Autistic Behaviors Among Girls with Fragile X Syndrome

Michèle M. M. Mazzocco, Wendy R. Kates, Thomas L. Baumgardner, Lisa S. Freund, and Allan L. Reiss
Kennedy Krieger Institute, Johns Hopkins University School of Medicine

Reports of autistic behaviors were examined for 30 school-age girls with fragile X (fraX) and 31 age- and IQ-matched controls through a structured interview administered to each girl's parent(s). IQ scores were obtained for each participant; anxiety, neuroanatomical, and molecular-genetic data were derived for girls with fraX. Girls with fraX had significantly more autistic behaviors than controls. These behaviors were qualitatively similar to those reported for boys with fraX, but were not correlated with IQ. Anxiety in girls with fraX was positively correlated with abnormal social and communication behaviors; posterior cerebellar vermis area was negatively correlated with measures of communication and stereotypic/restricted behaviors. Severity of stereotypic/restricted behaviors was negatively correlated with the prevalence of active non-fraX chromosomes. Thus anxiety and posterior cerebellar area measures had distinct associations with subsets of autistic behaviors; these associations may have important implications for understanding the neurobiology of autism.

Almost as soon as an association between fragile X syndrome and autistic disorder was first proposed (Brown et al., 1982), controversy regarding the validity of this association emerged (Cohen et al., 1991; Einfeld, Molony, & Hall, 1989; Fisch et al., 1986). However, there is little if any controversy

1A portion of this research was presented at the 4th Consensus for Biological Bases and Clinical Perspectives on Autism, in Troina, Sicily, October 6, 1995. This research was supported by the following grants from the National Institutes of Health: Grants 5 K02 MH01142-02, 5 RO1 MH50047-03, and 5 RO3 MH52311-02 from the National Institute of Mental Health; and grants HD31715-02 and P50 HD25806 from the NICHD.

2Address all correspondence to Michèle Mazzocco, Behavioral Neurogenetics and Neuroimaging Research Center, Kennedy Krieger Institute, 707 N. Broadway, Baltimore, Maryland.
regarding the contention that fragile X syndrome, a hereditary cause of developmental disability (approximate prevalence rate is 1:1000; Sherman, 1991), is associated with a variable yet well-described phenotype (Hagerman, 1991) that includes autistic-like behaviors (Hagerman et al., 1986; Kerby & Dawson, 1994; Levitas et al., 1983; Reiss & Freund, 1990, 1992). Reports on the frequency of autistic disorder among children with fragile X range from 0 to 60% (Brown et al., 1982; Hagerman, 1991; Hagerman et al., 1986; Levitas et al., 1983; Wisniewski et al., 1985). The variability in part reflects variation in the definitions and criteria for autism on which individual studies have been based. The predominant prevalence figure of autism among males with fragile X, based on DSM-III-R criteria (American Psychiatric Association [APA], 1987), is approximately 16% (Cohen et al., 1991), and exceeds the prevalence of 5% reported for children with mental retardation (Gillberg, 1995).

Among the arguments against a specific association between fragile X and autism is the proposal that the mental retardation (MR) seen in both disorders may be their common link and, perhaps, a causal mechanism (Einfeld et al., 1989). This proposal is supported by reports of greater behavioral similarity between autistic and MR children than between autistic and non-MR children (Adrien, Ornitz, Barthelemy, Sauvage, & Lelord, 1987). Additional support comes from numerous reports of males (Levitas et al., 1983) and females (Hagerman et al., 1986; Mazzocco, Freund, Baumgardner, Forman, & Reiss, 1995) with fragile X and autism who are also mentally retarded. Nevertheless, autistic features have been reported to occur with greater frequency among mentally retarded boys with fragile X relative to mentally retarded boys with autism (Baumgardner, Reiss, Freund, & Abrams, 1995; Kerby & Dawson, 1994). The specificity of such autistic features, as described by Reiss and Freund (1990, 1992), includes social interaction deficits, stereotypies, abnormal communication, and abnormal responses to sensory stimuli; but does not include deficits in attachment to or interaction with caregivers. Findings of this pattern of behaviors have been replicated in additional studies of males with fragile X (e.g., Mazzocco et al., 1996). Regardless of the presence or absence of an autistic disorder or PDD diagnosis among children with fragile X, the study of autistic behaviors in fragile X syndrome may have important implications for understanding the neurobiological components of autism. These implications may be drawn on the basis of the behavioral similarities reported among the children with either disorder, including the behaviors described above. The majority (70–80%) of individuals affected by autism (Ungerer, 1985), and the majority of males affected by fragile X (Hagerman, 1991), have mental retardation; and the majority of individuals affected by either disorder are male. Similarities in cognitive profiles across non-MR females
with fragile X (Kovar, Pennington, Mazzocco, & Hagerman, 1997; Mazzocco, Pennington, & Hagerman, 1993) and non-MR children with autism (Ozonoff, Pennington, & Rogers, 1991) include deficits in executive function; although differences across these two groups are also noted. For instance, the emotion perception deficits reported among children with autism (Ozonoff et al., 1991) have not been reported in fragile X (Kovar et al., 1997; Mazzocco et al., 1993).

