

Studies of intellectual function in FXS often demonstrate a mean IQ decline of 4 to 9 standardized points over intervals ranging from several months to 13 years. For example, in a retrospective study, IQ decreases were observed in 9 of 11 (82%) females with FXS (mean decrease of 9 standardized points; Fisch et al. 1994). In a subsequent prospective study of a different group of 13 girls with FXS, significant IQ decreases were again observed in eight subjects (Fisch et al. 1996). Yet, a review of these studies suggests that not all individuals with FXS show declining IQs over time (Hagerman et al. 1989; Wright-Talamante et al. 1996). Based on a retrospective chart review of 50 males and females with FXS who had repeated standardized IQ testing separated by 7 months to 13 years, intellectual development appeared to depend, in part, on genotypic features such as the presence of incomplete methylation (Wright-Talamante et al. 1996). Accordingly, these investigators hypothesized that a subgroup of individuals with higher levels of FMRP may be less vulnerable to IQ decline. It has also been argued that declining IQ in individuals with FXS might also be explained by inherent properties of cognitive tests, which for older children, may place greater emphasis on skills that are known to be specific weaknesses in this disorder (Hagerman et al. 1989). As Hay (1994) has also pointed out, interpretation of standardized IQ data in individuals with FXS is extremely problematic because investigators have combined data from different tests, different sources and from different age-groups. This problem is compounded by the fact that most IQ tests have fewer items at the low ability levels, rendering the standardized IQ scores in individuals with FXS more imprecise and less reliable.

While prospective longitudinal data on intellectual functioning are quite limited in school-age children with FXS, recent studies have begun to address adaptive behavioral development in young, preschool-age boys with FXS (Bailey et al. 1998, 2001; Roberts et al. 2001). Although significant variability exists among individual subjects, results from these studies suggest that the rate of adaptive behavior development in preschool-age boys with FXS ranges from one-third to one-half that expected for typically developing children. With respect to more specific domains of adaptive development, expressive language appears to be more adversely affected than receptive language (Roberts et al. 2001). These well-designed longitudinal studies indicate that while development progresses at half or less the normal rate in young boys with FXS (up to age 8), the development in adaptive functioning does not appear to plateau or slow until after that time, most likely during pre- and early adolescence (i.e., 9–13 years of age).

Only a few studies have investigated the effect of FMRP on intellectual functioning in FXS. Some investigators (Loesch et al. 2004; Tassone et al. 1999a, b), but not others (Skinner et al. 2005), have reported a correlation between FMRP levels and intellectual functioning. Further, the association between FMRP and intellectual functioning appears to differ according to gender and methylation status. In a cross-sectional study examining the relationship between FMRP and IQ in males and females with FXS aged 1 to 60 years, FMRP levels explained 68% of the variance in IQ in 13 males with a partially methylated full mutation, 38% of the variance in 12 mosaic males and 24% of the variance in 19 females with the full mutation (Tassone et al. 1999a, b). However, given the low numbers of individuals included in these groups, and the large age range of the individuals, these data should be treated cautiously. In a larger cross-sectional study, FMRP levels accounted for 52% of the variance in IQ scores in 68 males and approximately 12% of the variance in the IQ scores of 56 females (Loesch et al. 2004). However, several methodological problems also limit the conclusions that can be drawn from this study. For example, cohorts from Australia and the USA were combined in the analysis, there were few observations in the data with FMRP levels between 20% and 60%, and the age range of the participants was extremely wide (4 to 76 years).

In summary, although there is general agreement that decline in IQ and, perhaps to a lesser extent, adaptive behavior occurs in school-age children with FXS, prospective, longitudinal information regarding the timing and specificity of these disquieting developmental characteristics is extremely limited. Further, the relationship between FMRP levels and intellectual functioning is unclear. The present study examined changes in the annual rate of intellectual development in a large sample of school-age children with FXS compared to their age- and gender-matched unaffected siblings. To overcome methodological problems associated with previous studies, we employed a single measure of IQ at each assessment point, and administered the same subtests of the test to the children at each age. We hypothesized that children with FXS would show significant slowing in intellectual functioning over time when compared to their typically developing age-matched peers, particularly in performance-based areas. We also hypothesized that lower levels of FMRP would be strongly associated with impairments in intellectual development, and that gender would not be associated with intellectual development in FXS when controlling for FMRP levels.

Method

Subjects

Participants were 290 children (145 pairs of siblings from 145 families). Each pair consisted of a child diagnosed with fragile X syndrome (FXS), and an unaffected biological sibling. Criteria for inclusion were: (1) a child aged 6–16 years who had the full mutation associated with FXS (>200 CGG repeats and evidence of aberrant *FMR1* methylation), (2) a biological sibling living in the same household aged 6–16 years who had tested negative for any form of *FMR1* mutation (<40 CGG repeats), (3) the children were both at school, and (4) the mother of the children was a carrier of the *FMR1* premutation (55 < CGG repeats < 200 and no evidence of aberrant *FMR1* methylation). If families had more than one typically developing sibling in the target age range, a same-gender sibling closest in age to the child with FXS was chosen to take part in the study. If families had more than one child with FXS in the target age range¹ a female child with FXS closest in age to a typically developing sibling was chosen. This was done in order to increase the number of females and gender-matched siblings in the sample. Sixty percent of the sibling pairs were gender-matched at time 1. There were no significant differences in the mean ages of the children with FXS and their unaffected siblings. All children had participated in a previous study investigating the development of children with FXS compared to their typically developing siblings (see Hall et al. 2007). Children were recruited from across the USA (west, 28.9%; south, 26.3%; northcentral, 24.6%; northeast, 15.8%) and Canada (4.4%) through the National Fragile X Foundation, flyers distributed to special interest groups, local contacts, and our research website. Written informed consent was obtained from the parents of all participants. Diagnostic status of affected and unaffected children, as well as the mother of the children, was confirmed by PCR and Southern Blot DNA analyses (Kimball Genetics, Inc).

