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Autism Spectrum Disorder in Fragile X Syndrome: Communication, Social Interaction, and Specific Behaviors

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The present study extends our previous work on social behavior impairment in young males with fragile X syndrome (FraX). Specifically, we evaluated whether the autistic phenomenon in FraX is expressed as a range of behavioral impairments as in idiopathic autism (Aut). We also examined whether there are behaviors, identified as items of the Autism Diagnostic Interview-Revised (ADI-R), that in FraX predispose to or differentiate subjects with autism spectrum disorder (ASD) diagnosis. Finally, regression models were utilized to test the relative contribution of reduced communication and socialization skills to ADI-R scores and diagnoses. A cohort of 56 boys (3–8 years) with FraX was examined in terms of scores on measures of cognition (IQ was a co-variate in most analyses.), autistic behavior, problem/aberrant behavior, adaptive behavior, and language development. We found that, indeed, in terms of problem behavior and adaptive skills, there is a range of severity from FraX + Aut to FraX + PDD (Pervasive Developmental Disorder) to FraX + none. ADI-R items representing "Play" types of interaction appear to be "susceptibility" factors since they were abnormal across the FraX cohort. Integrated regression models demonstrated that items reflecting complex social interaction differentiated the FraX + ASD (Aut + PDD) subgroup from the rest of the FraX cohort, while abnormalities in basic verbal and non-verbal communication distinguished the most severely affected boys with FraX + Aut from the milder FraX + PDD cohort. Models incorporating language, adaptive communication, and adaptive socialization skills revealed that socialization was not only the main influence on scores but also a predictor of ASD diagnosis. Altogether, our findings demonstrate that the diagnosis of ASD in FraX reflects, to a large extent, an impairment in social interaction that is expressed with variable severity in young males with FraX.

KEY WORDS: fragile X; autism; autism diagnostic interview (ADI); social withdrawal; socialization; communication

INTRODUCTION

Fragile X syndrome (FraX) is currently the most common cause of inherited mental retardation. Its prevalence rates are approximately 1:4,000 males and 1:6,000 females [Kaufmann and Moser, 2000]. FraX is associated with an unstable expansion of a CGG polymorphism within the 5′-untranslated region of the *FMR1* gene, located in the X chromosome [Kaufmann and Reiss, 1999]. Depending on the number of CGG repeats, affected alleles are classified as normal (5–40), intermediate or gray zone (~45–54), premutation (~55–200), or full mutation (>200) [Maddalena et al., 2001]. A mixed pattern of full mutation and premutation alleles is termed size mosaicism [Rousseau et al., 1994; Kaufmann and Reiss, 1999]. Full mutation alleles are typically associated with hypermethylation, resulting in gene silencing and a phenotype that, in males, includes cognitive impairment and other behavioral abnormalities. Individuals with mosaic patterns characteristically show a milder cognitive impairment than those with full mutation [Hagerman et al., 1994; Merenstein et al., 1996; Kaufmann and Reiss, 1999; Kaufmann et al., 1999], and greater development of adaptive skills [Cohen et al., 1996]. Although many aspects of the behavioral phenotype of males with FraX have been delineated [Reiss and Freund, 1992; Freund, 1994; Kerby and Dawson, 1994; Lachiewicz et al., 1994; Baumgardner et al., 1995; Freund et al., 1995; Kau et al., 2000], the variability and specificity of most manifestations are still under investigation [Rogers et al., 2001; Hatton et al., 2002].

Autism (Aut) is one of the most recognized and severe behavioral abnormalities observed in males with FraX [Hagerman et al., 1986; Baumgardner et al., 1995; Cohen, 1995; Bailey et al., 1998; Kaufmann and Reiss, 1999; Hagerman, 2002]. Prevalence rates of Aut in FraX have been estimated at 15–33% [Hagerman et al., 1986; Bregman et al., 1987; Dykens and Volkmar, 1997; Feinstein and Reiss, 1998; Bailey et al., 1998; Rogers et al., 2001]. Several recent studies have concentrated on the differentiation between boys with both FraX and Aut (FraX + Aut) and those with idiopathic Aut. Results suggest similar, although milder, profiles on several measures of autistic behavior in the FraX + Aut group [Bailey et al., 1998; Rogers et al., 2001; Demark et al., 2003; Kau et al., 2003]. Studies have also focused on general behavioral abnormalities

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of boys with FraX + Aut, in order to ascertain whether this group represents a specific subset of subjects with FraX [Hagerman et al., 1986; Cohen, 1995; Turk and Graham, 1997; Bailey et al., 1998, 2000; Rogers et al., 2001; Hatton et al., 2002]. Hatton et al. [2002] found that scores of total problem behaviors, as measured by the Child Behavior Checklist (CBCL) [Achenbach, 1991], were higher and correlated with scores on the Childhood Autism Rating Scale (CARS) [Schopler et al., 1988] in FraX + Aut. Moreover, Rogers et al. [2001] have shown that boys with FraX + Aut had lower developmental scores than those with just FraX (FraX + none) according to the Vineland Adaptive Behavior Scale (VABS) [Sparrow et al., 1984], and lower cognitive scores as determined by the Mullen Scales of Early Learning [Mullen, 1995].

In a previous study [Kau et al., 2003], we further explored the potential existence of a distinct social behavioral profile (SBP) in boys with FraX + Aut and examined whether FraX + Aut exhibited similar autistic behavior and SBP patterns to idiopathic autistic comparison groups with and without language delay. Regardless of age, we found that young males with FraX + Aut display a characteristic pattern of problem/aberrant behavior and deficits in adaptive behavior. This SBP includes not only abnormalities in socialization, such as increased social withdrawal, but also greater scores on measures for irritability, stereotypic behavior, and attentional problems. In correspondence with Rogers et al. [2001], boys with FraX + Aut showed reduced IQ and socialization, daily living, and motor skills. Despite their greater impairment when compared with the non-autistic FraX cohort, boys with FraX + Aut showed milder withdrawal than subjects with idiopathic Aut. The FraX + Aut cohort also differed from comparison autistic groups in terms of a lessened severity of scores on the Reciprocal Social Interaction (Recs) domain of the Autism Diagnostic Interview-Revised (ADI-R) [Lord et al., 1989, 1994]. This difference suggested that the diagnosis of Aut in FraX may result from a relatively greater contribution of the Communication impairment (Comm) and Repetitive Behaviors and Stereotyped Patterns (Reps) domains of the ADI-R.

