Compulsive, Self-Injurious, and Autistic Behavior in Children and Adolescents With Fragile X Syndrome

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Abstract

Compulsive, self-injurious, and autistic behaviors were examined in 31 boys and 29 girls with fragile X syndrome aged 5 to 20 years. Self-injurious behavior occured in 58% of boys and 17% of girls, whereas compulsive behavior occurred in 72% of boys and 55% of girls and did not appear to be associated with self-injurious behavior. Fifty percent of boys and 20% of girls met diagnostic criteria for autism on the ADOS-G. Girls who showed compulsive behavior had lower levels of FMRP than girls who did not show compulsive behavior, and boys with autistic symptoms had lowered levels of cortisol. Taken together, these data suggest that autistic and compulsive behaviors are highly prevalent in fragile X syndrome and that lowered levels of FMRP and cortisol may be biological markers for these behaviors.

Several authors have noted the extent to which problem behaviors shown by children with genetic disorders often appears to have a compulsive quality and, by implication, may be highly resistant to treatment (Bodfish et al., 1995; King, 1993). For example, the severe self-injurious behavior (SIB) shown by children with Lesch-Nyhan syndrome has often been described as involuntary and unpredictable (Dismang & Cheatham, 1970; Nyhan, 1994), and the individuals themselves have described how they are unable to refrain from engaging in the behavior (Christie et al., 1982). Many other behaviors that are characteristic of particular syndromes, often termed *behavioral phenotypes*, include hand-biting in fragile X syndrome (Symons, Clark, Hatton, Skinner, & Bailey, 2003), face-hitting in Cornelia de Lange syndrome (Bryson, Sakati, Nyhan & Fish, 1971) smiling and laughing in Angelman syndrome (Oliver, Demetriades, & Hall, 2002), skin-picking in Prader-Willi syndrome (Thornton & Dawson, 1990), and hand-wringing in Rett syndrome (Hagberg, Aicardi, Dias, & Ramos, 1983). Individuals diagnosed with particular genetic syndromes appear to show higher levels of these specific problem behaviors than would be expected in individuals matched for developmental age but without a diagnosis of a syndrome (Bodfish & Lewis, 2002). For example, hand-biting has been reported to occur in over 70% of individuals with fragile X syndrome (Symons et al., 2003) and in almost all cases of children with Lesch-Nyhan syndrome (Anderson & Ernst, 1994; Christie et al., 1982).

Although many problem behaviors can be seen as almost inevitable features of these syndromes, several researchers have documented the influence of environmental factors on behaviors shown by individuals with genetic syndromes (Anderson, Dancis, & Alpert, 1978; Hall, DeBernardis, & Reiss, 2006; Hall, Oliver, & Murphy, 2001; Oliver, Murphy, Crayton, & Corbett, 1993). These studies have shown that many syndrome-specific behaviors can be influenced by antecedent and/or consequent social–environmental events (e.g., task demands, contingent removal of task demands) and in some cases, these behaviors can be reduced in frequency by the manipulation of the environmental factors. For example, Anderson et al. showed that compulsive hand-biting in Lesch-Nyhan syndrome increased when social attention was presented contingently and decreased when the behavior was ignored or when

attention was delivered contingent on the absence of hand-biting. Similarly, Hall et al. (2006) found that hand-biting in children with fragile X syndrome was more likely to occur during antecedent social demands, suggesting that the behaviors may have been maintained by negative reinforcement. These data suggest that despite the apparent compulsivity of many syndrome-specific behaviors, there may be a complex interplay between genetic and environmental factors in their genesis and maintenance.

Despite the apparent compulsive quality of many syndrome-specific behaviors, very few investigators have actually documented the extent to which problem behaviors in genetic syndromes are associated with compulsive behaviors. In a postal survey of children and adults with Cornelia de Lange syndrome, Hyman and colleagues (Hyman, Oliver, & Hall, 2002) found that 88% of individuals showed at least one form of compulsive behavior and that those individuals who showed compulsive behaviors were also more likely to exhibit self-injury. Both behaviors became more prevalent with age, leading the authors to speculate that over time, certain forms of self-injury may begin to activate neurochemical systems in the brain and, thereafter, develop compulsive properties. Compulsive behaviors have also been shown to be highly prevalent in individuals with Prader-Willi syndrome (Dykens, Leckman, & Cassidy, 1996), although in this syndrome, the association between compulsive behaviors and self-injury has not yet been investigated.

