The Influence of Environmental and Genetic Factors on Behavior Problems and Autistic Symptoms in Boys and Girls With Fragile X Syndrome

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ABSTRACT. *Objective.* **Fragile X syndrome, caused by mutations in a single gene of the X chromosome (FMR1), is associated with neurobehavioral characteristics including social deficits with peers, social withdrawal, gaze aversion, inattention, hyperactivity, anxiety, depression, and autistic behavior. However, there is considerable variability in the behavioral and psychiatric problems among children with this condition. The purpose of this study was to measure genetic and environmental factors influencing behavior problems and autistic symptoms in children with fragile X syndrome.**

Design. **We conducted an in-home evaluation of 120 children (80 boys and 40 girls) with the fragile X full mutation and their unaffected siblings, including measurements of the FMR1 protein (FMRP), quality of the home environment, maternal and paternal psychopathology, effectiveness of educational and therapeutic services, and child behavior problems.**

Results. **Results of multiple regression analyses showed that for boys with fragile X, effectiveness of educational and therapeutic services and parental psychological problems predicted internalizing and externalizing types of problems, while the quality of the home environment predicted autistic behavior. For girls with fragile X, the results emphasized significant effects of FMRP on behavior, in particular social withdrawal and anxious/depressed behavior.**

Conclusions. **These findings are among the first to link FMRP expression to behavior. They also emphasize the significance of home- and school-based environmental variables in the neurobehavioral phenotype and help to lay the foundation for studies designed to identify specific interventions for reducing problem behavior in children with fragile X syndrome.** *Pediatrics* **2001;108(5). URL: http://www.pediatrics.org/cgi/content/full/108/5/e88;** ***fragile X syndrome, FMR1 protein, home environment, special education, autistic behavior.***

ABBREVIATIONS. FMRP, FMR1 protein; M, mean; SD, standard deviation; WISC-III, Wechsler Intelligence Scale for Children–Third Edition; CBCL, Child Behavior Checklist; SCL-90-R, Symptom Checklist-90-Revised; HOME, Home Observation for Measurement of the Environment; SCORS, Special Curriculum Opportunity Rating Scale; NS, not significant.

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Fragile X syndrome, caused by mutations in a single gene on the long arm of the X chromosome, occurs in 1 of every 2000 to 5000 live births and is the most common known inherited cause of mental retardation. The cytogenetic fragile site on the X chromosome from which the syndrome derives its name is typically caused by the presence of >200 cytosine-guanine-guanine triplet repeats within the promoter region of the FMR1 gene, which prevents normal transcription. This "transcriptional silencing" of the gene and the subsequent diminished or absent production of the FMR1 protein (FMRP) results in aberrant brain development and function.[1,2] Because females have 2 X chromosomes, production of FMRP is maintained to varying degrees by the presence of the unaffected X chromosome. Variability in the production of FMRP also may be the result of a condition known as mosaicism, in which transcriptional silencing of the gene occurs in some, but not all cells, either because of varying sizes of the repeat expansion or variation in methylation patterns. Mosaicism can occur in both females as well as males with fragile X. Individual differences in FMRP production in the brain as a result of these processes are thought to account for a significant proportion of the variability in cognitive outcome in individuals with fragile X.

In addition to cognitive impairment, individuals with fragile X display a characteristic profile of behavioral and psychiatric difficulties. Most well-controlled studies demonstrate that persons with fragile X are at increased risk for particular maladaptive behaviors, including hyperarousal, social anxiety and withdrawal, and attention problems.[3–6] In males, these behaviors include social deficits with peers, abnormalities in communication, unusual responses to sensory stimuli, stereotypic behavior, social avoidance, gaze aversion, inattention, impulsivity, and hyperactivity.[7–15] Young girls with fragile X also exhibit maladaptive behaviors, including problems with depression, social withdrawal, and hyperactivity[6,16,17]; however, these symptoms tend to be less severe than in boys with fragile X. Consequently, individuals with fragile X are at increased risk for

psychiatric disorders, most notably anxiety, mood, and attention deficit/disruptive behavior disorders. Furthermore, many behavioral characteristics of children with fragile X are similar to those of children with autism. The proportion of persons with fragile X who meet criteria for autism is estimated to be 7% to 25%.[5,18–21] Although this overlap is not high, children with fragile X who do not meet diagnostic criteria for autism nevertheless demonstrate autistic-like behaviors such as stereotypy, avoidance of eye contact, social shyness, perseverative speech, and tactile defensiveness.[18]

Although a common behavioral profile has been described, there is considerable variability in behavior and psychiatric problems among children with fragile X syndrome, ranging from severely autistic behavior to normal functioning. It is important to describe the full range of this variability and to account for sources of this variation in children with this condition.[22] This will serve to avoid the promulgation of inaccurate information and stereotypes, provide parents and professionals with a broader picture of possible developmental trajectories, and hopefully illuminate sources of variation that are relatively fixed versus those which are amenable to intervention. As is the case with cognitive functioning, it is possible that the variability in behavior is, in part, attributable to molecular genetic variables associated with fragile X. Although FMRP has been consistently associated with intelligence,[23–25] only 1 study to date has investigated the influence of FMRP on behavior in individuals with fragile X.[23] In this study, a negative correlation between FMRP and the prevalence of 10 typical fragile X behaviors was observed, but only in males with mosaicism. The association between FMRP and other types of behavior problems common in individuals with fragile X syndrome, including autistic behavior, is not known.