Similarities in neuroanatomical anomalies have also been described between individuals with fragile X and individuals with autism. Among the findings reported for individuals with the fragile X mutation are reports of hypoplasia of the posterior cerebellar vermis, particularly lobules VI and VII; enlarged volumes of the fourth ventricle (Reiss, Aylward, Freund, Joshi, & Bryan, 1991; Reiss, Freund, Tseng, & Joshi, 1991), and age-related changes in both the hippocampus and the superior temporal gyrus (Reiss, Lee, & Freund, 1994). Additionally reported for males are findings of enlarged lateral ventricles and increased volumes of the caudate nucleus (Reiss, Abrams, Greenlaw, Freund, & Denckla, 1995) and the hippocampus (Reiss et al., 1994). Findings among females include increased volume of the thalamus (Reiss, Abrams, Greenlaw, et al., 1995). (See Abrams & Reiss, 1995 and 1996, for a review of these findings.) Through neuroanatomical studies of individuals with autism, researchers have identified several brain structure anomalies that are consistent with those reported for individuals with fragile X. These include hypoplasia of the cerebellar vermis and enlarged volumes of the fourth and lateral ventricles (Courchesne et al., 1994). Reports on investigations of cerebellar vermis hypoplasia in autism have been inconsistent. However, through a reanalysis of data across several studies, Courchesne et al. (1994) demonstrated that cerebellar hypoplasia is seen in a large subset of individuals with autism (85%), and that cerebellar hyperplasia is seen in a smaller subset. Additional evidence of similarity between the two disorders is the increased cell packing density found in the hippocampus of individuals with autism, which is seen in postmortem studies (Bauman, 1991) but not in MRI studies (Saitoh, Courchesne, Egaas, Lincoln, & Schreiber, 1995).

Despite this evidence for similarity across autism and fragile X syndrome, it should be noted that neuroanatomical studies of autism, in general, have yielded conflicting results. This can be attributed to the etiological heterogeneity of autism (Ciaranello & Ciaranello, 1995; Lot speich & Ciaranello, 1993). In comparing the neuroanatomy of individuals with autism to those with fragile X (a syndrome characterized by a homogeneous etiology), conclusions about similarities in findings may therefore be limited to a subset of individuals with autism.
The study of the neurobiology of fragile X syndrome may provide a model for possible neurobiological mechanisms underlying autistic behavior, in at least a subset of individuals with autism. The mutation associated with fragile X syndrome has been identified as an expansion of the cytosine-guanine-guanine (CGG) nucleic acid repeat (Verkerk et al., 1991) within the FMR1 (fragile X mental retardation—1) gene at location Xq27.3. Expansions in excess of 200 CGG repeats are associated with methylation and with clinical expression of the fragile X syndrome, and are classified as “full mutations.” Smaller expansions are classified as “premutations,” and do not appear to be associated with a behavioral phenotype (Feng, Lakkes, Devys, & Warren, 1995; Macpherson, Bullman, Younigs, & Jacobs, 1994; Mazzocco et al., 1993; Reiss, Freund, Abrams, Boehm, & Kazazian, 1993; Rousseau et al., 1994). Although the precise mechanism of the FMR1 gene on brain development and function is unknown at this time, studies of FMR1 expression and intellectual functioning in females with fragile X indicate an important association between these two variables (Abrams et al., 1994; Reiss, Baumgardner, Freund, Abrams, & Denckla, 1995). In this paper, the association between FMR1 expression and autistic behaviors is examined among girls with the fragile X full mutation.

If a specific pattern of autistic behaviors is associated with the FMR1 full mutation, that pattern should be similar in quality—although not necessarily in severity—across males and females with fragile X. This prediction is consistent with the qualitative similarity and quantitative differences reported for the psychological difficulties reported for children with fragile X. These difficulties appear in the areas of social functioning, stereotypic behavior, visual spatial skills, visual motor coordination, and attention. An important feature of the difficulties reported among females with fragile X is their presence among individuals with full-scale (FSIQ) scores in the normal range. In this study, autistic behaviors among girls with fragile X were examined, relative to behaviors among girls in a peer comparison group, among girls with MR and girls with normal FSIQ scores. The primary aim of the study was to establish whether a pattern similar to that reported for boys would also emerge among girls. Moreover, the study was carried out to address the association between MR and autistic behavior in fragile X among a group of girls with a wide range of FSIQ scores, and the association between degree of autistic behavior and FMR1 gene expression among girls in the fragile X group. Finally, the association between autistic behavior and neuroanatomical area of the posterior cerebellar vermis, among girls with fragile X only, was explored in view of findings that the size of this region is significantly decreased among individuals (male and females) with fragile X (Reiss, Aylward, et al., 1991) or autism (Courchesne et al., 1989).
METHOD

