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Autism Spectrum Disorder in Fragile X Syndrome: Differential Contribution of Adaptive Socialization and Social Withdrawal

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The present study extends our previous work on characterizing the profile of social behavior abnormalities in boys with Fragile X (FraX) and autism spectrum disorder (ASD) using clinically oriented behavioral rating scales and standardized instruments. The goal was to further distinguish behavioral parameters contributing to the diagnostic classification of FraX + ASD. The study design included two cohorts of boys with FraX (3–8 years), a larger *main* cohort for cross-sectional analyses (n = 56, 24 with ASD), and a *longitudinal* subset (n = 30, 11 with ASD) of the *main* cohort with up to 3 yearly observations. The focus was on the relative contribution of delayed adaptive socialization and social withdrawal, including item components of their corresponding rating instruments, to the diagnosis of ASD in boys with FraX. Using a combination of regression analyses, we demonstrated that: (1) as delayed socialization, social withdrawal is also a correlate of FraX + ASD; (2) items of social withdrawal scales representing avoidance were the main predictors of ASD status, particularly in older boys; (3) adaptive socialization skills reflecting rules of social behavior and recognition and labeling of emotions, linked to verbal reasoning abilities, were selectively associated with FraX + ASD; (4) adaptive socialization is the primary determinant over time of ASD status in boys with FraX; and (5) integrated adaptive socialization-social withdrawal models allow the identification of distinctive FraX + ASD subgroups. Altogether, our findings suggest that two distinct but interrelated social behavior abnormalities, one linked to impaired cognitive processes (delayed socialization) and the second one to disturbance in limbic circuits (avoidance), play a role in the development of ASD in boys with FraX.

Key words: fragile X syndrome; autism; autism spectrum disorder; adaptive socialization; social withdrawal; social avoidance; social cognition

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INTRODUCTION

Fragile X syndrome (FraX) is the most frequent form of inherited severe cognitive deficit [Kaufmann and Moser, 2000; Sherman, 2002]. In most cases, the mutation consists of an unstable expansion of a CGG trinucleotide repeat within the 5′ UTR region of *FMR1*, which when it reaches the 200–2,000 repeats range (vs. 5–40 normal), termed full mutation, leads to hypermethylation and silencing of the gene. In general terms, severity of FMRP deficit is correlated with severity of physical and neurobehavioral phenotype [Hagerman et al., 1994; Kaufmann and Reiss, 1999; Loesch et al., 2003]. Despite this, FMRP levels only account for a small proportion of the variance in different specific aspects of

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neurobehavioral development and have virtually no influence on the severity of autistic behavior in FraX [Bailey et al., 2001a,b; Kaufmann et al., 2003a].

Autism is a frequent and severe neurobehavioral phenotype in FraX [Hagerman, 2002]. Estimates of the prevalence of FraX + Autism range between 16 and 47%, a reflection of differences in study design and methodology for assessing autistic features [Hagerman et al., 1986; Baumgardner et al., 1995; Bailey et al., 1998; Feinstein and Reiss, 1998; Rogers et al., 2001; Demark et al., 2003; Kau et al., 2004; Kaufmann et al., 2004]. An important factor influencing studies of autism in FraX is the fact that up to 90% of males with FraX display a variety of behavioral abnormalities that can be interpreted as atypical social interaction or autism spectrum behaviors such as perseveration, hand flapping, self-injury, avoidant eye contact, and social anxiety [Merenstein et al., 1996; Bailey et al., 1998; Hatton et al., 1999; Hagerman, 2002; Kaufmann et al., 2004]. We have recently reported that, when applying instruments specifically designed for evaluating autistic behavior such as the Autism Diagnostic Interview—Revised (ADI-R), most boys with FraX show autistic features [Kaufmann et al., 2004]. Nonetheless, severity and profile of these autistic behaviors enable a differentiation between individuals with FraX who meet Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [APA, 1994] criteria for autism spectrum disorder (ASD) and those who do not [Kaufmann et al., 2004]. The importance of precisely delineating the profile of boys with FraX + Autism or FraX + ASD is underscored by the fact that these diagnoses are relatively stable over time [Sabaratnam et al., 2003] and that children with FraX + ASD display other severe cognitive and behavioral abnormalities [Kau et al., 2000; Bailey et al., 2000, 2001a; Hatton et al., 2002, 2003; Sabaratnam et al., 2003; Roberts et al., 2005a].