In addition to possible effects of FMRP, variations in the environment, including characteristics of the family, home, and educational setting, may ameliorate or exacerbate behavioral and psychiatric problems associated with fragile X. For example, it is possible that psychological characteristics of parents, including a background genetic predisposition to psychopathology, may contribute to child problems in addition to the specific effects of the fragile X mutation. Examination of the home and school environment also may yield important information about the nongenetic sources of problem behavior. This approach is important because identification of environmental effects on behavior (ie, effectiveness of educational or therapeutic services, characteristics of parents or parenting, family emotional climate) will lead to more targeted and effective interventions. Furthermore, although biologically based treatments to reverse some of the effects of fragile X are possible, including pharmacological therapy and protein replacement, the impact of the decrement in FMRP is likely to occur very early in development when the nervous system undergoes its most rapid growth and long before significant behavior problems are detected. Finally, the effects of fragile X extend beyond the individual child to the family system. The stress associated with the presence of a child with developmental disability affects the family as a system as well as the parents and siblings individually.[26]

To date, no study has comprehensively examined the influence of both genetic and environmental factors on behavioral outcomes in a large sample of children with fragile X syndrome. We believe that investigation of both child-based genetic factors as well as family- and school-based environmental factors in families affected by fragile X is necessary to ultimately identify specific points at which interventions aimed at reducing problem behavior will be treatment- and cost-effective.

In the current study, we sought to establish a better understanding of the association between specific genetic and environmental factors and behavioral outcome in children with fragile X in comparison to their siblings unaffected by fragile X. The inclusion of the sibling comparison group is important because of the need to establish relations between predictors and behavioral outcomes in children unaffected by fragile X, but who predominantly share the same environment. The study, based on a full day in-home evaluation, used molecular analysis of the fragile X protein, cognitive evaluations of parents and children, direct observation of the home environment including parent and child behavior, and parent report of child behavior.

METHODS

Participants

Families having at least 1 child with the fragile X full mutation and 1 child without fragile X were recruited for the study. To eliminate potential confounding effects of maternal problems associated with having a full mutation, only families in which mothers had a premutation were included in the current analyses. In addition, each fragile X–sibling pair of children had the same biological mother and father. The diagnoses of children with fragile X, their siblings, and mothers were confirmed by southern blot DNA analysis.

Participants were 120 children with the fragile X full mutation (40 girls and 80 boys; 5 girls and 9 boys were mosaic) and their unaffected siblings (62 girls and 58 boys). All mothers and 85% of fathers participated in the study. Children were between 6 and 17 years of age (fragile X: mean [M] = 10.76, standard deviation [SD] = 2.83; unaffected siblings: M = 11.20, SD = 3.10). The sample of children was 91.7% white, 2.5% Hispanic, 2.5% black, 1.7% Asian, 0.8% Pacific Islander, and 0.8% multi-ethnic. Families in 36 United States and Canada, in urban, suburban, and rural areas, were represented in the sample. The parents' highest level of education was 0.8% partial high school, 10.8% high school diploma, 34.2% partial college, 30.8% college degree, and 23.3% graduate degree. Potential participants were excluded on the basis of other known medical problems or signs of current illness.

Families were recruited from an existing fragile X registry, responses from advertisements placed in various fragile X association newsletters, a national fragile X e-mail list, the Stanford Psychiatry Department research website, and through referrals from other researchers, the National Fragile X Foundation, clinicians, and families.

Procedures

To determine a family's eligibility, results of previous fragile X testing were requested. DNA testing for the FMR1 mutation was conducted on all probands and previously untested family members. FMRP percentage was obtained for all children with fragile X in the study (see below). For these tests, families were mailed a testing kit, allowing the blood draw(s) to be conducted before the visit and in their own physician's office or at a community clinic.

Blood samples were sent directly from the blood draw site to the genetics testing facility by overnight mail.

Measures

Fragile X Diagnosis and FMRP Analysis

Southern blot analyses were performed by Kimball Genetics, Inc (Denver, CO) as detailed by Taylor and colleagues.[27] FMRP immunostaining, an indirect alkaline phosphatase technique, was used according to Willemsen et al.[28–30] To measure FMRP, slides were analyzed under the microscope, distinguishing lymphocytes from other blood cell types by morphology. Granulocytes stain nonspecifically and therefore only lymphocytes are counted. 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.

Intelligence

Children were administered the Wechsler Intelligence Scale for Children–Third Edition[31] (WISC-III; The WISC-III is a standardized intellectual assessment for children ages 6–16 years yielding Verbal, Performance, and Full Scale IQ scores.)

Behavior Problems

The Child Behavior Checklist[32] (CBCL) is a well-standardized and widely used instrument, with several factors including withdrawn behavior, social problems, anxiety and depression, somatic complaints, aggressive behavior, delinquent behavior, as well as overall internalizing and externalizing behavior scores. Autistic behavior was measured using the Autism Behavior Checklist,[33] which includes 57 behavioral characteristics of autism in 5 areas: sensory, relating, body and object use, language, and social and self-help. The child's mother was the respondent for both of these measures.