Participants

The girls included in this study were enrolled through the context of a larger study on neurobiological components of learning disability (LD) and behavior in girls. There were 30 girls with fragile X (fra X) who ranged in age from 6 years 1 month to 16 years 2 months ($M = 10$ years 7 months, $SD = 3$ years 2 months). Only girls with the full mutation were included in the study, which was confirmed by DNA analyses. Thirty-one girls from a (non-fragile X) peer comparison group were group-matched (with the fra X group) on age and FSIQ. The girls in this group included non-fraX girls with LD, and siblings of probands in the larger study. The age of these 31 girls ranged from 6 years 6 months to 16 years 5 months ($M = 10$ years 3 months, $SD = 2$ years 10 months). The girls in the fra X group had FSIQ scores that ranged from 66 to 116 ($M = 87.4$, $SD = 14.1$); girls in the peer comparison group had FSIQ scores that ranged from 68 to 120 ($M = 92.5$, $SD = 16.6$). Ten of the girls with fra X and 9 of the girls in the peer comparison group had scores in the MR ($<70$) or borderline (70–79) range.

Participant Ascertainment

Nine of the girls with fra X were ascertained through male siblings who were probands identified at the Johns Hopkins Hospital/Kennedy Krieger Institute or Children's Hospital National Medical Center in Washington, DC. The remaining subjects were identified through local cytogenetic laboratories, or recruited through announcements in various fragile X associations.

Six of the girls in the peer comparison group were unaffected sisters of a girl in the fra X group. The remaining girls in the peer comparison group were recruited through announcements in public and private schools, and area newspaper, or through a review or medical records of the Kennedy Krieger Institute. The chart review was used to identify girls who could be matched on IQ with girls who had fra X and who had low average to borderline IQ scores. Each girl in the peer comparison group received genetic testing for fra X.

Parents of potential participants were told that the study was designed to identify social, emotional, and cognitive development in girls; and that a benefit of the study was a contribution toward understanding how genetic factors influence a child's development. Parents of all potential participants were asked why they wanted their daughter to participate. Many parents reported wanting to learn more about how to help their child learn. A girl was
excluded from the study if she had an identifiable genetic condition (other than fragile X) such as early treated phenylketonuria or tuberous sclerosis; a neurodegenerative disorder; or CNS injury (e.g., from head trauma).

**Procedure**

Each girl was individually administered the Wechsler Intelligence Scale for Children–Revised (WISC-R; Wechsler, 1974). The WISC-R was used instead of the WISC-III because data collection began prior to publication of the WISC-III. Each girl’s parent(s) was interviewed with respect to problem behaviors that correspond to the 16 diagnostic criteria established in the DSM-III-R.

**Parent Interview.** A structured interview, the Neuropsychiatric Developmental Interview (NDI; Reiss & Freund, 1990), was administered to each parent to determine whether their child met any of the DSM-III-R criteria in the past and/or currently. This 36-item interview was developed to identify which specific autistic-like behavior(s) a child manifests, and the severity of each behavior at the age when the behavior was most frequent. Each NDI item is a straightforward question regarding the presence and severity of a specific behavior. For example, one item is, “has (child’s name) ever become upset or preoccupied when there are changes in the environment, such as moving a lamp or piece of furniture to a different place? How often does this occur?” The severity ratings range from zero (behavior not present) to 5 (child almost always engages in the behavior). Ratings of 3 and 4 correspond to the behavior occurring 50 or 75% of the time, respectively. For the purpose of establishing a diagnosis of autistic disorder, a rating of 3.5 or greater was considered evidence that the individual met the corresponding behavioral criteria. In addition to questions pertaining to the criteria for autism, the NDI includes five additional questions pertaining to social behavior. Scores from subsets of questions pertaining to each of the 16 criteria were totaled. The 16 resulting criteria scores were examined regardless of diagnosis to assess range of severity of a specific behavior as a function of group status (i.e., fra X or peer comparison group). Each of the 16 criteria correspond to one of three types of behaviors which include (a) impaired social interaction, (b) impaired verbal and nonverbal communication and imagination, and (c) restricted activities and interests (APA, 1987).

**Neuroimaging.** MRI scans were obtained for 28 of the girls with fra X. The midsagittal image chosen for analyses was derived from a T1-weighted sagittal acquisition as described by Reiss and colleagues (Reiss, Aylward, et al., 1991; Reiss, Freund, et al., 1991). The variable used for
the analyses included area of lobules VI and VII of the posterior cerebellar vermis, as a ratio to intracranial area.

**DNA Analyses.** A lymphocyte-derived Southern blot DNA analysis at the FMR1 gene was carried out for each girl in the fraX group, by EcoRI + Eag I digest with the StB 12.3 probe (Oberle et al., 1991). The Activation Ratio (AR) was also assessed for each subject, to reflect the number of cells with the normal X chromosome as a ratio of the total number of X chromosomes (normal and fragile X). (See Abrams et al., 1994 for a description of the methodology used to derive AR.)