We have demonstrated that skills and problem behaviors representing social aspects are relatively more affected in boys with FraX + ASD [Kau et al., 2004; Kaufmann et al., 2004]. For instance, among communication and socialization skills, delay in adaptive socialization and not language/communication impairment is the primary correlate of ASD diagnosis in males with FraX [Kaufmann et al., 2004]. Furthermore, among problem behaviors, scores on social withdrawal and not on other aberrant behaviors (e.g., attentional difficulties) are distributed in a continuum along the spectrum of severity of autistic behavior [Kaufmann et al., 2004]. These findings are in line with the notion that in idiopathic ASD, there is a wide range of abnormalities in social behavior [Eigsti and Shapiro, 2003; Pelphrey et al., 2004]. The present study intended to expand our previous work, by focusing on the relationship between social withdrawal and ASD in FraX and on the identification of specific behaviors underlying delayed socialization and withdrawn behavior in boys with FraX. We attempted to answer the following questions:

1. Is social withdrawal, as delayed adaptive socialization, also correlated with ASD status in FraX?
2. Are there specific behaviors representing "true" social withdrawal, and not social indifference, correlated with ASD status in FraX?
3. What specific aspects of delay in adaptive socialization skills are correlated with ASD in FraX?
4. What are the relative influences of delayed adaptive socialization and social withdrawal on ASD status in FraX?

MATERIALS AND METHODS

Subjects

This study included exclusively boys with FraX diagnosis, both with (FraX + ASD) and without ASD (FraX-only). Two cohorts were evaluated: a larger cross-sectional *main* cohort of 56 subjects and a *longitudinal* subset of the *main* cohort that included 30 subjects who were annually assessed for a total of 3 years. Details about the characteristics of the participants, including the number of subjects, diagnostic profiles, and data completeness are presented in Table I. The subjects were recruited as part of a study of cognitive and social skills in young males with FraX at the Kennedy Krieger Institute (Baltimore, MD). All participants were screened for FraX by standard Southern blotting and PCR techniques [Rousseau et al., 1991], in conjunction with a clinical evaluation. In the *main* cohort, 14 (25%) of the subjects exhibited allele size mosaicism for the *FMR1* mutation (combination of full mutation and premutation alleles) and 1 was mosaic for methylation (mixture of completely and partially methylated full mutation alleles) [Maddalena et al., 2001]; the remaining 41 subjects had completely methylated *FMR1* full mutation. Two of the allele size mosaics were in the FraX + ASD group. The ethnic composition of both cohorts were predominantly white (~95%), with ~3% Hispanic and ~2% Black. Of the subjects' mothers with available data on *FMR1* mutation, approximately 92% had premutation and the remaining 8% full mutation. Despite this, the mean parental (primarily maternal) FSIQ scores were within the normal range (105.7 ± 15.0). The study was approved by the Johns Hopkins Medicine Institutional Review Boards. Written informed consent was obtained from the parents or legal guardians of all participating subjects, after the procedures were fully explained.

Instrumentation

Our previous work on ASD in FraX [Kau et al., 2004; Kaufmann et al., 2004] led to the research strategy

TABLE I. Characteristics of Participants*

	Subjects (n)		Age (months) mean (SD)		FSIQ[a] mean (SD)	
Cohorts	Adaptive behavior	Problem behavior	Adaptive behavior	Problem behavior	Adaptive behavior	Problem behavior
Main cohort	56	53	57.1 (13.9)	57.1 (13.8)	55.2 (16.5)	56.6 (16.8)
With ASD	24	22	57.0 (15.4)	57.7 (15.4)	45.8 (15.5)	46.9 (15.7)
Without ASD	32	31	57.1 (12.9)	56.7 (12.9)	63.4 (14.0)	63.6 (14.1)
Longitudinal cohort	30	29	58.4 (12.9)	59.2 (12.5)	57.8 (15.6)	58.9 (14.8)
With ASD	11	10	56.2 (16.3)	58.2 (15.6)	45.5 (15.5)	47.2 (15.1)
Without ASD	19	19	59.7 (10.9)	59.7 (10.9)	65.0 (10.5)	65.0 (10.5)