Parental Psychological Symptoms

The Symptom Checklist—90 Revised[34] (SCL-90-R) is a 90-item self-report of current psychological symptoms. The SCL-90-R yields 9 primary symptom dimensions (somaticism, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism) and 3 global indices (global severity, positive symptom distress, and positive symptom total).

Assessment of Home Environment

The home environment was assessed using the Home Observation for Measurement of the Environment[35] (HOME). The HOME is a semistructured interview and observation done in the family home. Factors include parent responsivity, encouragement of maturity in the child, acceptance of the child, learning materials present in the home, effort to provide cultural, recreational, or artistic enrichment, family companionship, and the quality of the physical environment of the home. For purposes of interrater reliability, 2 examiners made independent ratings of observational items on the HOME during visits of 22 homes. Then, approximately 2 weeks after the visit, 1 of the examiners (who tested the children and did not administer the parent interviews) contacted the parent by phone to administer the interview items. Interrater reliability for the HOME total score was high (intraclass correlation = 0.84).

Family Economic Status

Household income was adjusted for regional differences in housing and cost of living. Parent report of gross annual household income was divided by the median household income in the family's area as defined by the home's zip code. The zip code median income was determined by Decisionmark Corporation (Cedar Rapids, IA) and based on the 1990 US Census data and the Census Bureau's 1998 estimates and 2003 projections. This data was obtained from the world wide web via www.homes.com.

Educational and Therapeutic Services

Because of a dearth of measures designed to assess the effectiveness of special education services, a new measure was developed for this purpose. The Special Curriculum Opportunity Rating Scale (SCORS; unpublished data) includes a 15-item Q-sort allowing the parent to rank the cognitive and behavioral skills that a child needs to develop. The parent then ranks the same 15 items according to how much the skills have actually improved in the past 6 months. The items include academic, emotion management, planning, social, speech and language, and other skills needed for development. The correlation of the two 15-item Q-sorts is a measure of the effectiveness of a child's educational and therapeutic services to meet his or her current developmental needs. Test-retest reliability 1 to 2 weeks apart with 15 children was adequate for the developmental needs (r = .69) and improvement (r = .68) Q-sorts. Initial validation studies demonstrate that the SCORS has good convergent and discriminant validity within this fragile X sample.

Data Analysis

We first sought to examine the behavioral profiles of boys and girls with fragile X syndrome and their siblings. Specifically, we wished to identify the behavior domains reported to be most problematic for children with fragile X, as well as the domains that are relatively less affected by the syndrome. To accomplish this, we conducted a multivariate analysis of variance using group (boys with fragile X, girls with fragile X, male comparison siblings, and female comparison siblings) as the independent variable and the syndrome and composite scales of the CBCL as the dependent variables.

Next, in preparation for the multiple regression analyses, we examined bivariate correlations between planned independent variables and dependent variables for purposes of data reduction and model simplification.

To examine the variance in behavior problems accounted for by environmental and biological/genetic factors, we conducted hierarchical multiple regression analyses separately for boys with fragile X, girls with fragile X, and comparison siblings. A hierarchical, rather than simultaneous approach, was taken to determine the relative contributions of biological/genetic versus environmental factors on child behavior problems as pertaining to a priori hypotheses. Biological/genetic, or innate characteristics of the child, including gender (applicable to analyses involving comparison siblings), IQ, and FMRP percentage, were entered on the first step. Child IQ was included in this step to account for variation in behavior that is attributable to developmental disability.[36] (FMRP and effectiveness of services were not relevant to the analysis of comparison siblings and not included in regression analyses pertaining to this group.) Environmental factors, including the quality of the home environment (HOME total score), parental psychopathology (mean of father and mother SCL-90-R Global Severity Index t scores), and effectiveness of educational and therapeutic services (SCORS correlation) were entered in the second step. Parental psychopathology was included in this step to account for the well-documented association between parent and child problems, and also to examine whether this association holds in the case of children with fragile X syndrome. For each group, regressions on total behavior problems, internalizing behavior problems, externalizing behavior problems, and autistic behavior, were performed. Follow-up analogous regressions on the withdrawn, anxious/depressed, thought, and attention problem subscales of the CBCL were performed given their clinical relevance for fragile X as shown in previous studies and the current data set (Fig 1).

RESULTS

Descriptive Statistics

Descriptive statistics of each independent variable by group are presented in Table 1. Boys with fragile X had a mean full scale IQ of 46.56 (moderate mental retardation range); however, the variability was attenuated by a floor effect (39% of boys with fragile X obtained the lowest possible IQ score, 40). Girls with fragile X had a mean full scale IQ of 75.48 (borderline intellectual functioning range) and considerable variability, with scores ranging from the moderate mental retardation to superior range. Siblings had IQ scores similar to the WISC-III normative sample

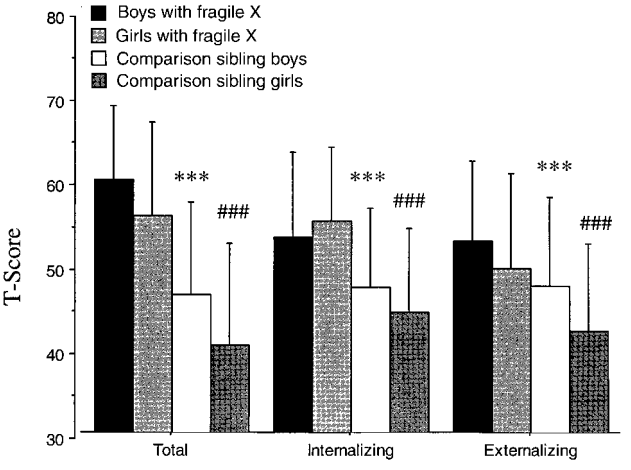

Fig 1. CBCL syndrome *t* scores of children with the fragile X full mutation (79 boys and 40 girls) and their siblings (58 boys and 61 girls). *** Fragile X boys > sibling boys, $P < .001$; ### fragile X girls > sibling girls, $P < .001$. Error bars represent 1 SD.