**RESULTS**

Preliminary *t* tests verified the lack of group differences on age or FSIQ, which was expected owing to the matching procedure employed for selection of the peer comparison participants, *ps > .20.*

**NDI Assessment of DSM-III-R Criteria for Autism**

Only one girl from either group met full DSM-III-R criteria for autistic disorder. Although the number of girls with a diagnosis of PDD was greater among the fraX group (17%) than among the control group (6%), this difference did not reach statistical significance, Fisher’s Exact *p = .25.* Nevertheless, the average severity ratings were compared for behaviors corresponding to each of the 16 DSM-III-R diagnostic criteria (APA, 1987).

Of particular interest was which behaviors occurred more frequently, and with greater severity, among girls with fraX versus girls in the peer comparison group. Thus it was important to examine the number of girls who received ratings of 3 (behavior occurs 50% of the time) or greater for items within a particular category of behavior (i.e., impaired social interaction, impaired communication/imagination, or restricted behaviors/interests). Chi-square statistics failed to reveal a significant effect of group on the number of girls who received any positive endorsements across all NDI item(s), nor on the number of girls who received at least one positive endorsement on any autism-specific NDI item(s). However, the number of behaviors endorsed per girl differed as a function of group status (fraX vs. peer comparison groups). Girls in the fraX group received more positive endorsements on the NDI overall, *t*(59) = 2.38, *p < .05*, and on NDI autistic-specific items, *t*(59) = 4.087, *p < .001*, relative to the comparison group. The girls with fraX also had a higher number of endorsements with severity ratings ≥3 across all NDI items, *t*(59) = 3.56, *p < .001, and across NDI autism-specific items, *t*(59) = 3.30, *p < .01* (see Table I).
Table I. Behavioral Endorsed on the NDI, As a Function of Participation Group

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of girls with endorsements for</th>
<th>Fragile X</th>
<th>Peers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any NDI rating ≥3</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Any NDI autistic behavior rating ≥3</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>M (SD) number of endorsements for</td>
<td>Autistic behaviors</td>
<td>7.17 (5.09)</td>
<td>2.94 (2.67)</td>
</tr>
<tr>
<td></td>
<td>Autistic behavior ratings ≥3</td>
<td>6.63 (5.97)</td>
<td>2.45 (2.66)</td>
</tr>
</tbody>
</table>

*p < .01.  
*p < .001.

Behavioral Severity Ratings for Specific Behaviors

Due to the lack of normal distribution of severity ratings per behavior category, nonparametric statistics were employed to examine individual types of behavior severity ratings. Z statistics from Mann-Whitney tests were used to examine the pattern of ratings across NDI questions. In cases of tied rankings, the tied Z and p values were used.

The two groups of girls were differentiated by subsets of NDI items that pertained to specific DSM-III-R criteria for autistic disorder, as shown in Table II. Severity ratings were higher among the girls with fraX, relative to peers, on questions indicative of impairment in the following: social play (DSM-III-R item A4), Z = −2.43, p < .02; ability to make peer friendships (A5), Z = −2.70, p < .01; communication (B1), Z = −2.10, p < .05; nonverbal communication (B2), Z = −2.39, p < .02; speech production (B4), Z = −3.52, p < .001; and ability to initiate or sustain conversations with others (B6), Z = −2.15, p < .05. Girls with fragile X had higher ratings of behaviors pertaining to restricted repertoire of activities, including stereotyped body movements (C1), Z = −4.02, p < .0001; preoccupation with parts of objects (C2), Z = −2.81, p < .01; distress in changes in environment (C3), Z = −2.28, p < .05; and restricted range of interests (C5), Z = −2.83, p < .01.

The number of group differences alone suggests a high prevalence of autistic behaviors among girls with fraX. An important clarification to this conclusion is the examination of the magnitude of these group differences, which revealed that in most cases, the mean severity rating for both groups of girls was below 1.00. Group means ranged from 0 to 1.30 among the peer comparison group, and from 0.20 to 4.53 among the fraX group.

Parents’ responses to five additional NDI questions that pertained to social interaction were also examined (Table III). The frequency of ratings ≥3 was greater among girls with fraX, relative to the frequency reported
Table II. Number of Girls Endorsed with Behaviors Corresponding to DSM-III-R Criteria for Autistic Disorder, As a Function of Participant Group

<table>
<thead>
<tr>
<th>DSM-III-R criteria behaviors</th>
<th>Fragile X (n = 30)</th>
<th>Peers (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Behavior present</td>
<td>Rating ≥ 3</td>
</tr>
<tr>
<td>Social interaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>A2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>A3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>A4</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>A5</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Communication/imagination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>B2</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>B3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>B4</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>B5</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>B6</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Repertoire of activity/interests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>C2</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>C3</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>C4</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>C5</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

for peers, for three of these behaviors. Fifty percent of girls with fraX and 16% of the peers were rated as being nervous with strangers, Fisher's Exact $p < .01$; 40% of girls with fraX and 10% of peers were rated as being uninterested in others, Fisher's Exact $p < .01$; and 47% of girls with fraX and 16% of peers were described as being uncomfortable with others, Fisher's Exact $p < .05$. There were no significant differences in frequency for ratings of being easily overexcited or having rapid mood changes.