The present study intends to expand upon the aforementioned investigation [Kau et al., 2003] by further analyzing the behavioral and autistic features of the FraX + Aut phenotype. Our first aim was to determine whether the SBP of FraX + Aut is exhibited as a continuum within the various levels of the spectrum of autistic behavior in boys with FraX or only present in the most severely affected subjects. This is important in light of evidence that the idiopathic autistic phenomenon presents as a range of behaviors of different severity [Fattal-Valevski et al., 1999; Lord et al., 2001; Spiker et al., 2002]. For diagnostic, management, and pathogenetic purposes, we also attempted to identify specific behaviors, as characterized by instruments such as ADI-R, which distinguish boys with FraX + Aut and differentiate them from those with idiopathic Aut. Finally, this study examined factors that predispose or contribute to the diagnosis of autism spectrum disorder (ASD) in boys with FraX. These included autistic-like behaviors present throughout the FraX cohort, which make FraX subjects susceptible to ASD diagnosis, and specific deficits in skills that best predict a diagnosis of ASD in FraX. The latter focused specifically on the role of language delay, a distinctive feature of the FraX behavioral phenotype [Pulsifer, 1996], in ADI-R scores and diagnoses by analyzing a statistical model that incorporated measures of communication and socialization skills. Subsequently, the following questions formed the basis of our investigation:

1. Does the social behavioral profile shown in our FraX + Aut cohort exist as a continuum between non-autistic FraX subjects, FraX + PDD (Pervasive Developmental Disorder), and FraX + Aut?
2. What are the autistic behaviors, as measured by the ADI-R, which contribute the most to ADI-R scores and ASD diagnosis in FraX? Could any of these behaviors be considered susceptibility factors (to ASD) within FraX?
3. Are ADI-R scores and diagnosis of ASD or Aut in our FraX cohort primarily a result of a reduction in communication skills, or are they better explained by a deficit in socialization skills?

MATERIALS AND METHODS

Subjects

The present study includes boys with FraX syndrome both with (FraX + ASD) and without ASD (FraX + none). A total of 56 boys with FraX (mean age 57.1 ± 13.9 months, mean IQ 55.2 ± 16.5) were recruited as part of a study of cognitive skills and social behavior of young males with FraX at the Kennedy Krieger Institute (Baltimore, MD). All participants were screened for FraX by standard Southern blotting techniques [Rousseau et al., 1991], in conjunction with clinical examination; 14 (25%) of the subjects exhibited typical or size mosaicism for the *FMR1* mutation (combination of full mutation and premutation) and 1 was a mosaic for methylation (mixture of completely and partially methylated full mutation alleles) [Maddalena et al., 2001]. Two of the typical mosaics were in the FraX + Aut cohort. Twenty-four of the subjects (~43%) met the criteria for ASD, as determined by the ADI-R and the DSM-IV. The ASD category encompasses the diagnoses of Aut (25%) and PDD (~18%). The ethnic composition of the sample was predominantly white (~95%), with approximately 3% Hispanic and 2% Black. The maternal education of the subjects varied with approximately 53% with post-high school degrees and 26% with graduate degrees. The majority of the families were middle class and ~35% of low socioeconomic level. A substantial proportion of the mothers of the FraX subjects had premutation; however, mean parental (primarily maternal) IQ scores were within the normal range (105.77 ± 15.05). A summary of the characteristics of the subjects in this study is shown in Table I. This study was approved by the Johns Hopkins Medical Institutions' Institutional Review Board and written informed consent was obtained from all parents or legal guardians of the subjects, after the procedures were fully explained.

Instrumentation

Cognitive evaluation. The Stanford Binet-IV (SB-IV) [Thorndike et al., 1986] or the Bayley Scales of Infant Development II (BSID-II)—Mental Scales [Bayley, 1993] was administered to assess the cognitive abilities of the subjects. The SB-IV was used for all subjects who were able to establish a true basal. The remaining 23 subjects (~41%) were evaluated by the BSID-II, in order to obtain the Mental Developmental Index (MDI). If the child's chronological age was

TABLE I. Characteristics of Participants

Diagnosis	Subjects (n)	Age (months) mean (SD)	IQ[a] mean (SD)
Fragile X	56	57.1 (13.9)	55.2 (16.5)
With autism	14	59.2 (17.0)	43.1 (14.1)
With PDD	10	54.0 (13.3)	49.6 (17.3)
Without ASD	32	57.1 (12.9)	62.3 (13.7)

ASD, autism spectrum disorder.
[a]IQ equivalent by BSIQ-II or FSIQ by SB-IV.

greater than 42 months, a BSID-II-estimated IQ was calculated by dividing the mental age-equivalent by the chronological age and multiplying the ratio by 100. These scores, including the test composite score from the SB-IV, the MDI, and the BSID-II-estimated IQ, all of which represent levels of overall cognitive abilities, were labeled as "IQ" for data analysis purposes.