(M = 107.55; SD: 12.21). FMRP ranged from 1.5% to 74% (M = 12.09, SD: 11.57) in boys with fragile X and from 14% to 77.7% (M = 51.03, SD: 18.57) in girls with fragile X. In terms of environmental measures, the quality of the home environment (M = 46.33, SD: 7.03; range: 24–57) as well as family income (M = 1.16 or 116% of the median household income for home's zip code; range: 0.13–4.33) varied widely among families. Mothers and fathers of boys, but not girls, with fragile X had SCL-90-R scores significantly higher than the measure reference mean of 50 (mothers of boys with fragile X, M = 52.64, SD: 9.67, 1 sample $t(79) = 2.44$, $P < .05$; fathers of boys with fragile X, M = 52.85, SD: 9.28, 1 sample $t(68) = 2.54$, $P < .05$). Notably, children of nonparticipating fathers ($N = 18$) had significantly more autistic symptoms than children of participating fathers, U = 481.5, $P < .01$. Differences between these 2 groups of children in CBCL composite scale scores were not significant.

Mothers reported much variability in the effectiveness of educational and therapeutic services; however, girls (M = .41, SD: 0.33) received more effective services than boys (M = .25, SD: 0.40), $t(118) = 2.08$, $P < .05$).

CBCL

Descriptive statistics of child behavior problems (*t* scores) of boys and girls with fragile X and their comparison siblings are shown in Table 2 and graphically in Figs 1 and 2. The percentages of children in each group whose scores fell in the borderline or clinical range (for syndrome scales, *t* score >66 and for composite scales, *t* score >60) are also shown in Table 2. A multivariate analysis of variance with each of the CBCL scales as dependent variables and group as the independent variable (boys with fragile X, girls with fragile X, sibling boys, sibling girls) revealed groupwise differences for all scales (Fs = 2.97–77.37, all $P < .05$) except somatic complaints and delinquent behavior. Follow-up pairwise tests (Tukey) revealed that girls with fragile X had significantly more behavior problems than comparison sibling girls in all domains except somatic complaints, delinquent behavior, and aggressive behavior, all $P < .05$. Boys with fragile X had more problems than comparison sibling boys in all areas except somatic complaints, anxiety/depression, delinquent behavior, and aggressive behavior, all $P < .05$. Social problems, attention problems, and thought problems were the most pronounced problems reported by parents of boys and girls with fragile X. As seen in Table 2, 40.0% of girls and 41.8% of boys with fragile X had social problems in the borderline or clinical range. The percentages for thought problems were 25.0% and 54.4%, and attention problems were 47.5% and 62.0%, for girls and boys with fragile X, respectively. Finally, 47.5% of girls and 54.4% of boys with fragile X had total behavior problem scores in the clinically significant range, in comparison to 11.8% of children in the comparison sibling group.

Autistic Behavior

Boys with fragile X had moderate levels of autistic behavior (M = 42.16, SD: 23.36) similar to those of a sample of children with severe mental retardation (M = 43.95, SD: 18.92), but well below that of children diagnosed with autism (M = 77.49, SD: 20.01).[33] Girls with fragile X had mild levels of autistic behavior (M = 18.97, SD: 22.67) with as much variability as boys with fragile X. Autistic behavior in the sibling group is not reported because of lack of variability.

Multiple Regression Analyses

Child age and adjusted family income were not significantly correlated with any dependent variable in any of the 3 groups of interest, and, therefore, were not entered into the regression analyses. Correlations among parent SCL-90-R, the HOME, and the SCORS coefficient revealed a significant association between the parent psychopathology and the quality of the home environment, $r(120) = -36$, $P < .001$. Despite this modest correlation, these 2 factors were retained in the analyses according to a priori hypotheses. The final model consisted of gender (for the sibling group), full scale IQ, and FMRP percentage (for the fragile X groups) in the first step, followed by the HOME total score, mean parent SCL-90-R Global Severity Index *t*-score, and the SCORS correlation coefficient (measure of the effectiveness of services) in the second step. Results of regression analyses are shown in Table 3.