Table III. Nonautistic Endorsements on the NDI As a Function of Participant Group

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Fragile X (n = 30)</th>
<th>Peers (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nervous with stranger</td>
<td>15</td>
<td>5$^a$</td>
</tr>
<tr>
<td>Uncomfortable with others</td>
<td>14</td>
<td>5$^a$</td>
</tr>
<tr>
<td>Uninterested in others</td>
<td>12</td>
<td>3$^b$</td>
</tr>
<tr>
<td>Easily overexcited</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Rapid mood changes</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

$^a$ $p < .02$.

$^b$ $p < .01$. 
Autistic Behavior Ratings and Mental Retardation

Correlations between the NDI ratings and FSIQ scores were examined to determine whether those girls who exhibited more severe or more numerous autistic behaviors had lower FSIQ scores than girls with less severe or fewer autistic behaviors. Thus correlations were carried out, for each of the two groups of girls, between FSIQ and total severity ratings for NDI items and between FSIQ and each behavior category (impaired social interaction, impaired communication/imagination, and restricted behaviors/interests). Among the peer comparison group, the negative correlation between FSIQ and communication abnormalities was statistically significant, \( \rho = -.38, p < .05 \). In contrast, among girls with fraX none of the correlations was statistically significant, \( ps > .24 \).

The lack of an FSIQ–autistic behavior correlation suggests that MR is not the underlying mechanism for autistic behavior in girls with fraX. Additional support for this notion was derived from examination of the distribution of autistic ratings among the girls with fraX. Two of the 10 girls with fraX who had an FSIQ in the MR or borderline range had no positive endorsements on the NDI. The number of endorsements with severity ratings \( \geq 3 \) among the remaining 8 girls in this MR/borderline subgroup ranged from 3 to 25. The number of NDI endorsements with ratings \( \geq 3 \) ranged from 0 to 17 among the 20 girls with fraX who had an FSIQ score \( > 79 \). Among the majority of girls in the peer group, the number of NDI endorsements with a severity rating \( \geq 3 \) fell below the average number of such endorsements for girls in the fraX group. Among the girls with fraX whose number of NDI endorsements \( \geq 3 \) exceeded 1 standard deviation from their own group mean (i.e., \( > 8 \)), none had MR (their FSIQ scores ranged from 72 to 115).

Diagnostic Ratings and Neuroanatomical Data

Among girls with fragile X only, correlations were examined between area measure of lobules VI and VII, as a ratio of total intracranial area, and total scores for (a) all autistic behavior items from the NDI, (b) the 5 social (not autistic) behavior items from the NDI, and (c) each of the three categorical subsets of NDI scores pertaining to the DSM-III-R criteria for autism (i.e., impaired social interaction, impaired communication/imagination, and restricted behaviors/interests). The lack of variability in NDI ratings among the peers made this analysis inappropriate in the peer comparison group.

Although the neuroanatomical variable was normally distributed, the NDI rating scores were not; thus Spearman Rank Correlations were em-
ployed. Rhos corrected for tied ranks were used where appropriate. As shown in Table IV, Lobule VI + VII area was significantly correlated with both total autism or nonautism NDI ratings, and specifically with communication ratings and the total rating for stereotypic/restricted behaviors. Higher ratings (indicating a greater severity and/or more types of behaviors) on communication and stereotypic/restricted behaviors items were associated with greater degree of decrease in the vermal lobules VI+ VII region area.

**Activation Ratio (AR) and NDI Ratings**

Spearman rho correlations were derived to examine the association between AR and NDI behavior ratings. Rhos corrected for tied ranks were used where appropriate. AR was not significantly correlated with total autism or nonautism NDI ratings, nor with social interaction or communication/imagination ratings, rho absolute values < .28. In contrast, AR was significantly correlated with total rating for restricted behaviors/interests, rho = -.40, p < .05. Thus higher ratings (including a great severity and/or more types of behaviors) on these NDI stereotypic/restricted behavior items were associated with lower AR, the latter of which is an indirect indicator of FMR1 gene expression.