Measures

Demographic Questionnaire—Parents completed a family information form detailing the age and gender of family members, marital status, ethnicity, parent education, and family income. Table 1 shows a breakdown of the demographic characteristics.

Fragile X Mental Retardation Protein (FMRP)—Blood drawing kits and consent forms were mailed directly to each family in order to obtain FMRP levels for the child with FXS. Blood draws were performed by a local physician or lab and samples were mailed directly to Kimball Genetics using overnight mail. FMRP immunostaining, an indirect alkaline phosphatase technique, was used (Willemsen et al. 1997). Slides were analyzed under the microscope, distinguishing lymphocytes from other blood cell types by morphology. For each slide, 200 lymphocytes were scored, and the percentage of lymphocytes expressing FMRP was determined. Scoring was performed in blinded fashion with respect to DNA results. Mean FMRP levels were 13.05% (SD=12.86, range=1% to 74.5%) in boys with FXS and 53.51% (SD=18.39, range 14% to 95.5%) in girls with FXS. FMRP values were utilized from time 1 testing only after initial sampling of a subgroup of time 2 FMRP levels confirmed very high correlations with initial values. Fifty-eight (64.4%) of the boys with

¹Some children with FXS also had siblings with FXS, although diagnosis was not always confirmed.

FXS had the full mutation, 11 (12.2%) had a partially methylated full mutation, and 21 (23.3%) were mosaic for FXS. Of the girls with FXS, 48 (87.3%) had the full mutation and 7 (12.7%) were mosaic for FXS.

Intellectual functioning—The Wechsler Intelligence Scale for Children—Third Edition (WISC-III; Wechsler 1991) is a standardized measure of intellectual functioning for children aged 6 to 16 years. The WISC-III includes five verbal subtests (i.e., information, similarities, vocabulary, comprehension and arithmetic) and five performance subtests (picture completion, picture arrangement, block design, object assembly and coding). The WISC-III was administered to the children with FXS and their unaffected siblings by a trained researcher either in their homes or in our laboratory. In our previous study (Hall et al. 2007), we reported that 28.7% of boys with FXS received standardized IQ scores that were on the floor of the test. To avoid this problem, we used raw scores in the present study. Other advantages of raw scores included the capacity to track the precise rates of intellectual development in different cohorts of children, to determine how these rates change over time, and to measure the effects of key variables, such as gender, age, and FMRP levels, on these rates. Raw scores for each subtest were converted into percentage correct scores (range 0% to 100%). The five subtests from each domain were then averaged to yield a percentage correct verbal score and a percentage correct performance score.

Procedures

The WISC-III was administered to 145 pairs of children at time 1.² A subset of 80 (55.2%) pairs of children also received the WISC-III at time 2. Children with FXS were selected to receive a second assessment if at least 1 year had passed since the first assessment and if the child with FXS had a same-gender unaffected sibling who was still living in the household. In addition, to ensure that the number of females with FXS in the sample remained high, all families containing girls with FXS were prioritized for the second assessment. The average interval between the two assessments was 3.89 years (SE=0.18, range=0.97 to 7.41 years). Forty-one male sibling pairs and 39 female sibling pairs received a second assessment. There were no differences between those children who received only one assessment and those who received two assessments on any of the demographic variables. A \$100 honorarium was paid to each family upon completion of their participation at each time point.

Data Analysis

Statistical analyses were conducted with structural equation modeling (SEM) techniques (AMOS, Version 7.0; Arbuckle 2005) using maximum likelihood methods. SEM allows the investigator to estimate measurement and causal models, to assess the adequacy of each theorized model, and to compare competing models (Bollen 1989; Burns and Nolen-Hoeksema 1992; Tomarken and Waller 2003). Several fit indices were used, including the χ^2 , the χ^2/df , the Tucker–Lewis Index (TLI; Bentler and Bonett 1980), the Comparative Fit

²In the study by Hall et al. (2007), 150 pairs of siblings were included. However, five boys with FXS were unable to complete any of the subtests on the WISC-III at either time point and refused blood draws for the FMRP analysis. These boys, and their unaffected siblings, were therefore excluded from the data analysis in the present study.