Comparison Siblings

Child gender and intelligence were significantly associated with behavior problems in the sibling group. Male gender and lower IQ were related to increased behavior problems, accounting for 5% to 10% of the variance. After accounting for the influence of gender and IQ, environmental factors accounted for an additional 24% of the variance in total behavior problems. In particular, parental psychopathology and lower home environment quality were independently associated with behavior problems. The quality of the home environment predicted externalizing but not internalizing behavior problems,

TABLE 1. Descriptives of Independent Variables by Group*

	Comparison Sibling Boys (n = 58)		Comparison Sibling Girls (n = 62)		Boys With Fragile X (n = 80)		Girls With Fragile X (n = 40)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	10.88	3.01	11.50	3.17	10.94	2.69	10.42	3.10
Wechsler full scale IQ	106.52	12.19	108.52	12.26	46.56	9.39	75.48	22.30
FMRP (%)	NA	NA	NA	NA	12.09	11.57	51.03	18.57
HOME total	47.28	6.97	50.06	6.90	45.29	7.05	48.40	6.60
Effectiveness of services	NA	NA	NA	NA	.25	.40	.41	.33
Mother SCL-90-R	52.41	10.51	52.79	9.19	52.64	9.67	52.55	10.27
Father SCL-90-R	55.09	9.08	50.49	8.88	52.85	9.28	52.12	9.21
Adjusted family income	1.07	.56	1.24	.82	1.09	.58	1.30	.91

* The HOME scores are derived from separate interviews about each child. Therefore, the quality of the home environment may differ between children with fragile X and their siblings. Parent SCL-90-R scores and adjusted family income are not reported in the sibling group because they share the same households and parents with the children with fragile X. Note that 68 of 80 fathers of boys with fragile X and 34 of 40 fathers of girls with fragile X completed the SCL-90-R. Adjusted family income is total household income divided by the median household income in the zip code of the family's home.

TABLE 2. Descriptive Statistics and Percentages of Children With Behavioral Symptoms in the Borderline or Clinical Range by Group: CBCL Syndrome and Composite Scales

	Comparison Sibling Boys (n = 58)			Comparison Sibling Girls (n = 61)			Boys With Fragile X (n = 79)			Girls With Fragile X (n = 40)		
	Mean	SD	% Clinical	Mean	SD	% Clinical	Mean	SD	% Clinical	Mean	SD	% Clinical
Syndrome scales												
Withdrawn	52.36	4.51	1.7	51.90	3.96	0.0	58.24	8.25	21.5	59.76	7.65	17.5
Somatic complaints	53.98	5.68	5.1	53.11	5.27	4.9	53.78	6.09	6.3	54.33	6.97	10.0
Anxiety/depression	53.86	5.81	5.1	52.72	6.36	6.6	54.72	5.90	5.1	56.20	5.90	2.5
Social problems	52.86	5.58	6.9	51.61	4.05	1.6	65.56	8.75	41.8	64.78	11.90	40.0
Thought problems	52.07	4.95	5.1	51.82	4.81	3.2	66.13	8.56	54.4	58.93	9.66	25.0
Attention problems	52.86	4.90	3.4	52.39	5.18	6.5	68.58	9.69	62.0	63.38	10.68	47.5
Delinquent behavior	52.72	5.22	3.4	52.11	4.52	4.8	53.37	4.92	2.5	52.88	4.82	5.0
Aggressive behavior	53.72	6.53	8.5	52.02	4.44	1.6	56.42	7.52	12.7	55.08	7.75	12.5
Composite scales												
Internalizing domain	48.00	9.67	13.8	45.34	10.58	8.2	53.75	10.06	34.2	55.93	8.76	40.0
Externalizing domain	48.00	10.63	15.5	42.95	10.45	9.8	53.27	9.50	26.6	50.18	11.20	25.0
Total problems	46.95	11.03	13.8	41.43	12.39	9.8	60.59	8.72	54.4	56.63	11.06	47.5

whereas parental psychopathology was positively associated with both internalizing and externalizing problems among siblings of children with fragile X.

Boys With Fragile X

Behavior problems in boys with fragile X were consistently associated with environmental factors, and not with FMRP or IQ. Specifically, maternal report of more effective educational and therapeutic services was associated with fewer behavior problems and autistic symptoms, whereas parental psychopathology was significantly associated only with internalizing problems. And, as can be seen in Fig 3, autistic behavior increases linearly as the quality of the home environment decreases.

Girls With Fragile X

In contrast to boys with fragile X, for the most part, genetic rather than environmental factors were associated with behavior problems in girls. Although FMRP was more strongly associated with internalizing types of problems, IQ was more strongly associated with externalizing problems. As shown in Fig 4, internalizing behavior problems decrease linearly as level of FMRP increases. Overall, IQ and FMRP account for 34% of the variance in total behavior problems among girls with fragile X. Whereas parental psychopathology was associated with internalizing behavior in boys with fragile X, this factor was most strongly correlated with externalizing behavior in girls with fragile X. Finally, IQ was the only significant predictor of autistic behavior, accounting for approximately 33% of the variance.