Autistic behavior. Autism related features were assessed by the ADI-R [Lord et al., 1994], a highly standardized semi-structured interview conducted with the child's caregiver in order to obtain detailed descriptions of behavioral symptoms associated with criteria required for DSM-IV diagnoses of PDD and Aut. The ADI-R relies on descriptions of behaviors that demonstrate developmental deviance rather than developmental delay. The ADI-R provides a total score as well as separate scores in three distinct areas related to the diagnosis of Aut. These domains represent Reciprocal Social Interaction (Recs), Communication impairment (Comm), and Repetitive Behaviors and Stereotyped Patterns (Reps). The Recs threshold score (that score and any number higher) for the diagnosis of Aut is 11; the Comm threshold is a score of 9 for "verbal" subjects or 8 for "non-verbal" subjects; the Reps threshold is 3. Higher scores indicate greater impairment. A diagnosis of Aut is given, according to the ADI-R, if the participant meets the cut-off criteria for each of the three areas and the developmental deviance occurred before 3 years of age. A diagnosis of PDD is given if the participant meets the cut-off criteria for Recs (i.e., core domain for ASD) and one of the other two domains, according to DSM-IV and ADI-R standards. In order to fully appreciate the range of abnormal behaviors, scores of 3 (indicating highest level of abnormal behavior) on individual ADI-R items were not converted to a score of 2, as ADI-R scoring protocol suggests. This approach, however, did not affect ADI-R diagnosis for any of the subjects, yet provided the widest spectrum of scores for analytical purposes. The ADI-R was administered to the participant's caregiver by two trained interviewers, who were blind to group membership. Reliability of total scores across items for the three domains was established between the psychologist trainer and the two interviewers. Reliability intraclass correlation coefficients ranged between 0.88 and 0.94.

Problem/aberrant behavior assessment. Problem/aberrant behaviors were assessed using the CBCL and the Aberrant Behavior Checklist-Community (ABC-C) [Aman and Singh, 1986]. The CBCL is a widely used parent report instrument for assessing behavioral and emotional problems in children. Either the 2–3 years version [Achenbach, 1992] or the 4–18 years version [Achenbach, 1991] was used. There are six subscales in the 2–3 years version and eight subscales in the 4–18 years version. These subscales are primarily grouped in Externalizing or Internalizing domains. In the 2–3 years version, the Internalizing domain includes Withdrawn and Anxious/Depressed behaviors while the Externalizing domain is composed of Aggressive and Destructive behaviors. Separate Sleep and Somatic Problems subscales are also part of the 2–3 years CBCL. In the 4–18 years version, the Internalizing domain includes Withdrawn, Anxious/Depressed, and Somatic complaints, whereas the Externalizing domain contains Aggressive and Delinquent behavior subscales. Social, Thought, and Attention problems are included as separate subscales. *T* scores are generated for each individual syndrome subscale and for both the Internalizing and Externalizing behavior domain composites. A total *T* score, combining all subscales is also calculated. *T* scores for the individual syndrome subscales between 66 and 70 are considered to be in the borderline clinical range, while *T* scores above 70 are considered clinically significant. For the Internalizing and Externalizing domains and the Total Composite, *T* scores between 60 and 63 are considered to be in the borderline clinical range, while *T* scores above 63 are in the clinically significant range. This study focused primarily on CBCL measures related to the so-termed Social Behavior Profile of FraX + Aut, a series of parameters of adaptive and problem/aberrant behavior linked to autistic features in FraX [Kau et al., 2003]. The feasibility of applying CBCL to FraX populations was recently demonstrated by Hatton et al. [2002] and Kau et al. [2003].

The ABC-C is also a parent report measure, which assesses the prevalence of inappropriate and maladaptive behaviors in individuals between 3 and 18 years with developmental disabilities. It is composed of five subscales, Irritability, Lethargy/Social Withdrawal, Stereotypic Behaviors, Hyperactivity, and Inappropriate Speech. Raw scores for each subscale were used for analyses.

Adaptive behavior evaluation. Adaptive behavior was assessed using the VABSs, Survey Form, Interview Edition [Sparrow et al., 1984]. The VABS, which is administered by a semi-structured interview with the parent, provides a general assessment of developmentally adaptive behavior in a variety of areas, and is appropriate for infancy through 18-year-old age groups. The VABS provides an Adaptive Behavior Composite score and four individual domain scores. The domains include Communication skills, Daily Living skills, Socialization skills, and Motor skills. The age equivalents of all five scores were used in the data analysis.

Language skills evaluation. Language skills were assessed using the Preschool Language Scale-3 (PLS-3) [Zimmerman et al., 1992]. The PLS-3 is a standardized measure used to evaluate the semantics and language structure of children functioning at a birth to 6-year-old age level. Like many children with FraX, a majority of our sample demonstrated limited verbalization. Recognizing that language is a major area of impairment in these children, we chose to use a measure composed of subscales measuring both Auditory Comprehension (PLScomp) and Expressive Language (PLSexp), standardized for very young children. Many of the receptive tasks on the PLS-3 require limited verbalization and allowed us to examine the child's ability to comprehend language. In addition, many of the expressive tasks also require limited verbalization as the test is designed primarily for pre-verbal children.

Study Design and Data Analysis

Based on the characteristic demonstration of autistic features as a continuum, we divided the FraX subjects according to their diagnoses on DSM-IV/ADI-R into either three groups: FraX + Aut, FraX + PDD, and FraX + none, or two groups: FraX + ASD and FraX + none. The rationale for an ASD versus none comparison includes the conceptualization of PDD as a milder form of Aut, particularly when defined as a condition with core impairment in social interaction (DSM-IV/ADI-R criteria), and the need of a larger statistical sample with a wider range of impairments for our regression analyses. Variability in ADI-R scores within the ASD group will determine whether the subject is diagnosed as having PDD or Aut.