ASD, autism spectrum disorder; SD, standard deviation; FSIQ[a], full scale IQ, Stanford Binet Intelligence Scale-IV; Bayley Scales of Infant Development estimated IQ.
*Individuals with complete data.

illustrated in Figure 1. Social behavior and related parameters were analyzed along two axes, skills or abilities (e.g., adaptive socialization skills) and problem/aberrant behavior (e.g., social withdrawal), which were related to central "gold standard" measures of autistic behavior (e.g., ADI-R) and to DSM-IV diagnostic criteria. Supporting this scheme are our findings of parallel continua of severity of the aforementioned social behavior parameters and autistic behavior impairment by DSM-IV/ADI-R [Kaufmann et al., 2004], and current views on evidence-based research on idiopathic ASD [Ozonoff et al., 2005].

Autistic behavior. The diagnosis of ASD was established by DSM-IV criteria [APA, 1994], which were complemented by the ADI-R [Lord et al., 1994]. As in our previous studies [Kau et al., 2004; Kaufmann et al., 2004], we used the term ASD to refer to boys with FraX and either the more severe autism (Kanner type) phenotype or the less severe Pervasive Developmental Disorder (PDD-NOS) diagnosis. The ASD category did not include diagnoses such as Asperger syndrome or Childhood Disintegrative Disorder, which are included in DSM-IV under PDD-NOS. Autistic features and ASD diagnosis were also assessed by the ADI-R [Lord et al., 1994], a standardized semi-structured interview conducted with the child's caregiver to obtain detailed descriptions of behavioral symptoms associated with DSM-IV criteria for PDD-NOS or autism. The ADI-R provides a total score as well as separate scores in three distinct domains: Reciprocal Social Interaction (core domain), Communication Impairment, and Repetitive Behaviors and Stereotyped Patterns. Each domain is divided into subdomains, which include a variable number of items. ADI-R's diagnosis of autism requires cut-off criteria for each of the three abovementioned domains and developmental deviance before age 3, while diagnosis of PDD-NOS is given if cut-off criteria for the core Social Interaction domain and one of the other two domains are met. Additional information on ADI-R's structure and administration can be found in our earlier publications [Kau et al., 2004; Kaufmann et al.,

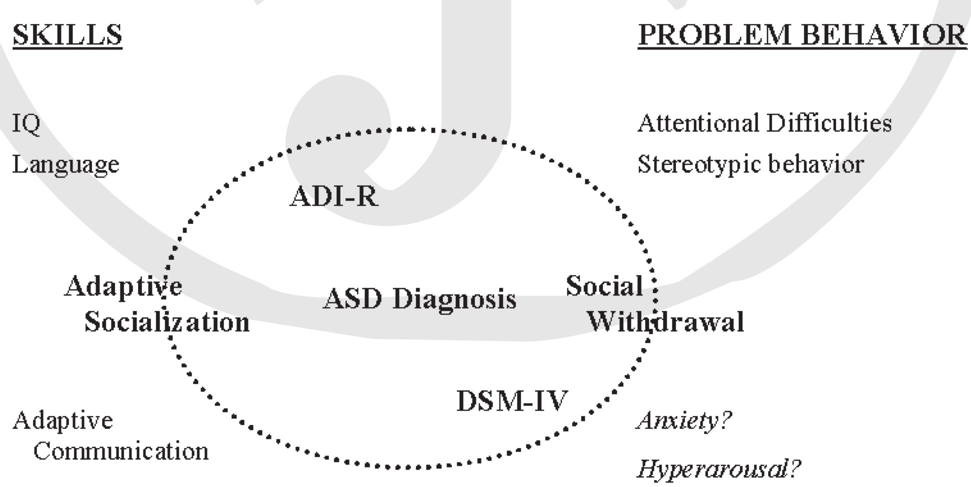

Fig. 1. Model of the research strategy applied in this study. ASD in FraX is defined on the basis of the DSM-IV and "gold standard" measures (i.e., ADI-R). Two axes of behavioral abnormalities, delay/impairment in skills, particularly those dealing with social interaction, and increase in severity of problem/aberrant social behaviors, are closely related to ASD diagnosis. Other factors are less influential or not yet defined in terms of their relationship to ASD.