Follow-up, analogous multiple regression analyses were conducted to examine genetic and environmental effects on specific types of behavior that are most problematic for children with fragile X, specifically withdrawn behavior, anxious/depressed behavior, social problems, thought problems and attention problems. For girls with fragile X, results of these analyses showed that, after accounting for the effect of IQ, FMRP was significantly associated with withdrawn ($\beta = -0.37$, $P < .05$) and anxious/depressed behavior ($\beta = -0.32$, $P < .05$) but not social ($\beta = -0.25$, not significant [NS]), attention ($\beta = -0.21$, NS), or thought problems ($\beta = -0.18$, NS). Interestingly, although environmental factors were not associated with total, internalizing, or externalizing domain scores in this group, 2 of these measures, parental psychopathology and effectiveness of ser-

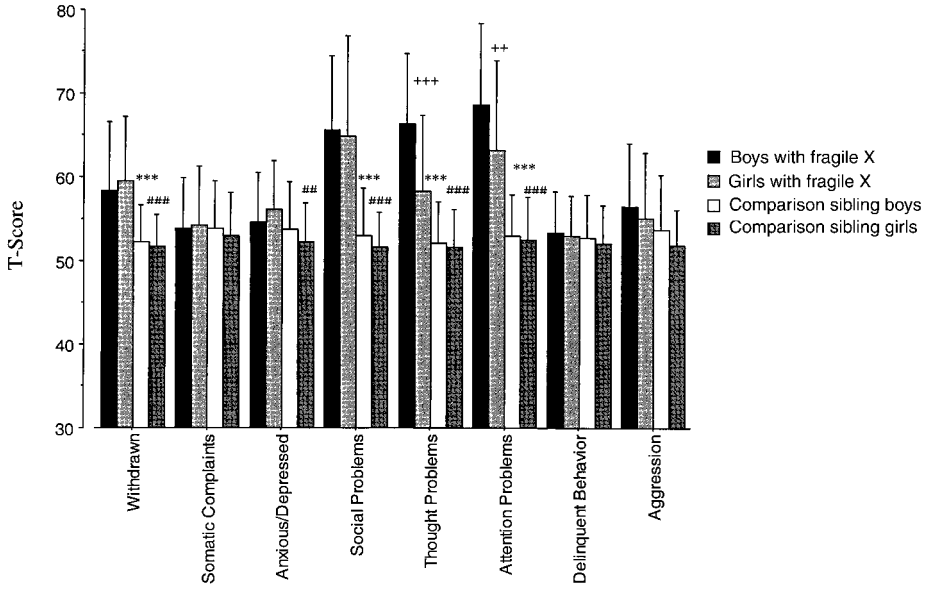

Fig 2. CBCL composite scale *t* scores of children with the fragile X full mutation (79 boys and 40 girls) and their siblings (58 boys and 61 girls). *** Fragile X boys > sibling boys, $P < .001$; ### fragile X girls > sibling girls, $P < .001$; ## fragile X girls > sibling girls, $P < .01$; +++ fragile X boys > fragile X girls, $P < .001$; ++ fragile X boys > fragile X girls, $P < .01$. Error bars represent 1 SD.

TABLE 3. Results of Hierarchical Multiple Regression Analyses Predicting Behavior Problems in Boys and Girls With Fragile X and Their Siblings*

	Comparison Siblings				Boys With Fragile X				Girls With Fragile X			
	TOT	INT	EXT	AUT	TOT	INT	EXT	AUT	TOT	INT	EXT	AUT
Step 1 (Bio/Genetic)												
Gender	.21*	.11	.22*	NA	NA	NA	NA	NA	NA	NA	NA	NA
Child FSIQ	−.22*	−.19*	−.16	NA	−.13	.10	−.14	−.21	−.44**	−.27	−.38*	−.55***
FMRP %	NA	NA	NA	NA	.03	−.11	.06	−.10	−.30*	−.32*	−.14	−.07
R^2 Change	.10**	.05	.08**	NA	.02	.02	.02	.06	.34***	.21*	.19*	.33**
Step 2 (Environmental)												
Home environment	−.20*	−.11	−.26**	NA	−.07	.07	−.15	−.36**	−.07	.05	−.13	−.12
Parental psychopathology	.40***	.29**	.40***	NA	.20	.28*	.15	.04	.16	.21	.38*	.19
Effectiveness of services	NA	NA	NA	NA	−.34**	−.30**	−.32**	−.24*	−.22	.05	−.15	−.10
R^2 Change	.24***	.12**	.29***	NA	.18**	.17**	.17**	.18**	.08	.04	.21*	.07
Total R^2	.34***	.17***	.37***	NA	.20**	.19**	.19**	.24**	.42**	.25	.40**	.40**
N	119	119	119		79	79	79	79	40	40	40	40

* Data are standardized regression coefficients. TOT, indicates CBCL Total Behavior Problems; INT, CBCL Internalizing Behavior Problems; EXT, CBCL Externalizing Behavior Problems; AUT, Autism Behavior Checklist total score. * $P < .05$. ** $P < .01$. *** $P < .001$.

vices, were associated with subscale scores. Specifically, increased parental psychopathology was related to increased anxious and depressed behavior ($\beta = 0.35$, $P < .05$), while increased effectiveness of services was associated with decreased attention ($\beta = -0.35$, $P < .05$) and thought problems ($\beta = -0.30$, $P < .05$) in girls with fragile X.

For boys with fragile X, examination of specific types of behavior on the CBCL did not change results pertaining to FMRP. FMRP was not associated with any of the 4 identified subscales. In terms of environmental measures, more effective educational and therapeutic services were associated with less withdrawn behavior ($\beta = -0.31$, $P < .01$), less anxious/depressed behavior ($\beta = -0.32$, $P < .01$), and fewer attention ($\beta = -.25$, $P < .05$) and thought problems ($\beta = -0.29$, $P < .05$), but not with social problems ($\beta = -0.13$, NS). Parental psychopathology was not associated with any specific behavioral subscale, although as described above, it was associated with the internalizing problems composite score in this group.