AR and cerebellar area were positively correlated, although the correlation was not statistically significant, rho = .276, p = .16. The lack of a significant correlation is not surprising in light of the multiple genetic and nongenetic factors that influence brain size (Reiss, Abrams, Singer, et al., 1995). A stronger correlation would be expected between AR and a score representing the deviation from predicted cerebellar vermis area. The importance of examining deviations from predicted scores among individuals with fraX has been well demonstrated with respect to FSIQ scores versus scores for deviation from predicted IQ (Abrams et al., 1994), but predicted cerebellar vermis areas were not available for the present study.
Anxiety Measures

In view of the specificity of the neuroanatomical–behavioral associations among girls with fraX, a final additional set of analyses was carried out to explore whether anxiety was related to specific categories of autistic behaviors in girls with fraX syndrome. Of interest was whether the specificity of behavioral associations differed across the neuroanatomical versus anxiety measures. A factor-analysis-derived composite score for anxiety/withdrawn behaviors (Anxiety Composite), as described by Freund, Reiss, Baumgardner, and Denckla (1997), was used as a self- and parent-report measure of anxious symptomology. This composite score was derived from scores obtained from parent-completed Child Behavior Checklist, the Quay anxiety/withdrawal score, scores from the Child Depression Index, and the total score from the Revised Children’s Manifest Anxiety scale. High scores on the Anxiety Composite indicate greater degree of anxiety.

Spearman rho analyses were derived, and rhos corrected for tied ranks were used where appropriate. The Anxiety Composite was significantly correlated with total autism or nonautism NDI ratings, rho = .45, p < .02; and rho = .52, p < .01, respectively; and with social interaction NDI ratings, rho = .39, p < .05; and communication ratings, rho = .43, p < .05. The correlation between Anxiety Composite and stereotypic/restricted behaviors (rho = .35) was not statistically significant, p = .056, although it was in the predicted direction and close in value to the significant correlations. Analyses of the association between Anxiety Composite and AR, FSIQ, and posterior cerebellar vermis area did not yield significant correlations, ps > .35.

DISCUSSION

In this study, autistic behaviors were examined among girls with or without fragile X who had FSIQ scores that ranged from MR to normal intellectual function. The findings support the notion that a pattern similar to that reported for males with fragile X is seen among girls with the disorder. A significant related finding was the lack of an association between MR and autism among the girls with fragile X. The degree of stereotypic/restricted behaviors was associated with both the degree of FMR1 gene expression, and the amount of decreased area in lobules VI and VII of the cerebellar vermis. These results are discussed with respect to whether the findings (a) contribute toward the specification of the behavioral phenotype of individuals with fragile X, (b) are consistent with the notion that fragile X and autism are associated, and (c) have implications for understanding the neurobiology of autism.
Autistic Behavior in Girls with Fragile X

Autism as a Behavioral Phenotype of Fragile X

Autistic Behaviors Among Boys and Girls with Fragile X

The girls with fragile X in this study exhibited a pattern of autistic-like behaviors very similar to the pattern described for boys with fragile X (Reiss & Freund, 1990, 1992). This pattern includes abnormalities in social and imaginative play, nonverbal communication, language form, and stereotypic/restricted behaviors. Autistic behaviors that were not endorsed for girls with fragile X were those pertaining to impairment in the awareness of others' feelings, lack of seeking comfort, and impaired imitation skills. The DSM-III-R criteria for which a statistically significant group difference emerged (on parental endorsements) included only those criteria for which significant group differences have also been reported for boys with fragile X versus controls (Reiss & Freund, 1990, 1992), with the exception of a significant difference (among the girls in this study) in the ability to establish and maintain peer relationships. Although group means on parental responses to the NDI suggested additional differences across the two groups of girls, the fact that the group differences were often based on mean ratings below 1 indicate that the more meaningful analyses of NDI responses were those assessing frequency of a positive rating of a behavior as a function of group. These frequency analyses were consistent with previously reported data for boys with fragile X.

Although qualitatively similar to behavior profiles reported for boys, the frequency of these autistic behaviors was lower among the girls in this study than were frequency ratings reported for boys. For instance, abnormal nonverbal communication (including poor eye contact) has been reported for 72% of boys studied by Reiss and Freund, versus 50% of the girls in this study. Qualitatively similar but quantitatively different profiles are consistent with the overall behavioral and intellectual profiles reported elsewhere for males and females with fragile X (see Baumgardner, Green, & Reiss, 1992, for a review).

The Fragile X-Autism Association

The presence of autistic behaviors among individuals with fragile X, and the consistency in the specificity of these behaviors across studies, suggests an association between these disorders. This association is implicated despite the low frequency of girls with fragile X for whom an autistic disorder diagnosis emerged, particularly in view of the behaviorally defined nature of autistic disorder. Further evidence of an association between these two disorders—or more specifically between fragile X and specific
types of autistic behaviors—is drawn from the consistency in types of autistic behaviors seen across studies of males with fragile X, and across studies of males and this study of females. This association does not imply that either disorder is a common etiology of the other, but does suggest that fragile X, a disorder with a specific genetic etiology, is an important model for understanding the neuropathology underlying certain autistic behaviors.