2004]. Although this study did not distinguish between ASD subgroups, of the 24 (~43%) subjects with FraX + ASD in the *main* cohort, 14 (25%) fulfilled criteria for autism, and 10 (~18%) for PDD-NOS. Similar proportions of ASD subjects were present in the *longitudinal* cohort.

Cognitive evaluation. Depending on the level of functioning, cognitive abilities were evaluated using either the Stanford Binet Intelligence Scale-IV (SB-IV) [Thorndike et al., 1986] or the Bayley Scales of Infant Development II-(BSID-II)-Mental Scales [Bayley, 1993]. The SB-IV was used for all subjects who were able to establish a true basal (n = 34); the remaining 22 subjects (~39%) were evaluated with the BSID-II, in order to obtain the Mental Developmental Index. Both SB-IV-calculated and BSID-II-estimated IQ were labeled as "IQ" for analytical purposes. Full scale (FSIQ), verbal (VIQ), and nonverbal (NVIQ) IQ scores or their equivalents were calculated for both SB-IV and BSID-II according to Sattler [1990] and Mayes and Calhoun [2003].

Language skills. They were assessed, as reference parameters, 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, which provides scores on Auditory Comprehension (i.e., Receptive Language) and Expressive Language.

Adaptive behavior. It was assessed by the Vineland Adaptive Behavior Scales, Survey Form, Interview Edition (VABS) [Sparrow et al., 1984]. The VABS is a semi-structured interview with the parent, which provides a general assessment of developmentally adaptive behavior in a variety of areas from infancy to 18 years. The VABS yields standard and age-equivalents scores on global function (i.e., Adaptive Behavior Composite) and on four individual domains: Adaptive Communication; Adaptive Motor Skills; Daily Living Skills; and the main focus of our analyses, the 50-item Adaptive Socialization domain (VABSsoc). Our analyses, which used exclusively VABS' standard scores, focused on six VABSsoc "informative" items that were identified by hierarchical regression analyses of the FraX cohort as described in the Results section: VABSsoc24 (*Label happiness, sadness, fear, and anger in oneself*), VABSsoc30 (*Follow school or facility rules*), VABSsoc31 (*Respond verbally and positively to good fortune of others*), VABSsoc32 (*Apologize for unintentional mistakes*), VABSsoc36 (*Do not talk with food in mouth*), and VABSsoc38 (*Respond appropriately when introduced to others*).

Problem/aberrant behaviors. These behaviors were assessed using both qualified Child Behavior Checklist (CBCL) and Aberrant Behavior Checklist-Community (ABC-C) subscales. The CBCL is a widely used parent/teacher report for assessing behavioral problems between 2 and 18 years [Achenbach, 1991, 1992]. Depending on the child's age, several subscales are grouped under Internalizing [e.g., Withdrawn (CBCLw)] and Externalizing [e.g., Attention problem] domains. *T*-scores are generated for each individual subscale, as well as for the domains and a total Composite. Subscales *T*-scores between 66 and 70 and >70 are considered in the borderline clinical and clinically significant ranges, respectively. This study focused primarily on the CBCLw subscale, which we [Kau et al., 2004] and others [Hatton et al., 2002] have successfully applied to FraX populations. The ABC-C [Aman and Singh, 1986] is also a widely used parental report that we have applied to the study of ASD in FraX [Kau et al., 2004; Kaufmann et al., 2004] and Down syndrome [Capone et al., 2005]. The ABC-C evaluates inappropriate and maladaptive behaviors in 3 to 18-year-old individuals with developmental disabilities through five subscales: Irritability, Lethargy/Social withdrawal (ABCsw), Stereotypic behavior, Hyperactivity, and Inappropriate speech. The ABC-C yields only raw scores for each subscale.