DISCUSSION

The results of this study add depth to understanding the development of neurobehavioral dysfunction in fragile X syndrome through investigation of both genetic and environmental influences on behavior problems in children with this condition. These results demonstrate that characteristics of the child's environment, particularly for boys with fragile X, play a significant role in behavior problems beyond the genetic effects of the disorder itself. Although additional research is needed to increase the specificity of these environmental effects (ie, examining more specific characteristics of the home, family, and educational/therapeutic services), the current findings help to lay the foundation for studies designed to identify specific interventions for reducing problem behavior in children with fragile X syndrome.

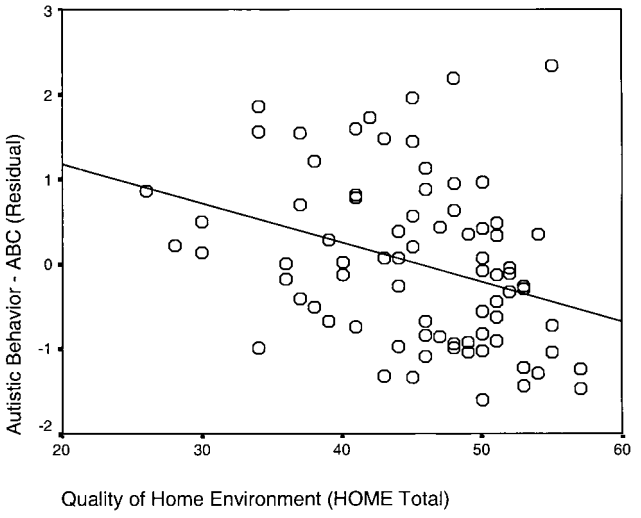

Fig 3. Statistically significant relation between quality of the home environment and autistic behavior residual scores in 79 boys with the fragile X full mutation. Residual scores represent autistic behavior scores after the effects of the other independent variables in the multiple regression are removed.

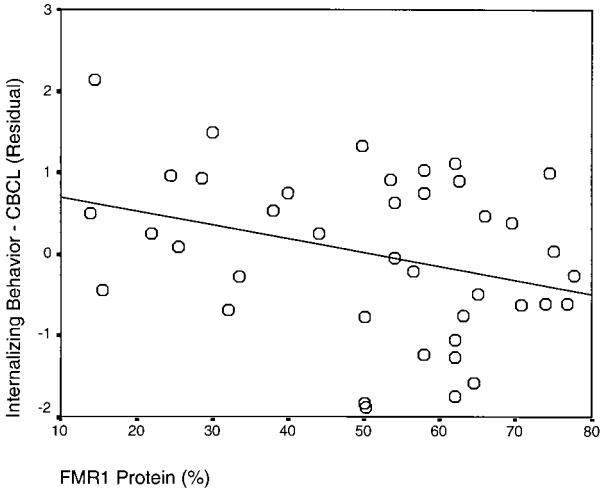

Fig 4. Relation between FMRP expression and CBCL internalizing behavior residual scores in 40 girls with the full mutation. Residual scores represent internalizing behavior scores after the effects of the other independent variables in the multiple regression are removed.

For boys with fragile X, results showed that 2 environmental factors, the effectiveness of educational and therapeutic services and maternal psychological problems, independently predicted behavior problems. In contrast, for girls with fragile X, the findings emphasized the separate effects of FMRP and intelligence, while environmental characteristics were not consistently associated with behavior problems. The reason for this dichotomy in the results is not clear. Gender and intellectual functioning are interdependent in fragile X. The wider spectrum of intellectual functioning and behavior problems in females with fragile X is likely to be due, in part, to variation in cellular X chromosome inactivation patterns and consequent FMRP production. Therefore, the differences observed in the current study may be a function of these genetic processes and level of functioning rather than gender. Future studies that include a sample of boys with higher levels of FMRP expression will clarify this issue.

Previous studies[23,25] and ongoing research in our own laboratory[37] have shown an association between FMRP and intellectual functioning in children with fragile X, suggesting a contribution of the FMR1 gene mutation to intellectual dysfunction. The present study extends this previous work by demonstrating a significant and independent association between FRMP and behavior problems in girls with fragile X, even after accounting for variation in intelligence. The association between FMRP and behavior is strongest in the internalizing behavior domain, particularly for socially withdrawn and anxious/depressed behavior. These results verify the hypothesis that reduced levels of FMRP place children at risk for specific neurobehavioral as well as neurocognitive effects. In addition, because the general effect of intelligence was removed in our analyses, the results show that the behavioral phenotype observed in females with fragile X is not simply secondary to a general cognitive deficit, but rather related to specific genetic effects of the FMR1 mutation.

The results of this study highlight several points at which intervention might be effective for children with fragile X. First, the association between the effectiveness of educational and therapeutic services and behavioral outcome indicates that from the mother's perspective, the fit between the child's developmental needs and the services he receives is important. In this sample, children who showed improvements in skills that their parents believed were important for them to develop had fewer behavior problems. This was the case for all types of behavior as well as autistic symptoms in boys with fragile X. Although the services were reported to be more effective for girls than boys, the impact of this effectiveness was seen only in reduced attention and thought problems for girls with fragile X. Why didn't the effectiveness of these services play a larger role for girls? Perhaps the focus of educational and therapeutic intervention for girls with the full mutation is on more observable problems such as learning problems and difficulties with attention, whereas internalizing problems are more difficult to detect and less amenable to behavioral intervention. Indeed, the lack of association between effectiveness of services and behavior problems may be an indication that the developmental needs of girls with fragile X are not being addressed adequately.