To our knowledge, there are no published studies in which researchers have investigated the co-morbidity of compulsive and problem behaviors in individuals with fragile X syndrome, the most common form of inherited developmental disability. Fragile X syndrome is caused by a mutation to the FMR1 gene on the long arm of the X chromosome; the mutation switches off production of the fragile X mental retardation protein (FMRP), the protein product of the gene (Devys, Lutz, Rouyer, Bellocq, & Mandel, 1993; Tamanini et al., 1997). This protein is an RNA-binding protein involved in regulating the production of proteins underlying the maturation and elimination of synapses during brain development (Greenough et al., 2001). Low levels of FMRP in individuals with the full mutation appear to cause or at least exacerbate the behavioral symptoms of fragile X syndrome (Reiss, Freund, Baumgardner, Abrams, & Denckla, 1995; Tassone et al., 1999), including deficits in executive functioning and memory, increased levels of anxiety, eye-gaze aversion, hand-biting, hyperactivity, and stereotypic behaviors (Cohen et al., 1988; Freund, Reiss, & Abrams, 1993; Lachiewicz, 1992; Lachiewicz & Dawson, 1994; Mazzocco, Baumgardner, Freund, & Reiss, 1998). Although the syndrome affects both genders (1 in 4,000 boys and 1 in 6,000 girls), boys with fragile X syndrome have significantly lower levels of FMRP and are, therefore, more affected by the disorder in terms of intellectual functioning. Therefore, FMRP can be seen as a useful biological marker for linking genes to behavior in this syndrome.

Reduced or absent FMRP in fragile X syndrome has also been found to be associated with abnormal regulation of two glucocorticoid-related proteins, GR-II and Ann-1 (Miyashiro et al., 2003; Sun, Cohen, & Kaufmann, 2001), leading some investigators to suggest that many of the cognitive and behavioral features observed in fragile X syndrome may result from dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis (Hessl, Rivera, & Reiss, 2004). The GR-II receptor reduction disrupts corticosteroid feedback regulation (Miyashiro et al., 2003), whereas Ann-1 mediates several hormonal regulatory responses, in particular, the inhibition exerted by glucocorticoids on the HPA axis (Jessop, 1999). Recent studies have also shown that the stress hormone cortisol, an indicator of HPA axis dysfunction, is significantly elevated in males with fragile X syndrome in comparison to typically developing children (Hessl et al., 2002). High levels of salivary cortisol were also found to be associated with increased levels of gaze aversion and hyperactivity in males with fragile X syndrome while they were undertaking a social stress test (Hall et al., 2006), suggesting that these specific behaviors are more likely to be evoked under periods of high stress.

Curin et al. (2003) have described HPA dysfunction in autism, a behaviorally defined syndrome that shares many of the phenotypic features with fragile X syndrome. Indeed, some investigators have speculated that there may be additional genetic mechanisms involved in individuals diagnosed with both conditions (Rogers, Wehner, & Hagerman, 2001). Although there is some debate concerning the extent to which these syndromes overlap, several researchers have indicated that the incidence of autism in fragile X syndrome may be as high as 25% (Bailey et al., 1998; Reiss & Freund, 1990). Recently, investiga-

tors have employed more refined state-of-the-art diagnostic measures (e.g., the Autism Diagnostic Observation Scale-Generic—ADOS-G), and these researchers have generally reported even higher incidences of autism in fragile X syndrome (up to 44%) than those using earlier diagnostic measures (Philofsky, Hepburn, Hayes, Hagerman, & Rogers, 2004; Rogers et al., 2001).

Given the apparent link between autism and fragile X syndrome, and the link between SIB and compulsive behavior, it is surprising that no investigators have examined the extent to which compulsive, self-injurious, and autistic behaviors are co-morbid in individuals with fragile X syndrome. In addition, no researchers have determined the extent to which levels of FMRP and cortisol may affect the occurrence of these behaviors. Our aim in the present study was, therefore, two-fold: (a) to describe the prevalence and comorbidity of self-injurious, compulsive, and autistic behaviors in fragile X syndrome and (b) to determine the extent to which the occurrence of these behaviors is influenced by levels of FMRP and salivary cortisol. Given the expected differences between male and females with fragile X syndrome in terms of intellectual functioning, FMRP, and cortisol levels, the results for each group were subjected to separate analyses.