Index (CFI; Bentler 1990) and the root mean square error of approximation (RMSEA; Browne and Cudeck 1993) with a 90% confidence limit. The TLI, CFI and RMSEA are sometimes recommended because they are less affected by sample size than the chi-square test (Tomarken and Waller 2003). CFI or TLI values close to 1.0 indicate a good fit, as do RMSEA values below 0.05 (Browne and Cudeck 1993). Changes in chi-square values relative to changes in degrees of freedom (chi-square difference tests) were used to compare nested models. The significance levels of model parameters were determined by examining the critical ratios (CR), a statistic comparable to a *t* statistic with infinite degrees of freedom (Arbuckle 2005). Missing data models were estimated using the direct full-information maximum likelihood (FIML) method (Arbuckle 1996).³

Results

Table 2 lists the means and standard errors for the scores obtained on each subtest of the WISC-III at time 1 and time 2 for children with FXS and their unaffected siblings. The percentage correct scores of both groups of children increased significantly between time 1 and time 2. As expected, mean percentage correct scores on each subtest of the WISC-III were significantly lower in children with FXS (particularly in boys) than in unaffected children at both time 1 and time 2 (all *p* values <0.001). In order to compare the scores between the two groups at time 1 and time 2, we calculated a mean difference score for each subtest at each time point (i.e., scores for each child with FXS were subtracted from the corresponding scores obtained by their unaffected sibling). For both males and females with FXS, the largest difference scores were obtained for the block design and arithmetic subtests at time 1 and time 2. The table also shows that the difference scores on most of the subtests increased from time 1 to time 2. For example, at time 1, when the children were approximately 11 years old, boys with FXS obtained an average score of 16.37% correct on the Information subtest whereas their unaffected siblings obtained a score of 56.59% on the same subtest—a difference of 40.22 percentage points. At time 2, boys with FXS obtained a mean score of 24.81% and unaffected siblings obtained a mean score of 70.51%—a difference of 45.7 percentage points. The gap between the two groups of siblings therefore widened from time 1 to time 2. However, in this analysis, the differences in the ages of the children at each time point and the differences in length of follow-up interval are not accounted for.

In order to examine the development of intellectual functioning in children with FXS and their unaffected siblings, while controlling for age and time interval between test administrations, as well as for gender and FMRP effects, we developed a structural equation model of the data. In this analysis, we used the average of the Verbal subtests and the average of the performance subtests. Table 3 lists the means and standard errors for the measures included in the structural equation model, and the correlations between the variables.

³This method can provide consistent parameter estimates in the presence of missing data, even when the data are not missing completely at random. Three alternative methods of estimating models with missing data include mean substitution, listwise deletion, and pairwise deletion. These methods are less efficient and provide consistent estimates only under the stronger assumption that any missing data are missing completely at random.

Measurement Model

The measurement model is presented in Fig. 1. In this model, circles represent unobserved variables (factors and error terms) and rectangles represent observed variables, including scale scores. One-headed arrows represent directional effects, and two-headed arrows represent correlations. T1 and T2 represent time 1 and time 2, respectively. Verbal and performance scales at time 1 load on the intellectual ability factors at time 1 for children with FXS and their unaffected siblings. Verbal and performance scales at time 2 load on the corresponding intellectual ability factors at time 2. E1 to E8 are the error terms for the verbal and performance scales.⁴ The correlated error terms for the scales at the two time points in both groups of children represent the hypothesis that these error terms contain systematic variance that is stable over time. If these correlations are statistically significant, this would indicate that the variance of the verbal and performance scales can be decomposed into three components: a general intellectual ability factor, and two additional verbal and performance factors that are orthogonal to the general intellectual ability factor and to each other as well. FXS AGE and SIBLING AGE are the ages of the FXS and unaffected siblings at time 1 respectively. INTERVAL is the number of years between assessments, FMRP is the FMRP percentage level in the child with FXS, and GENDER is the gender of the child with FXS (coded 0 for females and 1 for males)⁵. The measurement model was identified by setting one unstandardized factor loading for each factor (i.e., verbal T1 and verbal T2) to 1.0. The unstandardized factor loadings for the error terms were also set to 1.0.

The fit of the measurement model was excellent [$\chi^2(30, N=145)=23.57, p=0.79; \chi^2/df=0.79; TLI=1.01; CFI=1.00; RMSEA=0.00 (0.00-0.04)$]. We conducted two nested tests in which several restrictions were applied to the measurement model. In the first nested test, the regression coefficients for the performance T1 and performance T2 variables were declared to be 1.0 in the intellectual ability factors. In addition, within each factor, the intercepts and error variances for the verbal and performance variables were set to be the same. The increase in chi-square in this nested model was not significant [$\chi^2(12, N=145)=12.46, p=0.41$], indicating that the intellectual ability factors of the affected and unaffected children were super-parallel at both time points.⁶ In the second nested test, the means and variances of the ages of the siblings at time 1 were declared to be equal. The increase in chi-square in this nested model was not significant [$\chi^2(2, N=145)=0.53, p=0.77$], indicating that the ages and variances of the children with FXS and their unaffected siblings were not significantly different. The overall fit of the measurement model with these additional restrictions was excellent [$\chi^2(44, N=145)=36.56, p=0.78, \chi^2/df=0.83; TLI=1.01; CFI=1.00; RMSEA=0.00 (0.00-0.04)$].

The R^2 values for the verbal T1 and performance T1 scales were 91% in the children with FXS, and 88% in their unaffected siblings. The corresponding R^2 values for the verbal T2 and performance T2 scales were 86% in the children with FXS and 79% in their unaffected siblings. These high R^2 values indicate that the intellectual ability factors accounted for the

⁴Some investigators prefer to call these terms “other causes,” since they may contain systematic variance as well as random measurement errors.

⁵There were no significant differences in intellectual ability scores between male and female unaffected siblings. Therefore, the gender of the unaffected siblings was not included in the model.

⁶In a super parallel factor, the regression coefficients, error variances, and intercepts for all the indicators are equal.

majority of the variance in the verbal and performance scales at both time points in both cohorts of children.