Second, parental psychopathology was associated with behavior problems of children with fragile X, as well as their siblings. A large body of previous research has demonstrated consistent links between parent psychopathology, most notably affective disorders, and child behavior and psychiatric problems in families presumably unaffected by a genetic syndrome.[38] Interestingly, in the case of children with fragile X, parental psychopathology is associated with child behavior, over and above the genetic effects of the syndrome. The mechanisms underlying the transmission of risk for behavioral disturbance from parent to child may be genetic, environmental,

or both. Similar to physical traits such as height or weight, transmission of genetic risk for personality or psychological characteristics is likely to occur even in the presence of a genetic syndrome that affects this dimension of function. Furthermore, the parent-child dyad in the case of fragile X is likely to include cyclical patterns of behavioral influence in which the child's difficult behavior affects the parent's psychological status, which in turn affects the manner in which the parent interacts with their child. In addition, it is possible that the behavior of children with this genetic condition has an impact on the quality of the environment by affecting the level of stress in family members, time and financial resources for the home, and so on. Thus, this effect could be viewed as bidirectional. Clearly, additional work examining this finding is needed.

Third, the effects of the home and educational environment on autistic behavior in boys with fragile X are notable. One might expect biological or genetic factors to be the primary determinants of autistic behavior in this population. However, as shown by the current results, environmental factors had a significant impact on the degree of autistic behavior. It is known that individuals with autism benefit from increased structure, predictability in daily routines, an organized physical environment, as well as specific, targeted instruction.[39] Therefore, one interpretation of these findings is that boys with fragile X also benefit from a more structured, enriched home environment and targeted instruction. Increased cultural enrichment, activity, and structure may serve to keep the children focused and learning, reduce the frequency of repetitive play, and increase the frequency of meaningful social exchanges. Conversely, a decline in the enrichment, organization, or social climate of the home may increase the frequency of autistic behavior in children with fragile X, which may increase risk for meeting criteria for the diagnosis of autism.

This study had limitations influencing the interpretation of the findings. The lack of ethnic diversity limits the generalizability of the results to nonwhite families with children with fragile X. A second limitation of the study is maternal bias in reporting of environmental factors and child behavior. Mothers reported child behavior problems, effectiveness of services, and their own psychological symptoms, which could have inflated the relation between these predictors and the outcome measures. For example, one concern is that a mother's psychological status influenced her responses on the educational effectiveness measure. Follow-up correlations, however, revealed that these factors are unrelated. Future analyses will examine data from teacher-report of the services actually provided and behavior problems. Third, although the Autism Behavior Checklist was useful for efficiently measuring autistic symptoms, it relied on parent report and is not tied to *Diagnostic Statistical Manual, Fourth Edition* or *International Classification of Diseases, 10th Revision* criteria. The Autism Diagnostic Interview[40] and the Autism Diagnostic Observation Schedule[41] are now considered better measures of autism. Fourth, the cross-sectional design of the study does not make it possible to examine causal relationships between variables. For example, the association between effectiveness of services and behavioral outcome does not necessarily indicate that effective education and therapy leads to decreases in problem behavior. It is possible that children with fewer problems happen to receive better services, for example. Similarly, children with more behavior problems may elicit psychological distress in their parents and make the home and family more difficult to manage. Additional investigation of the specifics of home and family characteristics may yield important insights into the interaction between genetic and environmental influences on behavior in children with fragile X. Longitudinal studies will be needed to examine these causal links.

The results of this study should help to lay the foundation for future research designed to identify specific points at which intervention will be most effective in reducing behavior problems in children with fragile X syndrome. Based on the results presented here, an increased focus on interventions aimed at the family and school level, as well as the individual level of the child might be most effective. Because of the challenges and stresses associated with raising and teaching children with developmental delay and significant behavior problems, these interventions should include parent and teacher training to manage specific behavior problems, assisting and supporting parents with ways to manage and cope with stress, enhancing communication between parents and educators/therapists about the specific needs of the child at home and at school, and in especially distressed families, providing adequate respite care. Also, the well being of siblings of children with fragile X should not be overlooked. As evident from the results of this study, siblings who do not have the fragile X mutation are nevertheless influenced by the quality of the home environment and their parents' psychological health, which may be linked to stresses associated with having a child with a disability.

Finally, it should be emphasized that the features of the neurobehavioral phenotype occurring in children with fragile X are similar to characteristics of important child psychiatric disorders such as pervasive developmental disorder, attention-deficit/hyperactivity disorder and anxiety disorders. Basic mechanisms underlying these behaviorally defined disorders are certain to be different and more heterogeneous than those observed in fragile X. However, using fragile X as a more homogeneous model system for elucidating relations among genetic, environmental, and psychiatric factors provide conceptual and methodological insights that are applicable to investigations into the etiology and pathogenesis of more complex diagnostic entities. Accordingly, this research also has relevance that extends beyond the realm of fragile X syndrome.

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