Method

Participants

Participants were 60 children and adolescents with fragile X syndrome (31 males, 29 females) between 5 and 20 years of age (males: $M = 13.21$, $SD = 3.16$; females: $M = 13.06$, $SD = 3.93$). The diagnosis of fragile X for each child was confirmed by Southern Blot DNA analysis as detailed by Taylor et al. (1994); 7 (22.6%) males and 4 (13.8%) females were mosaic for fragile X syndrome. Twenty-two (71.0%) males and 13 (44.8%) females were taking medication at the time of the assessment. Medications primarily included stimulants (40% of the sample), antidepressants (30%), antipsychotics (5%), antihypertensives (5%), and anticonvulsants (5%). Twenty-three percent of participants were taking more than one class of medication. None was taking medications known to affect cortisol levels, such as birth control pills, estrogen, androgen hormones, spironolactone (Aldactone), phenytoin (Dilantin), hydrocortisone (Cortef, hydrocortone), prednisone (Deltasone), and quinacrine (Atabrine).

Participants were recruited from across the United States (95%) and Canada (5%) as part of a larger longitudinal study investigating the developmental outcomes of school-age children with fragile X syndrome. Inclusion criteria were (a) the child had received a diagnosis of fragile X syndrome (CGG repeat length greater than 200), (b) the child had a same-gender biological sibling who did not have fragile X syndrome (CGG repeat length was less than 40), and (c) the child's mother was a carrier of the fragile X mutation (CGG repeat length was greater than 50 but less than 200). Families were recruited through our research database, the National Fragile X Foundation, flyers distributed to special interest groups, local contacts, and our research website. Written informed consent was obtained from the parents of all participants, and assent was obtained from the child if he or she understood the procedure.

Measures

To document the prevalence and phenomenology of compulsive, self-injurious, and autistic behaviors in fragile X syndrome, we adopted measures that had previously been employed for this purpose in behavioral research in individuals with mental retardation (Bodfish et al., 1995; Hyman et al., 2002; Lewis & Bodfish, 1998). The Compulsive Behavior Checklist (CBC) and Self-Injury Checklist (SIB-C) are reliable and valid instruments that have no item overlap. To measure autistic behavior, we used the ADOS-G because this measure employs direct observational methods, is widely used and validated in individuals with autism, and has been used previously in children diagnosed with fragile X syndrome (Philofsky et al., 2004; Rogers et al., 2001).

Compulsive behavior. The CBC (Bodfish et al., 1995) contains 25 types of compulsions, grouped under five categories: Ordering (e.g., arranges objects in a certain pattern), Completeness/Incompleteness (e.g., insists on closing open doors, open cupboards), Cleaning/Tidiness (e.g., cleans body part excessively), Checking/Touching (e.g., touches or taps item repeatedly), and Deviant Grooming (e.g., inappropriately cuts hair, eyebrows, or pubic hair). The instructions on the front sheet were: "Checkmark behaviors present and underline words that apply." The dependent variables were the number of types of compulsions displayed (of the 25 listed) and the number of categories represented (of the 5 listed). Psychometric analysis has established good interrater reliability

(84.8%), test–retest stability (83.3%), and validity by direct observation (91.4%) (Bodfish et al., 1995).

Self-injurious behavior. The SIB-C (Bodfish et al., 1995) contains seven types of SIB: hits self with body part, hits self against surface of object, hits self with object, bites self (e.g., bites hand or wrist or arm), pulls hair or skin, rubs or scratches self, and inserts finger or object (e.g., eye-poking). The instructions on the front sheet were "Place a checkmark next to the item for any of the following list of behaviors which the subject displays in a repetitive manner (repeats the same movement/behavior twice or more in succession)." The dependent variable was the number of types of SIB displayed. Psychometric analysis has established good interrater reliability (89%), test–retest stability (92%), and validity by direct observation (90.2%) (Bodfish et al., 1995).