The correlation between the intellectual ability factors at time 1 and time 2 was $r(145)=0.94$, $p<0.001$ in children with FXS, and $r(145)=0.87$, $p<0.001$ in the unaffected siblings. The correlations between the error terms for the verbal T1 and verbal T2 scales were $r(145)=0.77$, $p<0.001$ in children with FXS and $r(145)=0.71$, $p<0.001$ in their unaffected siblings. The corresponding values for the error terms for the performance T1 and performance T2 scales were 0.69 and 0.52, ($p<0.001$ for both). These findings indicated that the error terms for the verbal and performance scales did not simply contain random errors of measurement. In addition, these systematic variance components of the error terms were not correlated with gender, FMRP, age at the first evaluation, or by the passage of time following the first evaluation.

As expected, the ages of the FXS children at time 1 (FXS AGE) were correlated with the intellectual ability factor at time 1 [$r(145)=0.43$, $p<0.001$] and with the intellectual ability factor at time 2 [$r(145)=0.21$, $p=0.02$] in children with FXS. The ages of the unaffected children at time 1 (SIB AGE) were also correlated with the intellectual ability factors at time 1 and time 2 in unaffected siblings [$r(145)=0.92$ and 0.79 respectively, $p<0.001$ for both]. These results indicated that the intellectual ability of both groups of children increased with age at both time points. However, the strength of this association appeared to be substantially stronger in the unaffected siblings.

The gender of the children with FXS (GENDER) was strongly and negatively correlated with the intellectual ability factor at time 1 [$r(145)=-0.58$, $p<0.001$] and with the intellectual ability factor at time 2 in children with FXS [$r(145)=-0.64$, $p<0.001$], as expected. This result reflected the fact that the boys with FXS had significantly lower abilities than the girls with FXS. Level of FMRP was positively correlated with the intellectual ability factor at time 1 [$r(145)=0.55$, $p<0.001$] and with the intellectual ability factor at time 2 in children with FXS [$r(145)=0.64$, $p<0.001$], reflecting the fact that individuals with FXS who had higher levels of FMRP had higher intellectual ability scores. GENDER was also significantly negatively correlated with FMRP [$r(145)=-0.79$, $p<0.001$], reflecting the fact that girls with FXS had significantly higher levels of FMRP than boys.

The time between the two assessments (INTERVAL) was significantly correlated with the intellectual ability factor at time 2 in unaffected siblings [$r(145)=0.55$, $p<0.001$], but the correlation between INTERVAL and the intellectual ability factor at time 2 in children with FXS did not achieve statistical significance [$r(145)=-0.09$, $p=n.s.$]. This finding indicated that longer time intervals between assessments were significantly associated with higher intellectual ability scores in the unaffected children, but the association in the children with FXS was marginal at best.

Structural Equation Model

The correlations in the measurement model provide no information about the causal links between age, gender, FMRP, interval and the intellectual ability factors, or the sizes of these effects. Therefore, the structural equation model in Fig. 2 was estimated. In this model,

GENDER, FXS AGE and SIBLING AGE are exogenous variables, and the four intellectual ability factors, FMRP and INTERVAL are endogenous variables. E1 to E8 are the error terms for the indicator variables, and E9 to E12 represent the errors terms for the four intellectual ability factors. E0 is the error term for the FMRP variable. In this model, the causal effects of FMRP on the intellectual ability factors in children with FXS at the two time points are represented as well as the effects of GENDER on FMRP. This means that GENDER, in theory, can have direct effects on the intellectual ability factor of the FXS children at time 1 and indirect effects on the intellectual ability factor at time 2. The model also allows the investigator to determine whether GENDER has any significant effects on intellectual ability that are not mediated by FMRP levels.

The model in Fig. 2 was estimated with all the same constraints as the final measurement model: both intellectual ability factors in each pair of siblings were set to be super-parallel at both time points; and the means and variances of the ages of the pairs of siblings were set to be the same. The fit of this model was excellent [$\chi^2(66, N=145)=61.17, p=0.65, \chi^2/df=0.93$; TLI=1.01; CFI=1.00; RMSEA=0.00 (0.00–0.04)].

Effects of GENDER and FMRP—The unstandardized regression coefficient for the effect of GENDER on FMRP was -39.74 ($SE=2.61, p<0.001$), and the R^2 for FMRP was 62%. This indicated that the FMRP levels in boys with FXS were, on average, 39.74 percentage points lower than the levels of girls with FXS, and that GENDER accounted for approximately two thirds of the variance in FMRP levels in the FXS children. The unstandardized value for the direct effect of GENDER on the intellectual ability factor at time 1 in children with FXS was -14.65 ($SE=2.87, p<0.001$). This result indicated that boys with FXS had ability scores at time 1 that were 14.65 percentage points lower, on average, than the girls with FXS, when controlling for the effects of age and FMRP. However, the total direct and indirect effect of GENDER on the intellectual ability factors in children with FXS was -20.84 and -24.09 respectively. This indicated that the boys with FXS had ability scores at times 1 and 2 that were 20.84 and 24.09 percentage points lower, on average, than the girls with FXS, when controlling for the effects of age and interval between assessments.