Several statistical approaches were used to analyze the data. Differences between the FraX + Aut, FraX + PDD, and FraX + none cohorts on ADI-R scores, and on other behavioral measures of relevance to SBP, were analyzed by non-parametric tests because of the relatively small sample size and lack of significant age differences requiring an ANCOVA-like approach. Characterization of autistic behaviors, as measured by the ADI-R was performed by non-parametric and regression analyses. *P* value adjustments for multiple comparisons was conducted by the Bonferroni Multiple Comparison Procedure. Operationally, susceptibility ADI-R items were defined as those that were similar in FraX + Aut, FraX + PDD, and

FraX + none subgroups (i.e., not different by Kruskal–Wallis) and of clinical significance (score >1.0), indicating autistic characteristics in FraX not necessarily related to the presence of clinically diagnosed ASD. We chose this cutoff because a score of 1.0 on each individual question of any section of the ADI-R, when added together, will exceed the ADI-R cutoff for the diagnosis of Aut [Lord et al., 1994]. Differential or ASD "specific" ADI-R items were identified by a series of hierarchical regression analyses of the ADI-R subdomains effects on their respective domains and, in turn, of the individual items within those subdomains to see which factors best predicted ADI-R total scores (stepwise linear or continuous regression) and ASD/Aut diagnoses (stepwise logistic regression). Since each ADI-R subdomain is not composed of the same number of items, scores were adjusted accordingly. Informative or "specific" subdomains/items were introduced into "integrated" regression models, which accounted for multiple comparisons and determined their "final" relative contribution to the variance in ADI-R total scores and predictive value of ASD/Aut diagnoses. This integrated approach is in line with previous evaluations of the predictive value of ADI-R for diagnosing Aut [Lord et al., 1997]. Some items on the ADI-R, specifically in Recs-2, require an age of 4 or higher for assessment, which excluded 13 subjects from the analyses on this section; therefore, the resulting findings should be considered with caution.

In order to ascertain whether a deficit or delay in communication skills or a primary impairment in socialization skills was a major determinant of autistic behaviors in FraX, we conducted a series of regression analyses examining the predictive value of two verbal parameters [language comprehension (PLScomp), language expression (PLSexp)], a mixed verbal/non-verbal measure [adaptive communication (VABScom)], and a socialization skills parameter [adaptive socialization (VABSsoc)] upon ADI-R total scores and ASD/Aut diagnoses. To further determine the specificity of the regression models, IQ was introduced as a co-variate or a forced variable. Moreover, we conducted additional regression analyses substituting VABSsoc by its residual, after regressing PLScomp, PLSexp, and VABScom on VABSsoc. This stringent approach maximized the possibility of revealing abnormal social behaviors that are less influenced by cognitive impairment, and prevented confounding effects secondary to the high correlation between IQ and VABS scores and between VABSsoc and communication-related variables [Schatz and Hamdan-Allen, 1995; Freeman et al., 1999; Glasser et al., 2003]. All the primary analyses presented in the second and third section of Results were performed with IQ as co-variate. The fourth section of Results addresses the effects of IQ upon the statistical models. For all the regression analyses; F values $\geq$ 3.96–4.00 corresponding to P values $\leq$ 0.05 were considered significant.

RESULTS

ASD as a Continuum in the Fragile X Cohort

We previously characterized distinctive and specific features of social behavior impairment in boys with FraX (termed social behavior profile, SBP), by comparing subjects with FraX + Aut with a group of FraX boys with the DSM-IV/ADI-R diagnosis of PDD or neither Aut nor PDD (FraX + none). The SBP was characterized using data from several measures of problem/aberrant behavior and adaptive behavior, such as the ABC-C, CBCL, and VABS, and the profile suggested primary but not exclusive impairments in social interaction [Kau et al., 2003]. These findings raised the question that, if in FraX the diagnosis of Aut is associated with a core deficit in socialization (as opposed to a primary communication impairment) as defined by DSM-IV and ADI-R, does the SBP exist as a continuum between FraX subjects without ASD diagnosis and those with FraX + PDD and FraX + Aut? This spectrum of autism-related behaviors, with less severe features in subjects with PDD when compared with Aut, has already been demonstrated in idiopathic Aut and constitutes the basis for ASD diagnosis by ADI-R [Fattal-Valevski et al., 1999; Lord et al., 2001; Spiker et al., 2002]. As a first step, we compared the ADI-R profiles of our three FraX subgroups, FraX + Aut, FraX + PDD, and FraX + none. As exhibited in Table II, in concordance with the diagnostic algorithm of ADI-R (see Materials and Methods), in Recs FraX + PDD was found to be significantly different from FraX + none but not from FraX + Aut. In spite of the fact that the definition of PDD requires meeting cut-off criteria for Comm or Reps, the FraX + PDD cohort, and FraX + none did not differ significantly in either domain. While the Recs scores of FraX + PDD were slightly below those of FraX + Aut, the FraX + PDD group had lower mean scores than the FraX + Aut cohort on Comm (significantly) and Reps (trend level).

In terms of the SBP, we confirmed our initial assumption that the FraX + PDD group had an intermediate level of impairment in social interaction. As depicted in Table III, FraX + PDD subjects had scores representing lower deficit than FraX + Aut and higher impairment than FraX + none in all the SBP variables that are linked to social behavior (i.e., CBCL's Withdrawal, ABC-C's Lethargy/Social Withdrawal, VABS' Socialization, and VABS' Daily Living). Similarly, for another measure of significance of autistic behavior, ABC-C's Stereotypic Behaviors, FraX + PDD subjects had intermediate scores. In contrast, mean scores for problem and adaptive behavior measures of less direct relevance to ASD (i.e., CBCL's Attention, ABC-C's irritability, VABS' Communication) were either higher than FraX + Aut or lower than FraX + none. Significant differences in scores were only found in primary measures associated with socialization (ABC-C's Lethargy/Social Withdrawal and VABS' Socialization), as indicated in Table III.

Contribution of Specific Behaviors to ADI-R Scores and Diagnoses

To further characterize the phenomenon of ASD in FraX, analyses were performed to determine which ADI-R items, if any, represent susceptibility factors for FraX subjects, making them more likely to receive high ADI-R scores regardless of whether the subject is diagnosed with PDD or Aut. Two items in Recs, and one in Comm, were both relatively similar (i.e., not significantly different) in the FraX + Aut, FraX + PDD, and

TABLE II. ASD Continuum in Fragile X

ADI-R's domain	FraX + Aut mean (SD)	FraX + PDD mean (SD)	FraX + none mean (SD)
Reciprocal social interaction	17.5 (3.8)	15.2 (3.3)[b]	7.6 (3.8)[b]
Communication	12.3 (2.7)[a]	7.5 (3.2)[a]	7.5 (3.8)
Repetitive and stereotyped behaviors	5.2 (1.3)	3.7 (1.7)	2.8 (1.8)

[a]Significant difference Mann–Whitney between FraX + Aut and FraX + PDD.
[b]Significant difference Mann–Whitney between FraX + PDD and FraX + none.