Autism Diagnostic Observation Schedule- Generic. The ADOS-G (Lord, Rutter, DiLavore, & Risi, 2002) is a semi-structured interview administered directly to the child by a trained experimenter. The assessment lasts approximately 30 min to 1 hour, during which the experimenter employs a number of toys and social "presses" designed to elicit symptoms of autism. Items include joint interactive play, make-believe play, and creating a story. The experimenter administers one of four modules, depending on the child's level of development: Preverbal (Module 1); Phrase Speech (Module 2); Children/Adolescents With Fluent Speech (Module 3); and Adolescents/Adults With Fluent Speech (Module 4). Selected individual items (scored 0, 1, or 2) are summed to yield three summary scores: Communication (4 to 5 items); Reciprocal Social Interaction (7 items); and Total Communication plus Reciprocal Social Interaction Score (11 to 12 items). Using the cut-off points described in the manual, we employed these summary scores to determine whether the child fell into "autism," "spectrum," or "none" categories. According to the manual, interrater reliability for the Communication, Reciprocal Social Interaction, and Total Communication plus Reciprocal Social Interaction Score is .93, .84 and .92, respectively. Test–retest stability is .78, .73, and .82, respectively (Lord et al., 2002). Experimenters were trained to greater than 90% interrater agreement on all protocol and algorithm scores.

Intellectual functioning. The Wechsler Intelligence Scale for Children–Third Edition—WISC-III (Wechsler, 1991) and the Wechsler Adult Intelligence Scale–Third Edition—WAIS-III (Wechsler, 1997) are standardized measures of intellectual functioning for children ages 6 to 16 years and adults ages 17 years and over, respectively. Each test contains 5 verbal subtests and 5 performance subtests, yielding a Full-Scale (FSIQ) standard score. Standard scores have a population mean of 100 and an *SD* of 15.

Procedure

Two researchers visited the participant's home and conducted the intelligence test in the morning (approximately 10 a.m.) and the ADOS-G in

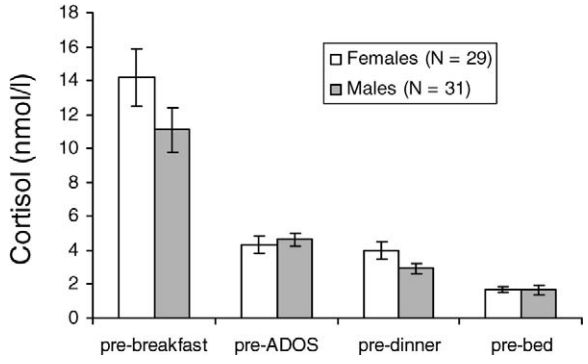

Figure 1. Cortisol concentrations obtained from saliva in males and females with fragile X syndrome at four time points throughout the evaluation day.

Table 1. Prevalence of Forms of Self-Injury and Compulsive Behavior in Persons With Fragile X (in %) by Gender

Behavior	Males (n = 31)	Females (n = 29)
Self-injury		
Hitting self	16.2	0.0
Biting self	45.2	6.9
Pulling hair or skin	3.2	3.4
Rubbing or scratching	22.6	13.8
Eye poking	0.0	0.0
Compulsions		
Ordering	54.8	44.8
Completeness	48.4	31.0
Cleaning/tidiness	32.3	24.1
Checking/touching	41.9	13.8
Grooming	19.4	20.7

the afternoon (approximately 3 p.m.). Children who were preverbal (7 boys, 4 girls) received Module 1 of the ADOS-G; those with phrase speech only (8 boys, 2 girls) received Module 2; children/adolescents with fluent speech (8 boys, 11 girls) received Module 3; and adolescents/adults with fluent speech (8 boys, 12 girls) received Module 4. Mothers filled out a demographic questionnaire and the CBC and SIB-C checklists.

Salivary cortisol was measured at four approximate time points throughout the evaluation day: pre-breakfast (8 a.m.), pre-ADOS-G (3 p.m.), pre-dinner (5 p.m.), and pre-bedtime (9 p.m.). On each occasion, participants were asked to place a 4-cm cotton roll (Sarstedt, Inc.) into their mouth for 1 to 2 min and to think of their favorite food. After each sample had been taken, the salivette was placed in a container in the household freezer, and the container subsequently mailed via overnight mail to the research office. The saliva samples were stored at −20° C until analysis; after thawing, the samples were centrifuged at 2000 g for 10 min and 100 ul of saliva was used for duplicate analysis. Cortisol levels were determined by employing a competitive solid phase time-resolved fluorescence immunoassay with flouromeric end-point detection (DELFIA) (Trier, Germany). The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding interassay coefficients of variation were between 7.1% to 9.0%.

Blood drawing kits and consent forms were mailed directly to each family in order to obtain FMRP levels for the child with fragile X syndrome. Blood draws were performed by a local physician; samples were mailed directly to Kimball Genetics (Denver, CO) using overnight mail. The FMRP immunostaining, an indirect alkaline phosphatase technique, was used according to Willemsen et al. (1997). Slides were analyzed under the microscope, distinguishing lymphocytes from other blood cell types by morphology. For each slide, 200 lymphocytes were scored, and the percentage of lymphocytes expressing FMRP was determined. Scoring was blind with respect to DNA results.