Considering that the CBCLw and ABCsw cover a relatively wide spectrum of social behaviors and that, to our knowledge, there is no available standardized instrument for measuring selective abnormalities in this area, we performed an informal analysis of CBCLw and ABCsw items in order to determine the nature of social withdrawal in FraX. We used our clinical experience (neuropsychologists: C.C., R.M.G.; child psychiatrist: D.B.B) to independently label the CBCLw and ABCsw items as whether they represented social avoidance (SA)- and/or social indifference (SI)-like behaviors. The appropriateness of this item classification was, to some extent, confirmed by examining the factor scale of the CBCLw and ABCsw, as reported in their respective manuals. Although this informal analysis inevitably required forcing some items into the best possible category, which needs to be validated against a reference measure, the high level of inter-rater agreement indicated that the strategy could be generalized. For the CBCLw, which includes nine items, CBCLw75 (*Shy or timid*), CBCLw88 (*Sulks a lot*), and CBCLw-111 (*Withdrawn, does not get involved with others*) were labeled as SA items; CBCLw42 (*Would rather be alone than with others*) and CBCLw65 (*Refuses to talk*) as both SA and SI (SA + SI) items; and CBCLw80 (*Stares blankly*) and CBCLw102 (*Underactive, slow moving, or lacks energy*) as SI items. The other two CBCLw items (CBCLw69, CBCLw103) were considered to represent neither SA nor SI. Using the same principle, out of 16 ABCsw items, 12 were deemed to represent SI: ABCsw3, ABCsw12, ABCsw20, ABCsw23, ABCsw32, ABCsw37, ABCsw40, ABCsw42, ABCsw43, ABCsw53, ABCsw55, and ABCsw58. In our analyses, only ABCsw40 (*Is difficult to reach or contact; has autistic tendencies; unresponsive to social* *interactions*) was informative in terms of ASD analyses. The remaining four ABCsw items reflected either SA [ABCsw5 (*Seek isolation from others, do not engage in interaction with others, uncommunicative*), ABCsw30 (*Isolate from others, have difficulty in socializing; may interact with others only when encouraged*)], or SA + SI [ABCsw16 (*Withdrawn, prefer solitary activity, socially isolative*), ABCsw42 (*Prefer to be alone; unsociable, shy, withdrawn; actively isolates self from others*)].

Study Design and Data Analysis

A larger cross-sectional, termed *main*, cohort was the basis for most analyses. Selected and confirmatory analyses were performed on a smaller *longitudinal* (3 yearly evaluations) subset of the *main* cohort. In order to optimize the analysis of age effect, for the *longitudinal* cohort, only data at baseline and at the third evaluation were considered in this study. Several statistical approaches were performed in this study. In addition to characterizing the basic features of the FraX cohorts/subgroups by descriptive statistics, we employed hierarchical multivariate linear models to determine the relative contribution of items to total scores of CBCLw, ABCsw, and VABSsoc, and for evaluating the relationship between VABSsoc and IQ measures. Since the main questions in this study related to the "predictive value" of social withdrawal and adaptive socialization parameters for ASD diagnosis, the primary analyses consisted of logistic regression models with CBCLw, ABCsw, VABSsoc, and their corresponding items, as independent variables and ASD diagnosis (Yes vs. No) as outcome. Age and IQ, either as VIQ or NVIQ, were separate or combined co-variates. To provide a more clear narrative, the Results section emphasizes basic logistic models without co-factors and mainly reports on Chi square and *P*-values of logistic likelihood ratio tests. The latter determined the relative contribution of each independent continuous variable to a Yes ASD vs. No ASD classification model (i.e., fitted model including each variable vs. a model not including each variable), and were in general agreement with Chi square and *P*-values obtained from the Wald test. When appropriate, corrections for multiple comparisons were performed. For additional information about analytical strategies, see our previous study [Kaufmann et al., 2004].

RESULTS

Social Withdrawal Is Also Correlated With ASD Diagnosis in Boys With FraX

As reported by Kau et al. [2004] and Kaufmann et al. [2004], among problem behavior subscales, boys with FraX + ASD show selectively higher scores on social withdrawal subscales. They also display relatively greater delay in socialization among adaptive behavior skills; moreover, when compared with language and adaptive communication skills, only delayed adaptive socialization is a predictor of ASD diagnosis [Kaufmann et al., 2004]. Using the same analytical approach, we examined whether there was a selective association between social withdrawal and ASD diagnosis in boys with FraX for the *main* (cross-sectional) FraX cohort (see Materials and Methods). Logistic regression analyses demonstrated that, among subscales of CBCL and ABC-C, there was a selective correlation between CBCLw and ABCsw scores and ASD diagnosis. Specifically, CBCLw and ABCsw were the only significant correlates of the No ASD status when compared with other CBCL subscales with high *T*-scores (>55; Fig. 2a) or with the other four ABC-C subscales (Fig. 2b), respectively. The "predictive" value of social withdrawal scales was independent of age and cognitive function, since models including age and/or VIQ or NVIQ as co-variates did not differ in their outcome. An overall comparison of CBCLw and ABCsw revealed that ABCsw was a better predictor of ASD status, as reflected by the greater score difference between FraX + ASD and FraX-only groups (Table II) and by the stronger correlations between ABCsw items and ASD diagnosis (see below). Consequently, in the following sections of Results, all data regarding social withdrawal scales will focus on ABCsw profiles.