The lack of a correlation between autistic behaviors and FSIQ score further supports the notion that autistic behaviors are an important component of the fragile X phenotype. The distribution of FSIQ scores among this sample of girls with fragile X differed from a normal distribution, with the group mean falling in the low average range (87.4). Therefore, a higher frequency of autistic behaviors may have occurred had the group included more girls with MR. Nevertheless, the range of FSIQ scores spanned the MR, borderline, and average ranges, and one third of the girls with fragile X had FSIQ scores in the MR (3 girls) to borderline (7 girls) range (< 80). The frequency of autistic behaviors in the fragile X group was not accounted for by this group of 10 girls. Although these findings corresponds directly to autistic behaviors and not to autism per se, the indirect implications may pertain to the behaviorally defined autistic disorder. Moreover, the relative frequency of autistic behaviors among girls with FSIQ scores > 80 is in direct contrast to the contention that MR is the common link between autism and fragile X syndrome.

Implications for the Neurobiology of Autism

Cohen et al. (1991) proposed that a prominent feature of the fragile X male phenotype suggestive of a mechanism leading to autism or autistic behavior is social avoidance. Related to their proposal is the finding that social anxiety has been reported for girls with fragile X, including those girls from the present study (Freund, Reiss, Baumgardner, & Denckla, 1997). Among these girls, the association between a measure of anxiety and the NDI autistic behavior ratings contributes additional support for the notion that social anxiety underlies some autistic-like behaviors. Further studies of the qualitative aspects of this anxiety are needed in order to understand the nature of this association.

One important finding in the present study, with respect to the role of anxiety in fragile X and autistic behavior, was the lack of an association between the anxiety composite score and FSIQ, suggesting that anxiety has an impact on the social functioning of girls with fragile X that is independent of their level of intellectual functioning. The weakest association between the anxiety composite score and behavior occurred for the stereotypic restricted behavior ratings, suggesting that there may be specificity in the degree to which anxiety influences autistic behavior. The complemen-
tary finding that posterior cerebellar vermis area was not strongly associated with these stereotypic/restricted behaviors, and not with the social behaviors, suggests two potentially independent mechanisms leading to different types of autistic behaviors. The association between AR and both stereotypic/restricted behaviors and posterior cerebellar vermis area has important implications for a function of the FMR1 gene, and for neurobiological components of these specific autistic behaviors.

Findings across neurobiological studies of autism have yielded inconsistent findings, presumably due to the heterogeneous etiology of the disorder (Ciaramello & Ciaramello, 1995). As described earlier, cerebellar hypoplasia of posterior vermal lobules VI and VII has been reported for a subset of individuals diagnosed with autism (Courchesne, Townsend, Akshoomoff, et al., 1994). Additional evidence from autopsy studies include preliminary findings of Purkinje neuronal loss in the posterior vermis of several autistic patients (Arin, Bauman, & Kemper, 1991; Courchesne, Townsend, Akshoomoff, et al., 1994). The finding of cerebellar hypoplasia for autistic patients is consistent with findings from neuroimaging studies of individuals with the fragile X mutation, in which cerebellar hypoplasia of vermal lobules VI and VII were also found (Reiss, Aylward, et al., 1991; Reiss, Freund, et al., 1991). Moreover (as would be expected with an X-linked disorder), gender differences in the degree of cerebellar hypoplasia have been found between fragile X males and females, and those differences are consistent with the gender differences reported for autistic behaviors included within the fragile X phenotype. That is, with respect to the posterior fossa, the neuroanatomical differences between fragile X females and age- and FSIQ-matched controls are clearly present but are not as pronounced as those differences between fragile X males and controls (Reiss, Abrams, Greenlaw, et al., 1995), suggesting an “intermediate neuroanatomical effect” in females similar to the intermediate cognitive effects reported elsewhere (Kemper, Hagerman, Ahmad, & Mariner, 1986; Kovar et al., 1997; Mazzocco et al., 1993; Pennington, Schreiner, & Sudhalter, 1991) and the intermediate gender effect reported between the present study and earlier studies of autistic behaviors in males. Taken together, these findings provide additional support for the mounting evidence implicating cerebellar hypoplasia in the pathogenesis of some autistic spectrum behaviors in both individuals affected by the fragile X mutation and a subset of those diagnosed with autism.