The unstandardized values for the direct effects of FMRP on the intellectual ability factors at time 1 and time 2 were 0.16 ($SE=0.06, p<0.001$) and 0.13 ($SE=0.04, p<0.001$), respectively. The unstandardized regression coefficient of 0.16 at time 1 indicates that for each increase of 10 units in FMRP levels, there will be a 1.6 percentage point increase in ability scores at time 1 in the boys and girls with FXS. The total of the direct and indirect effects of FMRP on the intellectual ability factor at time 2 was 0.28, indicating that the intellectual ability scores will increase by 2.8 percentage points at the second time point for every 10 percentage point increase in FMRP levels, when controlling for the effects of age and interval. In children with FXS, FMRP levels explained 5% of the variance in intellectual ability scores at time 1 and 13% of the variance at time 2.

Effects of Age and Interval on the Intellectual Ability Factors—The unstandardized regression coefficient for the effect of age on the intellectual ability factors at time 1 was 2.83 ($SE=0.29; p<0.001$) in the children with FXS and 4.92 ($SE=0.21; p<0.001$) in their unaffected siblings. When these parameters were set to be equal, the increase in chi-

square was highly significant [$\chi^2(1, N=145)=33.41, p<0.001$]. This confirmed that at time 1, in the group of children with FXS, each year of age was associated with an increased score of approximately 2.8 percentage points, on average. In contrast, in the group of unaffected children at time 1, each year of age was associated with an increased score of approximately 4.9 percentage points, on average.

The value for the effect of INTERVAL on the intellectual ability factors at time 2 was 1.64 (SE=0.50, $p<0.001$) in the children with FXS and 3.52 (SE=0.38, $p<0.001$) in their unaffected siblings. When these parameters were set to be equal, the increase in chi-square was highly significant [$\chi^2(1, N=145)=9.87, p=0.002$]. This indicated that the values were different in the two groups, and that the scores on the test were increasing at the rate of 1.6 percentage points per year in the children with FXS and 3.5 percentage points per year in the unaffected siblings between the first and second evaluations.

Correlation Between Intellectual Ability Factors Over Time—The unstandardized regression coefficient for the effect of the intellectual ability factors at time 1 on the intellectual ability factors at time 2 were 0.96 (SE=0.05, $p<0.001$) in children with FXS and 0.66 (SE=0.04, $p<0.001$) in their unaffected siblings. The standardized regression coefficients were similar in the two groups: 0.87 in the children with FXS and 0.86 in their unaffected siblings. These results indicated that the temporal association of the intellectual ability factors was high in each group, since the factors at time 1 accounted for approximately 75% of the variance in the factors at time 2.⁷

The explanatory power of the model was considerable. The R^2 values for the intellectual ability factors at time 1 were 64% in the children with FXS and 84% in their unaffected siblings. The R^2 values for the intellectual ability factors at time 2 were 93% in the children with FXS, and 95% in the unaffected siblings.

Discussion

We used structural equation modeling to estimate the rate of intellectual development in a large school-age cohort of girls and boys with FXS and in their unaffected siblings at two time points separated by approximately 4 years. To overcome methodological problems associated with previous studies, we administered the same instrument at both time points and employed raw scores, converted into percentage correct scores, rather than standardized IQ scores. Although the intellectual functioning of both groups of children improved during the study, as evidenced by increasing percentage correct scores in both groups, the annual rate of intellectual development was substantially faster in the unaffected children, as compared with their siblings with FXS. Specifically, during the interval between evaluations, the unaffected children gained 2.2 times as many percentage points per year, as compared with the FXS group. Given that the IQ's of the unaffected children (derived as standardized scores) presumably remained stable over the course of this study, this widening gap between the two groups accounts for the apparent drop in IQ scores in children with FXS reported by

⁷The error terms between the intellectual ability factors are correlated at time 1 and time 2. The magnitude depends in large part on the R^2 values for the factors. At time 2, they are very high, so only a small amount of systematic variance is left in the error terms. Hence, the error terms are highly correlated.

previous investigators (e.g., Fisch et al. 1994, 1996). This apparent drop in the IQ's of children with FXS over time therefore reflects a greater slowing of intellectual development than unaffected children. This study supports the findings of Skinner et al. (2005) who showed that suboptimal intellectual growth may have been responsible for the declining nonverbal IQ scores of 45 young males with FXS. A unique contribution of this study is that children were directly compared to their unaffected siblings and that both nonverbal and verbal scores were assessed using a comprehensive test of intellectual ability in a large sample of school-aged males and females with FXS.

With gender and age included in the model, FMRP appeared to have significant effects on intellectual functioning in the children with FXS at both time points, but only accounted for 5% of the variance in intellectual ability scores at the first evaluation, and 13% of the variance at the second evaluation. We found that gender still had strong effects on the annual rate of intellectual development in the children with FXS when controlling for age and FMRP levels in the blood. This finding strongly suggests that other biological abnormalities that are correlated with gender may contribute to the slowed intellectual development in children with FXS. It is possible that a significant proportion of the variance in intellectual functioning in FXS may be attributable to other biological influences, such as genes whose expression are regulated by FMRP, gender by environment interactions, or gender-specific hormonal effects.

However, it should be pointed out that FMRP levels were highly correlated with gender. This is because FMRP ranges for males and females with FXS were largely non-overlapping and the distributions of FMRP levels within gender were also restricted in range. In the measurement model, when the effects of gender and age are removed, FMRP levels were correlated with intellectual ability at time 1 ($r=0.55$) and at time 2 ($r=0.64$) in children with FXS. This appears to be a potentially vexing conceptual issue for all studies of individuals with X-linked disorders where biological markers like FMRP are highly correlated with gender.