TABLE III. Social Behavior Profile Continuum in Fragile X*

Variable	FraX + Aut mean (SD)	N	FraX + PDD mean (SD)	N	FraX + none mean (SD)	N
Age	59.2 (17.0)	14	54.0 (13.3)	10	57.1 (13.0)	32
IQ	43.1 (14.1)	14	49.6 (17.3)	10	62.2 (13.7)	32
CBCL attention	73.3 (5.7)	9	62.8 (4.1)	5	66.4 (7.5)	24
CBCL internalizing	58.1 (6.8)	13	52.5 (11.0)	9	51.0 (7.9)	30
CBCL withdrawal	63.4 (6.4)	13	59.0 (7.8)	9	54.7 (6.8)	31
ABC lethargy/SW	10.6 (6.6)[a]	13	4.6 (1.9)[a]	7	3.1 (3.8)	30
ABC stereotypic	6.8 (4.6)	13	4.4 (2.4)	7	3.2 (3.3)	30
ABC irritability	10.5 (7.7)	13	10.7 (6.5)	7	9.1 (7.5)	30
VABScomposite	23.8 (12.1)	14	26.9 (8.7)[b]	10	37.3 (11.2)[b]	32
VABSsoc	20.4 (12.0)	14	27.4 (9.7)[b]	10	39.2 (13.0)[b]	32
VABScom	23.9 (16.7)	14	22.7 (10.3)	10	34.6 (13.1)	32
VABSmot	26.9 (8.7)	12	27.2 (6.1)	9	36.3 (8.8)	29
VABSdaily	24.7 (9.8)	14	28.8 (11.6)	10	39.3 (12.5)	32

*Scores listed include T-scores for CBCL, raw scores for ABC-C, and age equivalent levels for VABS.
[a]Significant difference, Mann–Whitney between FraX + PDD and FraX + Aut.
[b]Significant difference, Mann–Whitney between FraX + PDD and FraX + none.

FraX + none subgroups, and had mean scores above the clinically significant cut-off score of 1.0. The Recs items were 64 and 68/69, representing deficits in Imaginative Play with Peers and Group Play with Peers or Friends, respectively. The item in Comm was 63, which evaluates impairment in Imaginative Play.

Complementing the above-mentioned analyses, by regression models we determined behaviors, as measured by the ADI-R, which may be differential or specific to ASD diagnosis in FraX. Since Recs scores were the main distinction between FraX + PDD or FraX + Aut and FraX + none (see Table II), we considered items on Recs as the primary determinants of ASD diagnosis. On the other hand, Comm and Reps scores would mainly contribute to variability and, therefore, diagnosis of Aut within the FraX + ASD cohort. We found that, although all four Recs subdomains significantly influenced this domain's composite scores, individual subdomains Recs-2 and Recs-4, representing Failure to Develop Peer Relationships and Lack of Socioemotional Reciprocity, respectively, were the most significant predictors. Recs-2 and Recs-4 accounted for 85.5% of the variance in Recs and 74.6% in total ADI-R scores. Within these two subdomains, item 68/69, representing Group Play with Peers or Friends, and item 11, representing Use of Other's Body, were found to be the greatest predictor of Recs-2 and Recs-4, respectively. Moreover, both subdomains and the two items were predictive of ASD diagnosis, but not of Aut within the FraX + ASD group. The F values of the subdomain stepwise regression analyses are shown in Table IV.

In Comm, a similar approach was used for subdomains Comm-1, representing Lack of, or Delay in, Spoken Language and Failure to Compensate Through Gesture, and Comm-4, representing Lack of Varied Spontaneous Make-Believe or Social Imitative Play. Since Comm-2 and Comm-3 are only applicable to a subset of "verbal" FraX children, as determined by ADI-R criteria, these Comm subdomains were excluded from the analyses. Of the two subdomains analyzed, Comm-1 was found to be the most significant predictor of Comm composite (44.4% of variance) and total ADI-R (55.4%) scores. In turn, Comm-1 was most influenced by item 32, representing nodding, which, as Comm-1, significantly predicted ASD (entire FraX cohort) and Aut (FraX + ASD cohort) diagnoses. Of the four Reps subdomains, Reps-1, representing Encompassing Preoccupation or Circumscribed Pattern of Interest, and Reps-3, Stereotyped and Repetitive Motor Mannerisms, were the highest contributors to scores on this domain (69.2% variance of Reps and 20.3% of total ADI-R, and predictors of ASD diagnosis but not of Aut). Upon analyses of the items of these two sections, excluding one item which is only assessed in subjects older than 10 years of age, it was found that item 71, representing Unusual Preoccupations, was by far the greatest influence upon Reps and also significantly predicted the diagnosis of ASD, but not the diagnosis of Aut. The F values for Comm and Reps subdomain stepwise regression analyses are shown in Table IV.

Informative subdomains and items were then subjected to integrated regression models, in order to control for multiple individual comparisons. Moreover, these integrated models would be more relevant to a diagnostic type of setting. The first model, which incorporated five ADI-R subdomains (Recs-2, Recs-4, Comm-1, Reps-1, Reps-3), showed that in combination these groups of items accounted for 86.2% of the variance in ADI-R total scores in FraX (Table V). In terms of ASD diagnosis, Recs-2 and Recs-4 significantly predicted ASD diagnosis, while Reps-3 was predictive at a trend level. Although Comm-1 did not predict ASD diagnosis in the entire FraX cohort, this subdomain was the only one that significantly influenced the diagnosis of Aut within the FraX + ASD group. Similar evaluations of informative items, attempting to refine the described models, demonstrated that four items (68/69, 11, 32, 71) accounted for 48.7% of the variance in ADI-R total scores (Table VI). Items 11 and 71 were predictors of ASD diagnosis at a trend level and item 32 was, as Comm-1, the only significant predictor of Aut diagnosis but not of ASD (Table VI).