Cerebellar Dysfunction and Autistic Behavior

Cerebellar dysfunction could account for the development of autistic behaviors through difficulty with processing of sensory stimuli. The findings
reported here indicate a relation between cerebellar hypoplasia and those autistic behaviors characterized by a marked restriction in the behavioral repertoire of activities and interests in girls with fragile X. This repertoire of behaviors includes stereotyped, ritualistic, perseverative behaviors (such as hand flapping, rocking, and word repetition), a narrow range of interests, and preoccupation with/or oversensitivity to specific auditory, visual, and tactile stimulation. These behaviors may reflect the child’s adaptation to a deficit in the modulation of sensory arousal (Huebner, 1992). That is, in response to persistent overarousal, the child narrows and severely restricts her behavioral repertoire to produce a self-calming effect. Knowledge of the functional neuroanatomy of the cerebellar vermis supports the proposed relation between the behaviors and cerebellar dysfunction. As reviewed elsewhere (Courchesne, Townsend, & Saitoh, 1994; Reiss, Aylward, et al., 1991) the cerebellar vermis plays a role in mediating sensory stimulation and arousal through its connections to the somatosensory, auditory and visual cortices (Crispino & Bullock, 1984; Joseph, Shambes, Gibson, & Welker, 1978) and the brainstem reticular formation (Tang & Zhang, 1987), known to be involved in arousal. Therefore, hypoplasia of the cerebellar vermis could account for a neuroanatomical deficit in the mediation of sensory stimulation and arousal which could lead to a restricted behavioral repertoire.

Stereotyped, perseverative behaviors may also reflect a deficit in the child’s attentional system. Such behaviors are consistent with impairment in the child’s capacity to shift attention voluntarily and rapidly, a deficit which has been found independently in both autistic patients and patients with acquired cerebellar lesions (Courchesne, Townsend, Akshoomoff, et al., 1994), and in individuals with fragile X (Mazzocco, Hagerman, & Pennington, 1992). The findings reported here provide neuroanatomical support for the proposed relation between cerebellar maldevelopment and the impairment of the attentional system, as does the finding that cerebellar area predicts attention to novelty in rats (Anderson, 1994). Cerebellar maldevelopment may play a role in the impairment in the capacity to shift attention through cerebellar connections to the reticular activating system (Haines, 1995), the superior colliculus (Crispino & Bullock, 1984), and the posterior parietal lobe (Schmahmann, 1991).

Impairment in the child’s capacity to shift attention may further underlie the child’s difficulties in social communication. The findings reported here indicate a relation between cerebellar hypoplasia and autistic behaviors characterized by pragmatic deficits in nonverbal communication, speech production and capacity to initiate and maintain conversations. Courchesne, Townsend, Akshoomoff, et al. (1994) suggested that impairment in the ability of children with autism to shift attention may undermine shared attention in
early parent–child social interaction, thus disrupting the child’s development of social communication skills. Neuroanatomical connections between the cerebellar vermis and cortical areas involved in auditory processing (Crispino & Bullock, 1984; Huang & Burkard, 1986) further support this proposed relation. In fragile X syndrome, the nature of the communication profiles may differ from that of children with autism. For instance, the repetitive language reported among males with fragile X involves more perseveration and less echolalia than does the language of males with autism (Sudhalter, Cohen, Silverman, & Wof-Schein, 1990). Components of social communication skills may be influenced more by social anxiety than by linguistic deficits per se. Awareness of social expectations (Freund, Reiss, Baumgardner, & Denckla, 1997), and subtle aspects of social awareness (such as perspective taking and emotion perception) appear intact among girls and women with fragile X (Kovar et al., 1997; Mazzocco et al., 1993; respectively), despite reported deficits in their social behavior and competence. Specific aspects of social interaction and communication may represent important domains in which fragile X and autism differ.

Conclusion

The findings from this study support the contention of an association between autism and fragile X. Girls with fragile X were found to have autistic behaviors that were not seen in age- and IQ-matched controls. These autistic behaviors were similar in quality, but occurred with lower frequency, relative to reports to autistic behaviors in boys with fragile X. Among the girls in this study, autistic behaviors were not linked with MR, nor with FSIO scores overall, a finding that highlights the impact of the FMR 1 gene independent of global intellectual functioning. Anxiety was most strongly related to social and communication aspects of the girl’s autistic behaviors, whereas neuroanatomical measures of the posterior cerebellar vermis were most strongly associated with their degree of stereotypic and restricted behaviors. The stereotypic/restricted behaviors were negatively correlated with the prevalence of active non-fragile chromosomes as measured by AR, and with neuroanatomical area of the posterior cerebellar vermis (lobules VI and VII).

Maldevelopment of the cerebellar vermis may account for the pathogenesis of stereotyped, ritualistic, perseverative, behaviors; impaired social communication; and oversensitivity to sensory stimulation through the cerebellum’s role in integrating auditory, visual, and tactile stimulation, modulating arousal, and facilitating voluntary shifts in attention. The limited overlap between correlates of anxiety and correlates of cerebellar vermis areas may result from the presence of two distinct, separate pathways to
behavioral dysfunction; or from a distinction between primary effects associated with cerebellar vermis area and secondary effects on social behavior and anxiety. Regardless of which hypothesis is supported through future research, the findings from this study demonstrate that anxiety ratings and posterior cerebellar area measures have distinct associations with subsets of autistic behaviors. These associations have important implications for understanding the neurobiology of autistic spectrum behaviors, in both individuals with fragile X and individuals with autistic disorder.

REFERENCES


(FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*, 65, 905-914.


Copyright of Journal of Autism & Developmental Disorders is the property of Kluwer Academic Publishing and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.