The percentage of variance assigned to FMRP is considerably lower than the values in the range of 12% to 68% reported by some previous investigators. It is conceivable that the high values reported by previous investigators were the result of the rather small Ns they employed. Other methodological differences in the studies may also play a role. For example, in the Tassone et al. (1999a, b) study, a large age range of subjects was included (1 to 60 years) and subjects received a variety of different IQ tests (with different subtests and different normative groups). In addition, the relationship between FMRP and FXS was examined within gender and by methylation status so that the resulting sample sizes were very small. As noted by the authors themselves, the distributions of the data in their study were also highly skewed and contained outliers that may have contributed to an artifactual statistical effect.

It should be noted that the correlation between FMRP and intellectual ability could be explained by a causal effect of FMRP on intellectual ability, a causal effect of intellectual ability on FMRP, or by an unknown third variable with simultaneous causal effects on FMRP and on intellectual ability. In this paper, we have assumed that the direction of

causality is from FMRP to intellectual ability. In separate analyses, we have tested the other possibilities using non-recursive modeling techniques. These analyses clearly showed that these other possibilities can be rejected. However, one still must be cautious in describing these effects as “causal”. The results of our analyses indicated that decreased FMRP levels in children with FXS, or FMRP effects on downstream neurobiological systems, contributed to the impaired learning in these children.

This is the first time that the precise effects of FMRP on intellectual development have been estimated over time in boys and girls with FXS. If, in theory, the boys’ FMRP levels could be increased up to the normal range (approximately 90–100%), the expected increase in scores on both the verbal and performance scales would be approximately 12 percentage points at the first evaluation and 21 percentage points at the second evaluation.⁸ As a result, their mean percent correct verbal and performance scores would approximately double, increasing from 13 to 25 percentage points at time 1, and from 20 to 41 percentage points at time 2. In girls, if FMRP levels could be increased to the normal range, their scores on the verbal and performance scales would be expected to increase from 32 to 37 percentage points at the first evaluation, and from 44 to 55 percentage points at the second evaluation, values that are still well below the mean verbal and performance scores of the unaffected boys and girls at times 1 and 2 (58 and 73 percentage points respectively).

However, if FMRP levels in the CNS could be measured, the estimate of the effects of FMRP might be substantially greater. For example, blood levels of FMRP may be poorly correlated with FMRP levels in the brain. Indeed, several investigators have pointed out that FMRP in the blood is synthesized in leukocytes, a tissue of mesodermal origin. In contrast, brain FMRP is of ectodermal origin (Abrams et al. 1999; Tassone et al. 1999a; Willemsen et al. 1999). In addition, we measured the effects of FMRP at 11 and 15 years of age, when intellectual functioning was already rather severely impaired. The remediation of FMRP deficiencies shortly after birth might have even more powerful beneficial effects on intellectual development. In fact, one would hope that remediation of FMRP associated neurobiological abnormalities might boost the scores of children with FXS into the normal range. The remediation of cognitive deficits associated with FXS may depend on the timeframe at which the intervention is introduced, and it seems likely that the time-intervention function may be non-linear, with early remediation being significantly more effective.

We found that the greatest differences between children with FXS and their unaffected siblings occurred on the tests of Arithmetic and Block Design. These data confirm previous reports suggesting that these skills are specific weaknesses in individuals with FXS (e.g., Bennetto et al. 2001; Mazzocco et al. 1992). Interestingly, Hagerman et al. have pointed out that abstract reasoning and symbolic language skills are often stressed in the IQ tests of later childhood and adolescence. That is, in some IQ tests, subtests are added to the assessment battery, thereby increasing the task demands at later ages (see also Hay 1994) and thus

⁸In the boys with FXS, these values were obtained by multiplying the total effect of FMRP on intellectual ability at each time point times 80, an increase that would bring the mean FMRP level from 13% to 93%. Corresponding values for the girls were obtained by multiplying the total effect of FMRP on intellectual ability at each time point times 40, an increase that would bring the mean FMRP level from 43% to 93%.

leading to apparent declines in IQ. To avoid this problem, we believe that investigators should endeavor to administer the same set of subtests to the same individuals at each time point.

Another unexpected finding was that there was no significant difference in the annual rate of intellectual development in verbal versus performance abilities in the children with FXS. This is surprising given that previous studies have suggested that some verbal based abilities may be spared in children with FXS. The vast majority of the variance in the verbal and performance scales was accounted for by the general intellectual ability factor at both time points in both groups of children. However, while our study did detect the presence of separate verbal and performance factors that were orthogonal to the general intellectual ability factor, they only accounted for a small amount of variance in the verbal and performance scales.

Measurement of the annual rate of intellectual development may present researchers with a new tool for evaluating intellectual functioning in typically developing individuals as well as those who are affected with different types of mental retardation or brain dysfunction. Unlike the IQ score, which is static over time, the annual rate of intellectual development is a dynamic coefficient that reflects the rate of intellectual development at different ages. Previous investigators have reported decreases in IQ scores over time in children with FXS, but not in unaffected children, and the explanation for this phenomenon has been unclear. Our examination of changes in the annual rate of intellectual development over time puts the previous findings in a new perspective. The percentage correct scores of both groups of children increased significantly between time 1 and time 2. This means that children with FXS were actually improving and learning throughout the course of the study. However, we observed a slowing of the annual rate of intellectual development in the affected and unaffected children alike during the time interval between the evaluations. In fact, between the first and second evaluations, children with FXS were only improving at the rate of 1.6 percentage points per year, as compared with 3.5 percentage points in their siblings. The apparent decrease in standardized IQ scores in the FXS children actually results from the greater decreases in the annual rate of intellectual development over time in the FXS children, as compared with their siblings.