Contribution of Communication and Socialization Skills to ADI-R Scores and Diagnoses

We analyzed the impact of communication and socialization skills on ADI-R scores and ASD (entire FraX cohort) or Aut (FraX + ASD cohort) diagnoses, by a regression model that postulated that a reduction in communication [language comprehension (PLScomp), language expression (PLSexp), adaptive communication (VABScom)], and/or socialization abilities [adaptive socialization (VABSsoc)] would lead to higher Recs and ADI-R total scores and, consequently, to an increased likelihood of a diagnosis of ASD. Of the four independent variables in our model, VABSsoc was the single most important predictor of Recs and ADI-R total scores, accounting for 53.5% of the variance of Recs and 48.9% of total scores, as shown in Table VII. VABSsoc was also the strongest predictor of ASD diagnosis in the entire FraX cohort, and of Aut diagnosis within the FraX + ASD group. Surprisingly expressive language was also a significant contributor to ADI-R total scores

TABLE IV. Effects of ADI-R Subdomains on ADI-R Domains and Total ADI-R Scores

Variable/parameter	**Recs**		**Total**	
R squared	0.965		0.815	
Adj. R squared	0.963		0.800	
DF regression	4		4	
F regression	354.290		56.147	
P regression	<0.0001		<0.0001	
	Coefficient[a]	F	Coefficient[a]	F
Recs-1	0.797	53.133 (R)	0.866	4.618 (R)
Recs-2	1.188	125.105 (R)	2.365	36.479 (R)
Recs-3	0.860	80.751 (R)	1.103	9.744 (R)
Recs-4	1.799	190.932 (R)	2.482	26.742 (R)
Variable/parameter	**Comm**		**Total**	
R squared	0.552		0.650	
Adj. R squared	0.536		0.637	
DF regression	2		2	
F regression	32.714		49.181	
P regression	<0.0001		<0.0001	
	Coefficient[a]	F	Coefficient[a]	F
Comm-1	0.949	15.512 (R)	2.644	28.381 (R)
Comm-4	0.811	11.650 (R)	1.789	13.337 (R)
Variable/parameter	**Reps**		**Total**	
R squared	0.836		0.268	
Adj. R squared	0.824		0.255	
DF regression	4		1	
F regression	65.181		19.794	
P regression	<0.0001		<0.0001	
	Coefficient[a]	F	Coefficient[a]	F
Reps-1	0.542	32.358 (R)	0.184	1.849 (E)
Reps-2	0.411	30.646 (R)	0.073	0.283 (E)
Reps-3	0.484	37.447 (R)	0.213	2.507 (E)
Reps-4	0.437	15.241 (R)	2.482	19.794 (R)

R, regression coefficient; Adj., adjusted; DF, degrees of freedom; F, F value; *P*, *P* value; (E), F-to-enter (not significant in forward model); (R), F-to-remove (significant in forward model). F values $\geq$ 3.96–4.00 are the equivalent of $P \leq 0.05$.
[a]Standardized regression coefficients or partial correlations (for variables not in the model).

and ASD diagnosis, but in an inverse relationship to the anticipated one. The higher the scores on PLSexp, the higher the total ADI-R scores. We hypothesized that this might be caused by the ADI-R algorithm, which assigns additional sections in Comm and potentially higher scores for subjects determined to be "verbal" by this instrument's criteria. However, analyses showed this unusual relationship even for the "non-verbal" cohort. Due to the overwhelming influence of VABSsoc upon ADI-R scores, we removed this variable from the model in order to ascertain which communication variables had a greater effect on the measured autistic behaviors. Without VABSsoc, adaptive communication (VABScom) was the only significant contributor to both Recs and ADI-R total scores. However, even without VABSsoc, VABScom correlated only at a trend level with ASD diagnosis. Finally, considering the close relationship between communication and socialization, we conducted regression analyses with the residual scores of VABSsoc. These were obtained after removing (i.e., regressing) the influence of all three communication variables (PLScomp, PLSexp, VABScom) upon VABSsoc. When this

TABLE V. Effects of Integrated ADI-R Subdomains on Total ADI-R Scores

Variable/parameter	Total	
R squared	0.872	
Adj. R squared	0.862	
DF regression	4	
F regression	86.887	
P regression	<0.0001	
	Coefficient*	F
Recs-2	2.171	42.182 (R)
Recs-4	1.943	22.569 (R)
Comm-1	2.050	42.750 (R)
Reps-1	0.933	6.424 (R)
Reps-3	0.203	2.159 (E)

F values $\geq$ 3.96–4.00 are the equivalent of $P \leq 0.05$.
*Standardized regression coefficients or partial correlations (for variables not in the model).

TABLE VI. Effects of Integrated ADI-R Items on Total ADI-R Scores

Variable/parameter	Total	
R squared	0.523	
Adj. R squared	0.487	
DF regression	3	
F regression	14.607	
P regression	<0.0001	
	Coefficient[a]	F
Item 68/69 (group play with peers or friends)	0.182	1.331 (E)
Item 11 (use of other's body)	2.313	4.035 (R)
Item 32 (nodding)	4.783	14.591 (R)
Item 71 (unusual preoccupations)	3.850	7.847 (R)

F values $\geq$ 3.96–4.00 are the equivalent of $P \leq 0.05$.
[a]Standardized regression coefficients or partial correlations (for variables not in the model).