Statistical Analyses

Our plan was to conduct exploratory analyses of the dataset to generate hypotheses that could inform future direct tests of relations among problem behaviors, autistic features, and biological markers in fragile X syndrome. For this reason we have chosen not to correct for multiple comparisons, but instead report resulting effect sizes.

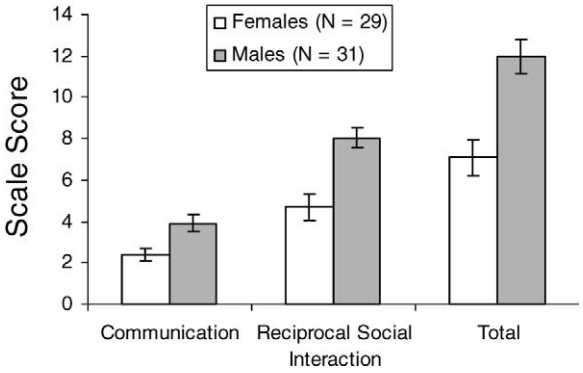

Figure 2. Profile of scale scores from the Autism Diagnostic Observation Schedule–Generic (ADOS-G) for males and females with fragile X syndrome.

Results

Salivary Cortisol, FMRP, and Intellectual Functioning

Figure 1 shows the mean levels of salivary cortisol obtained at each of the four evaluation points for each group. Repeated measures ANOVA indicated that there was a significant main effect of sample time, with cortisol levels declining significantly throughout the day, as expected, $F(3, 165) = 57.39$, $p < .001$. There was no main effect of gender, indicating that there was no difference between males and females in the profile of diurnal decline observed throughout the day.

In males, the mean percentage FMRP expressed in blood was 14.70% ($SD = 16.05$), range = 1% to 60.5%. In females, mean percentage FMRP was 52.02% ($SD = 16.97$), range = 15% to 84.5%. As expected, females had significantly higher levels of FMRP than males, $t(52) = 8.30$, $p < .001$, $d = 2.27$.

On the Wechsler Intelligence Scales (Wechsler, 1991/1997), WISC-III/WAIS-III, the mean IQ for male and female participants was 46.33 ($SD = 9.66$) and 70.76 ($SD = 20.91$), respectively. As expected, females had significantly higher IQs than did males, $t(57) = 5.79$, $p < .001$, $d = 1.50$.

Compulsive Behavior

Twenty-three boys (74.2%) and 16 girls (55.2%) showed at least one form of compulsive behavior on the CBC (see Table 1). The table shows that ordering and completeness compul-

sions were the most prevalent forms of compulsive behaviors for both genders. The mean total number of compulsions displayed in boys and girls was 3.77 ($SD = 3.48$) and 2.55 ($SD = 3.30$), respectively, a nonsignificant difference between the groups. The mean number of categories of compulsions displayed in boys and girls was 1.96 ($SD = 1.56$) and 1.41 ($SD = 1.66$) respectively, a nonsignificant difference between groups. Eighteen boys (58%) and 11 girls (38%) showed two or more categories of compulsive behavior. Correlation analyses indicated that there was no effect of age, FSIQ, or cortisol level on the prevalence of compulsions or on the number of compulsions displayed in either group. However, girls who showed compulsive behavior had significantly lower levels of FMRP ($M = 44.6\%$, $SD = 15.4$) than girls who did not show a compulsive behavior ($M = 60.0\%$, $SD = 15.29$), $t(25) = 2.60$, $p = 0.02$, $d = 1.00$. Girls with lower levels of FMRP were also more likely to show more categories of compulsive behaviors, $r(27) = -.45$, $p < .02$, and total number of compulsive behaviors, $r(27) = -.43$, $p < .03$. In boys with fragile X syndrome, there was no association between FMRP levels and the number of categories of compulsions, $r(27) = -.07$, or total number of compulsions, $r(27) = -.10$.