To the best of our knowledge, this is the first longitudinal study of intellectual development to include a large sample of both boys and girls with fragile X syndrome whose ages extend through the critical school age years. We hope that this information might also help clinicians develop treatment or training programs that will increase the success and well being of these children as they transition out of school, a critical time in the maturation of individuals with developmental disabilities. Further understanding of the specific intellectual skills that are most vulnerable to suboptimal development may provide clues as to how to best target interventions and improve clinical outcomes in the future.

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Fig. 1.

Measurement model illustrating the factor structure for the intellectual ability factors in children with FXS and their unaffected siblings. E1 to E8 are error terms for the scales. *Two-headed arrows* represent correlations, and *one-headed arrows* represent directional effects. Factor loadings are placed next to each factor indicator. R^2 values for each indicator can be obtained by squaring the standardized factor loadings. Only significant correlations are shown. * $p < 0.05$; ** $p < 0.01$

Fig. 2.

Structural equation model of the effect of GENDER, FMRP, FXS AGE, SIBLING AGE, and INTERVAL on the intellectual ability factors. E1 to E8 are the error terms for the factor indicators, while E9 to E12 are the error terms for the factors. E0 is the error term for FMRP. Unstandardized beta coefficients are indicated next to *one-headed arrows*, R^2 values are indicated next to the dependent variables, and correlations are indicated next to *two-headed arrows*. $^*p < 0.05$; $^{**}p < 0.01$

Table 1

Demographic characteristics

Variable	Means (SD) or percentages
FXS gender (% boys)	62.1
Sibling gender (% boys)	49.0
FXS age (years)	10.9 (2.9)
Sibling age (years)	11.1 (3.0)
Sibling (% older than FXS)	56.0
Age difference between sibling and FXS (years)	0.2 (3.4)
Other siblings in family	0.8 (0.9)
Mother age (years)	40.0 (4.9)
Mother education ^a	4.3 (1.0)
% Married	83.5
Income ^b	3.0 (0.9)
Ethnicity (% white)	82.7

^a 1=8th grade or less, 2=partial high school, 3=high school graduate, 4=partial college, 5=college graduate, 6=graduate degree

^b 1=less than \$20,000, 2=\$20,000 to \$50,000, 3=\$50,000 to 00,000, 4=00,000 to 50,000, 5=over 50,000

Table 2

Means and standard errors (in parentheses) for the percentage correct subtest scores on the WISC-III at time 1 and time 2 in boys and girls with FXS and their unaffected siblings