TABLE VII. Effects of Communication and Socialization on ADI-R Total Scores

Variable/parameter	Model 1		Model 2		Model 3	
R squared	0.555		0.417		0.427	
Adj. R squared	0.529		0.406		0.405	
DF regression	3		1		2	
F regression	21.216		37.919		19.362	
P regression	<0.0001		<0.0001		<0.0001	
	Coefficient[a]	F	Coefficient[a]	F	Coefficient[a]	F
PLScomp	−0.079	0.311 (E)	−0.093	0.451 (E)	−0.117	0.702 (E)
PLSexp	0.292	5.390 (R)	0.099	0.516 (E)	0.198	2.087 (E)
VABScom	0.003	0.001 (E)	0.154	1.267 (E)	−0.178	4.849 (R)
VABSsoc	−0.428	20.210 (R)	−0.415	37.919 (R)		
IQ (forced)	−0.270	15.175 (R)			−0.275	16.994 (R)

F values $\geq 3.96–4.00$ are the equivalent of $P \leq 0.05$.
[a]Standardized regression coefficients or partial correlations (for variables not in the model).

residual variable was introduced into the model, replacing the standard VABSsoc parameter, the "adjusted" socialization measure was still the greatest predictor of ADI-R total scores and diagnosis in the entire FraX cohort and in the FraX+ ASD group.

Effect of IQ on Autistic Behavior in Fragile X

Introduction of IQ as a co-variate or forced variable did not affect the analyses of ADI-R items found to be differential or specific to ASD diagnosis. In contrast, the regression model incorporating communication and socialization variables, in terms of their influence on ADI-R scores and diagnoses, yielded different outcomes depending on the presence or absence of IQ as a forced variable. Although adaptive socialization still accounted for a large proportion of variance in Recs (45.0%) and ADI-R total (40.6%), language comprehension (PLScomp) was the other significant variable related to ADI-R scores in FraX when IQ was not part of the model. With IQ as a co-variate, PLScomp had virtually no influence on ADI-R scores and PLSexp emerged as a significant factor.

DISCUSSION

The present study builds upon the findings of a previous investigation that characterized the co-morbidity of FraX and Aut. Our data demonstrate that, of the problem and adaptive behavioral abnormalities initially identified as linked to FraX + Aut, those representing deficits in social interaction are distributed as a continuum between FraX + Aut, FraX + PDD, and FraX + none. Items of ADI-R reflecting impairment in imaginative play and peer interaction appeared to be factors that increase the possibility (i.e., susceptibility) of ASD diagnosis in FraX. Autistic behaviors that differentiated FraX + ASD and FraX + none groups (i.e., specificity) included items dealing with peer relationships and socio-emotional reciprocity. Items under the Comm and Reps domains of ADI-R, in particular representing delayed verbal and non-verbal communication, also influenced ASD diagnosis and variability in scores within the FraX + ASD cohort. Finally, in a model that tested the relative contributions of communication and socialization deficits to ASD diagnosis, reduced adaptive socialization skills was the most important determinant of ADI-R scores and a strong predictor of ASD in FraX. Expressive language was the only communication variable that significantly influenced ADI-R scores; unexpectedly, better expressive language scores predicted more severe autistic behavior. The overall results appeared to be relatively specific, since they were not affected by IQ. Altogether, our findings demonstrate that the diagnosis of ASD in FraX reflects, to a large extent, impairment in social interaction that is expressed with variable severity in young males with FraX.

Previous studies have demonstrated that there is a distinctive sub-phenotype of FraX + Aut. Rogers et al. [2001] found that 33% of their FraX subjects met criteria for Aut according to ADI-R, ADOS-G (Autism Diagnostic Observation Schedule-Generic), and DSM-IV criteria. These authors, as well as Bailey et al. [1998], showed that boys with FraX + Aut display by standardized autism assessment tools (e.g., CARS, ADI-R) a similar profile to young males with idiopathic Aut. In a previous study [Kau et al., 2003], we corroborated these findings and demonstrated that the diagnosis of FraX + Aut was associated with a pattern of aberrant and adaptive behavioral abnormalities we termed social behavior profile (SBP). Although we provided evidence that Aut in FraX reflects impairment in social interaction, comparisons of the FraX + Aut cohort with language-delayed and non-selected groups of boys with idiopathic Aut suggested that communication impairment and stereotypic behavior might have a greater contribution to the diagnosis of Aut in FraX. The present study represents both an extension of this early characterization of autistic behavior in FraX and a direct evaluation of the relative contribution of deficits in communication and socialization to the diagnosis of ASD in FraX. In agreement with the concept that the autism phenomenon represents a range of behaviors, and perhaps also of other neurologic features [Lord et al., 2001], we found that most SBP parameters were distributed as a continuum from FraX + none to FraX + Aut. While all primary variables representing deficits in social interaction showed an Aut-PDD-none range of decreasing severity, other behavioral abnormalities of less direct relevance to ASD, such as attentional difficulties and irritability, did not. Furthermore, the most marked differences were between the FraX + none and FraX + PDD groups. These observations suggest that the association between FraX + ASD and SBP is even more specific to autistic behavior than initially concluded, on the basis of analyses of the most severely affected FraX + Aut cohort. Similar analyses of ADI-R items are not as informative since the diagnostic algorithm of ADI-R requires that, in order to meet diagnostic criteria, individuals with PDD should have high scores on Recs that approach those of subjects with Aut. Nonetheless, evaluations of ADI-R items, as indices of specific autistic behaviors, were quite revealing in terms of the components of the FraX's behavioral phenotype that lead to the diagnosis of FraX + ASD.

We found that two Recs and one Comm ADI-R items appear to confer susceptibility to the diagnosis of ASD in the FraX cohort. Interestingly, all three items represent impairments in "Play." While their contribution to the overall ADI-R score was limited, further evidence of their functional significance was

suggested by the fact that one of the items, 68/69 (Group Play with Peers or Friends), was also found to predict ASD diagnosis when contrasting FraX + ASD and FraX + none groups. Although in our hierarchical analyses, we identified individual items that predicted ASD diagnosis, in integrated models only the combination of items into their respective subdomains had a significant predictive value. Specifically, subdomains Recs-2 and Recs-4, dealing with complex social behaviors including relationships with peers, made a disproportional contribution to variance in ADI-R scores and to the diagnosis of FraX + ASD. In correspondence with the observations on mean ADI-R domain scores, Comm and Reps had only a minor influence on ASD diagnosis in FraX. However, as suggested by the significant difference between FraX + Aut and FraX + PDD on Comm domain scores, Comm-1 and its item 32 (nodding) in particular were the main discriminating factors for the diagnosis of Aut within the FraX + ASD cohort.