Self-Injurious Behavior

Eighteen boys (58.1%) and 5 girls (17.2%) showed at least one form of SIB on the Self-Injury Checklist (Table 1), with boys being significantly more likely to show SIB than girls, χ^2 (1, $N = 60$) $= 10.56$, $p < .001$. The table shows that biting self and rubs or scratches self were the most prevalent forms of SIB for both genders, with boys being more likely to exhibit "biting self" than were girls, χ^2 (1, $N = 60$) $= 11.22$, $p < .001$. The mean total number of SIBs displayed in boys and girls was .87 ($SD = .96$) and .24 ($SD = .64$), respectively, with boys showing more forms of SIB than did girls, $t(58) = 2.98$, $p = .004$, $d = .77$. Correlation analyses indicated that there was no effect of age, FSIQ, FMRP, or cortisol level on the prevalence of SIB or on the number of forms of SIB displayed in either group.

Autistic Features

Sixteen boys (51.6%) and 6 girls (20.7%) scored in the autism category on the ADOS-G. An additional 7 boys (22.6%) and 7 girls (24.1%) scored in the spectrum category. Boys were significantly more likely to score in either the spectrum or autism categories than were girls, χ^2 (1, $N = 60$) $= 5.38$, $p = .02$. Figure 2 shows a breakdown of the scores obtained by gender. There were significant differences between the groups on the Communication scale, $t(59) = 2.86$, $p = .01$, $d = .79$, the Reciprocal Social Interaction scale, $t(59) = 4.29$, $p < .001$, $d = 1.15$, and on the Total Score, $t(59) = 4.07$, $p < .001$, $d = 1.10$, with males obtaining significantly higher scores on these scales than did females. Correlation analyses indicated that there was no association between age, FMRP level, and ADOS-G Total Score in either group. However, in girls with fragile X syndrome, there was a significant association between FSIQ and ADOS-G Total Score, $r(28) = -.45$, $p < .05$, indicating that girls with lower IQs were more likely to show autistic behaviors. In males with fragile X syndrome, there was a significant negative association between ADOS-G Total Score and the pre-ADOS salivary cortisol sample, $r(30) = -.57$, $p < .01$. These data indicate that males who showed more autistic behaviors were more likely to have lower levels of salivary cortisol. There was no association between cortisol level and ADOS-G Total Score in girls with fragile X syndrome, $r(28) = -.04$.

Association Between SIB, Compulsive Behavior, and Autism

Individuals who showed SIB were no more likely to show compulsive behaviors than were individuals who did not. There was also no association between the number of forms of SIB and the number of compulsive behaviors displayed by boys or girls. Of the 16 boys and 6 girls who scored in the autism category on the ADOS-G, 12 boys and 3 girls exhibited compulsive behavior; 9 boys and 0 girls, SIB; and 6 boys and 0 girls, both self-injurious and compulsive behaviors. There was no association between the number of forms of SIB or compulsions displayed, and the total score on the ADOS-G. Individuals with autism or spectrum disorder were no more likely to show SIB or compulsions than were individuals without autism.

Discussion

In their review of research on the phenomenology and comorbidity of repetitive behavior dis-

orders in individuals with autism, Lewis and Bodfish (1998) noted that many studies suffered from methodological problems that included the use of instruments that had significant item overlap. To avoid this conceptual overlap problem, we studied a disorder with a well-known genetic basis that does not include repetitive behavior disorder (e.g., compulsions, self-injury, stereotypy) as a defining characteristic. We also employed measures of these behaviors that had high discriminant validity. For example, the ADOS-G scoring algorithm does not include items related to compulsive and self-injurious behaviors, or stereotypic behaviors and the rating scale we employed to measure compulsive behavior did not include items related to self-injury.

In this study, we found that 74% of boys and 55% of girls showed compulsive behavior, with ordering and completeness compulsions being the most prevalent forms of this type of behavior. To our knowledge, this is the first study to document the high prevalence of compulsive behaviors in fragile X syndrome and indicates that compulsive behavior may form a significant part of the behavioral phenotype of the syndrome. A high incidence of compulsive behaviors has also been documented in individuals with Cornelia De Lange syndrome (Hyman et al., 2002) and Prader-Willi syndrome (Dykens, Leckman, & Cassidy, 1996), syndromes in which SIB also frequently occurs. Interestingly, in this study, we did not find an association between compulsions and SIB, suggesting that in fragile X syndrome at least, the two behaviors may not be related. We note, however, that these are exploratory analyses that need to be confirmed experimentally (e.g., a gender group study where groups are properly matched for age and IQ).