Time 1					Time 2				
Subtest	Boys with FXS (N=90)	Unaffected siblings (N=90)	<i>p</i>	Mean difference score	Boys with FXS (N=41)	Unaffected siblings (N=41)	<i>p</i>	Mean difference score	
Information	16.37 (1.07)	56.59 (2.02)	<0.001	40.22	24.81 (2.40)	70.51 (2.83)	<0.001	45.70	
Arithmetic	9.85 (0.81)	60.41 (1.65)	<0.001	50.56	16.88 (2.23)	70.26 (2.32)	<0.001	53.38	
Comprehension	13.95 (1.55)	60.68 (2.00)	<0.001	46.73	23.54 (2.71)	73.72 (2.65)	<0.001	50.18	
Similarities	12.05 (1.30)	56.16 (1.99)	<0.001	44.11	19.25 (2.53)	69.46 (2.59)	<0.001	50.21	
Vocabulary	14.26 (1.03)	52.09 (1.96)	<0.001	37.83	21.50 (2.24)	66.28 (2.78)	<0.001	44.78	
Picture Arr.	7.43 (1.05)	48.23 (2.16)	<0.001	40.80	12.69 (2.54)	59.56 (3.22)	<0.001	46.87	
Object assembly	17.98 (1.46)	64.44 (2.08)	<0.001	46.46	23.97 (3.03)	76.04 (2.03)	<0.001	52.07	
Picture Comp.	22.19 (1.71)	67.44 (1.68)	<0.001	45.25	36.36 (3.48)	79.87 (1.75)	<0.001	43.51	
Coding (B)	10.78 (1.19)	40.98 (1.90)	<0.001	30.20	17.22 (1.90)	49.64 (2.48)	<0.001	32.42	
Block Design	6.67 (1.05)	61.34 (2.48)	<0.001	54.67	11.77 (1.95)	77.31 (2.68)	<0.001	65.54	
Subtest	Girls with FXS (N=55)	Unaffected siblings (N=55)	<i>p</i>	Mean difference score	Girls with FXS (N=39)	Unaffected siblings (N=39)	<i>p</i>	Mean difference score	
Information	37.18 (2.93)	54.78 (2.75)	<0.001	17.60	42.87 (3.41)	65.71 (3.42)	<0.001	22.84	
Arithmetic	31.09 (2.43)	61.07 (2.26)	<0.001	29.98	36.09 (3.48)	64.52 (2.29)	<0.001	28.43	
Comprehension	36.88 (3.17)	59.49 (2.70)	<0.001	22.61	39.75 (4.08)	68.95 (3.48)	<0.001	29.20	
Similarities	31.18 (3.07)	55.98 (2.41)	<0.001	24.80	36.57 (3.64)	64.29 (2.97)	<0.001	27.72	
Vocabulary	31.09 (2.34)	51.29 (2.31)	<0.001	20.20	35.06 (3.25)	62.32 (3.06)	<0.001	27.26	
Picture Arr.	26.53 (3.03)	49.74 (2.73)	<0.001	23.21	31.52 (4.45)	59.21 (4.18)	<0.001	27.69	
Object assembly	33.75 (3.06)	63.38 (2.66)	<0.001	29.64	42.87 (4.32)	74.79 (3.20)	<0.001	31.92	
Picture Comp.	45.45 (3.19)	67.23 (2.32)	<0.001	21.78	51.49 (3.96)	75.12 (2.82)	<0.001	23.63	
Coding (B)	28.94 (2.24)	41.33 (2.19)	<0.001	12.39	34.24 (2.73)	51.44 (2.50)	<0.001	17.20	
Block design	24.14 (2.98)	60.87 (2.89)	<0.001	36.73	32.18 (4.48)	72.21 (3.69)	<0.001	40.03	
Subtest	Children with FXS (N=145)	Unaffected siblings (N=145)	<i>p</i>	Mean difference score	Children with FXS (N=80)	Unaffected siblings (N=80)	<i>p</i>	Mean difference score	
Information	23.99 (1.52)	55.92 (1.62)	<0.001	31.93	33.34 (2.33)	68.03 (2.26)	<0.001	34.68	
Arithmetic	17.63 (1.34)	60.65 (1.33)	<0.001	43.02	25.96 (2.34)	67.28 (1.67)	<0.001	41.32	
Comprehension	22.33 (1.78)	60.24 (1.61)	<0.001	37.91	31.16 (2.60)	71.25 (2.22)	<0.001	40.08	
Similarities	19.06 (1.59)	56.09 (1.53)	<0.001	37.04	27.46 (2.42)	66.78 (2.01)	<0.001	39.32	
Vocabulary	20.42 (1.29)	51.80 (1.50)	<0.001	31.37	27.87 (2.11)	64.23 (2.09)	<0.001	36.35	
Picture Arr.	14.43 (1.51)	48.79 (1.69)	<0.001	34.36	21.50 (2.75)	59.38 (2.65)	<0.001	37.88	
Object assembly	23.76 (1.58)	64.05 (1.63)	<0.001	40.29	32.81 (2.84)	75.43 (1.93)	<0.001	42.63	
Picture Comp.	30.70 (1.85)	67.37 (1.36)	<0.001	36.66	43.44 (2.78)	77.41 (1.71)	<0.001	33.97	
Coding (B)	17.17 (1.33)	40.63 (1.49)	<0.001	23.46	25.18 (1.96)	50.57 (1.76)	<0.001	25.40	
Block design	13.06 (1.46)	61.16 (1.89)	<0.001	48.10	21.32 (2.67)	74.67 (2.32)	<0.001	53.35	

Chi-square tests of the assumption that there were no significant differences in each mean across groups, together with difference scores between the two groups, are also shown

Table 3

Correlation matrix

	Mean	SE	1	2	3	4	5	6	7	8	9	10	11	12
1. GENDER	0.62	0.04	1.00											
2. FMRP	28.09	2.05	-0.79	1.00										
3. FXS AGE	10.88	0.24	0.12	-0.10	1.00									
4. SIBLING AGE	11.07	0.25	0.08	-0.12	0.34	1.00								
5. FXS Verbal T1	20.71	1.39	-0.56	0.53	0.41	0.07	1.00							
6. FXS Perf. T1	20.05	1.37	-0.55	0.52	0.40	0.06	0.91	1.00						
7. FXS Verbal T2	27.87	1.71	-0.61	0.60	0.20	0.04	0.92	0.85	1.00					
8. FXS Perf. T2	28.01	1.77	-0.58	0.57	0.19	0.04	0.82	0.91	0.86	1.00				
9. SIBLING Verbal T1	56.44	1.44	0.05	-0.08	0.26	0.88	0.12	0.12	0.10	0.09	1.00			
10. SIBLING Perf. T1	56.44	1.35	0.05	-0.08	0.25	0.85	0.12	0.12	0.09	0.09	0.89	1.00		
11. SIBLING Verbal T2	71.59	1.39	0.11	-0.14	0.09	0.70	0.04	0.04	0.10	0.09	0.84	0.72	1.00	
12. SIBLING Perf. T2	71.74	1.26	0.11	-0.15	0.09	0.72	0.04	0.04	0.10	0.09	0.75	0.81	0.80	1.00
13. INTERVAL	3.96	0.17	0.25	-0.25	-0.04	0.17	-0.17	-0.17	-0.08	-0.08	0.13	0.13	0.48	0.49

GENDER gender of individual with fragile X (coded 0 for females, 1 for males), *FMRP* fragile X mental retardation protein, *Verbal* percentage correct score on verbal subtests, *Performance* percentage correct score on performance subtests, *T1* time 1, *T2* time 2, *FXS* fragile X syndrome, *SIBLING* unaffected sibling, *INTERVAL* years between time 1 and time 2