Since analyses of ADI-R items conferring susceptibility and specificity to the diagnosis of ASD have, to our knowledge, not been conducted in other conditions presenting with autistic behavior, it is difficult to fully appreciate the significance of these findings in FraX. Nonetheless, the presence of "susceptibility" items indicates that, as insinuated since the early descriptions of behavioral abnormalities [Hagerman et al., 1986; Baumgardner et al., 1995], certain elements of FraX's behavioral phenotype predispose to a diagnosis of ASD. Interestingly and contrary to these reports, we found these abnormalities involved complex communication and socialization and not fundamental social interaction (e.g., poor eye contact) or stereotypic behavior (e.g., hand flapping, perseverative speech) [Turk and Graham, 1997; Hagerman, 2002]. Similarly, items representing complex social interactions were the greatest contributors to ASD diagnosis in FraX (as opposed to a more balanced influence of Recs items demonstrated in the idiopathic ASD literature [Lord et al., 1997]). Although Recs' influence was anticipated, considering the diagnostic algorithm of ADI-R/DSM-IV, the only marginal effect of abnormal communication and stereotypic behavior upon ASD diagnosis further supported the notion that FraX + ASD represents a core deficit in socialization. Abnormalities in basic communication, as determined by ADI-R, emerged as the most important index of severity within the FraX + ASD cohort since Comm-1 was the only examined variable that (significantly) differentiated between the FraX + Aut and FraX + PDD subgroups.

The selective deficit in socialization in boys with FraX + ASD was underscored by the examination of the differential role of communication and socialization skills upon ADI-R scores and diagnoses. Our data demonstrate that deficits in adaptive socialization have a much greater role than any impairment or delay in communication in variability of ADI-R scores, not only in the entire FraX cohort but also in the group with autistic features (i.e., FraX + ASD). This feature was also relatively independent of cognitive level (i.e., IQ) and associated with an unexpected relationship between higher expressive language skills and ASD diagnosis. It is unclear whether scores on PLS may reflect rote expressive abilities more than conceptual expressive abilities; however, as seen in Table VII, adaptive socialization and expressive language appear to be related variables since exclusion of VABSsoc from the statistical model led to a substantial decrease in the predictive value of PLSexp. Supporting the unique relationship between socialization and ADI-R scores in FraX is the fact that receptive language had a role in the model only when IQ was not a co-variate. Even in the latter situation, and after removing the influence of all three communication variables upon adaptive socialization, VABSsoc was still the most influential variable. In a similar study of children with idiopathic Aut, Gillham et al. [2000] found that VABSsoc accounted for 48% of the variance in the diagnosis of Aut, almost the same proportion (48.9%) we found in our FraX cohort. Although not a major focus of this study, the finding that the main distinguishing factor between FraX + PDD and FraX + Aut was in ADI-R's Communication suggests that, FraX + ASD represents a relatively homogeneous deficit in social behavior in which severity is a function of the degree of disturbance in communication strategies and not in communication skills or "distracting" behaviors.

The presence and nature of ASD co-morbidity is one of the most controversial issues regarding the neurobehavioral phenotype of FraX [Baumgardner et al., 1995; Turk and Graham, 1997; Bailey et al., 1998; Rogers et al., 2001]. Through analyses of factors contributing to and differentiating ASD in FraX the present study concludes that FraX + ASD resembles idiopathic autistic behavior in its range of severity [Fattal-Valevski et al., 1999; Lord et al., 2001; Spiker et al., 2002] and on the influence of deficits in socialization [Gillham et al., 2000] and communication [Rutter and Schopler, 1987; Rapin and Allen, 1998]. This similarity to idiopathic ASD is in line with a recent quantitative magnetic resonance imaging study [Kaufmann et al., 2003a], in which boys with FraX showed a selective enlargement of the same cerebellar region (i.e., posterior-superior vermis) that is reduced, and in some occasions enlarged [Courchesne et al., 1994], in individuals with idiopathic Aut. These similarities, however, cannot exclude the possibility that the nature of autistic behavior in FraX might be different from that seen in idiopathic ASD. Shyness, social anxiety, and hyperarousal, recognized features of the FraX's behavioral phenotype [Hagerman, 2002], may influence social interactions [Cohen et al., 1989; Cohen, 1995; Hagerman, 1996; Mazzocco et al., 1997; Rogers et al., 2001] and/or affect ADI-R scoring. Future studies including observational instruments, such as the ADOS-G, or combining physiological measures of anxiety and arousal with behavioral analyses, will be needed in order to address this issue. We recognize that our study was also limited by sample size and composition (i.e., proportion of subjects with PDD and Aut), hindering the amount of variables that could be simultaneously evaluated and the interpretation of the statistical models. In addition to the difficulties in differentiating the examined communication and socialization skill variables, we also lacked a direct measure of non-verbal communication. The latter issue is especially important because deviance in non-verbal pragmatics is characteristic of children with ASD [Rapin and Allen, 1998; Joseph et al., 2002]. Data on other qualitative impairments of communication, such as tangential language [Sudhalter and Belser, 2001], would have also been of relevance to the analyses of communication and socialization. Furthermore, the use of two different tools for cognitive assessment, though inherent to the study of young individuals with cognitive impairment, is also a limiting factor in this investigation. Future investigations of autistic behavior in FraX should not only incorporate the above-mentioned elements but also extend to correlations with molecular, imaging, and other neurobiologic parameters. Our preliminary studies showing a relationship between increased histone acetylation and social withdrawal [Kaufmann et al., 2003b] in FraX demonstrate the feasibility of these approaches, and the potential value of the use of biological markers in ASD research.

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