We found that 58% of boys and 17% of girls showed at least one form of SIB, with self-biting being the most prevalent form, occurring in 45% of boys and 7% of girls. Previous researchers have also noted the high prevalence of self-biting in fragile X syndrome, particularly in boys (Symons et al., 2003). However, the children in those study samples tended to be much younger than those in the present study. For example, in a postal survey, Symons et al. found that hand-biting occurred in 72% of participants aged 2 to 12 years.

We found that 52% of boys and 21% of girls with fragile X syndrome scored in the autism category on the ADOS-G, a standardized and well-validated observational measure of autism. Previous researchers have documented much lower prevalence rates of autism in fragile X syndrome, ranging from 15% to 25%. Differences between these estimates and the current study are most likely due to the different demographic characteristics of the samples and the measures of autism employed. For example, Bailey et al. (1988) administered the Childhood Autism Rating Scale to boys with fragile X syndrome aged between 2 and 11 years and found the prevalence of autism to be 25%. Philofsky et al. (2004) reported that 44% of boys with fragile X syndrome aged 2 to 4 years scored in the autism range on the ADOS-G. To our knowledge, no previous researchers conducting studies with school-age children who have fragile X syndrome have reported the prevalence of autism using the ADOS-G, and none have reported the prevalence of autism in girls with fragile X syndrome on this measure.

Another interesting finding was the association between salivary cortisol and ADOS-G scores. Although we found no differences in average cortisol levels between males and females at each time-point, males who had more autistic symptoms had lower levels of salivary cortisol. These data support those of Curin et al. (2003), who showed that children diagnosed with autism had significantly lower levels of cortisol in comparison to a group of age-matched typically developing children. We also found no association between cortisol levels and the presence of SIB, also supporting the findings of Curin et al. The findings in this study do, however, contradict those of a previous study in which school-age males with fragile X syndrome were found to have significantly elevated levels of salivary cortisol in comparison to typically developing children (Hessl et al., 2002). Although the discrepancy in these findings needs to be explored in future studies, our results do suggest that in fragile X syndrome, degree of autistic symptoms may affect cortisol level, possibly because children with autism have impairments in reciprocal social interaction and, consequently, do not find social situations as stressful as do children with fragile X syndrome without autistic symptoms.

Although the presence of compulsive behavior was not associated with age, IQ, or cortisol level, we found that girls who showed compulsive behavior had significantly lower levels of FMRP than girls who did not. This finding suggests that there may be a potential link between compulsions and FMRP, the protein product of the

FMR1 gene. It is possible that the reason we found no association between compulsive behavior and FMRP levels in boys with fragile X syndrome was because of the restricted range of FMRP level in this group. No associations were found between FMRP levels and autistic behavior or SIB in either group of children.

We point out that at a simple level, reduced FMRP or hypermethylation of the FMR1 gene are both clear-cut biological markers of the disease state. Salivary cortisol, however, is a biological marker for the "state" of the human stress responsivity system (HPA) and, as such, is not specifically related to FMRP. We are conjecturing that when fragile X syndrome is present (as shown by the presence of the DNA or FMRP markers of disease), variation in the HPA system may moderate (and even perhaps mediate) the disease manifestations in the form of repetitive/stereotypic symptoms. Thus, salivary cortisol is perhaps best considered a marker reflecting the state of the HPA system that can further moderate effects of reduced FMRP.

Although the results of the present study suggest that low levels of cortisol and FMRP may be biological factors in the determinants of problem behaviors in fragile X syndrome, we did not examine the environmental determinants of these behaviors. Over the past few decades, researchers have shown that many behavior disorders in genetic syndromes are highly amenable to change by simple environmental manipulations (Iwata et al., 1994). For example, the frequency and severity of SIB in Lesch-Nyhan syndrome can be reduced by the presentation and/or removal of social–environmental antecedents and consequences (Anderson et al., 1978; Bull & LaVecchio, 1978); and in fragile X syndrome, the duration of hand-biting was shown to depend on levels of antecedent social interaction (Hall et al., 2006). These data suggest that operant learning processes (i.e., positive and negative reinforcement) may also be critical components in the genesis of these syndrome-specific behaviors. Future investigators should, therefore, begin to document the environmental determinants of these behaviors in children with fragile X syndrome before they become established in the child's repertoire. We hope that by isolating the environmental determinants of these behaviors early in the child's life, as well as documenting levels of biological markers such as cortisol and FMRP, biobehavioral interventions for these syndrome-specific behaviors may be possible in the near future.

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