Social Avoidance but not Social Indifference Is the Main Correlate of ASD in FraX

Using regression analyses, we evaluated which items (i.e., specific behaviors) within CBCLw and ABCsw were responsible for the relationship between these subscales and ASD diagnosis. We first determined which items made a greater contribution to total (i.e., combined) scores in each CBCLw and ABCsw. Items emerging as "informative" from these analyses were then tested in terms of their individual or combined (i.e., in a model with other items) correlation with ASD diagnosis. Five ABCsw items fulfilled these criteria for the *main* FraX cohort; four of them represented social avoidance (SA) alone [i.e., ABCsw5-SA, ABCsw30-SA] or SA and social indifference (SI) [i.e., ABCsw16-SA + SI, ABCsw42-SA + SI], and only one SI alone [i.e., ABCsw40-SI]. Some or all of these ABCsw items were individually correlated with ASD diagnosis, depending on whether the specific model included age and/or IQ as co-variate(s) or not. The five items were not only responsible for the significant correlation between ABCsw scores and ASD diagnosis, but also had a higher overall predictive value for ASD status than ABCsw total scores (~41% variance for the five ABCsw items vs. ~22% for scores reflecting all sixteen

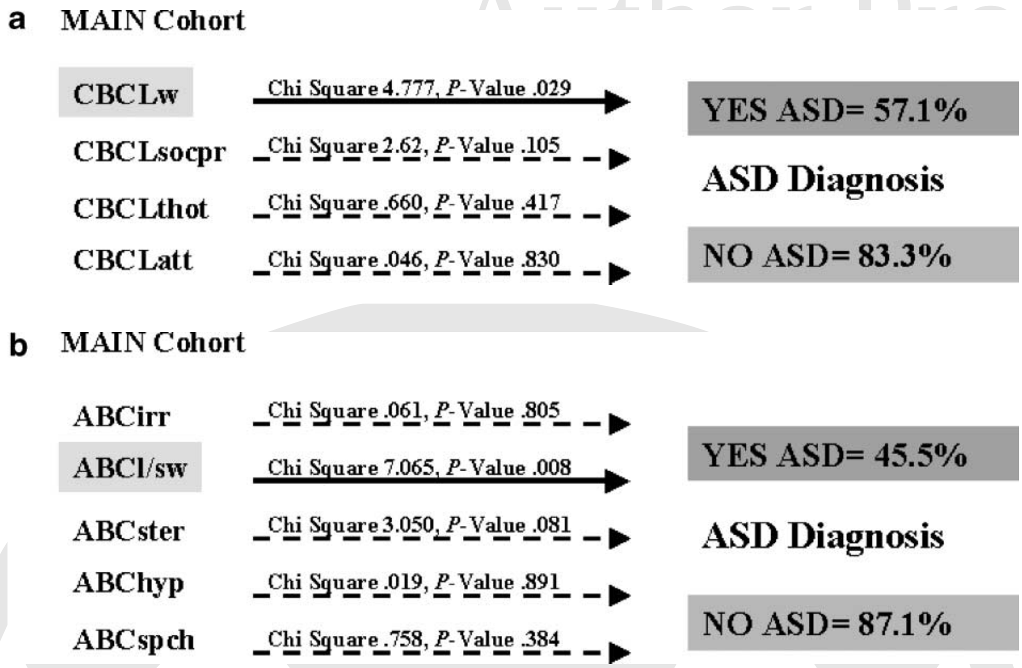

Fig. 2. Analyses of the relationship between social withdrawal subscales and ASD diagnosis for the *main* FraX cohort. **a**: Summary of logistic regression model including CBCLw and the other three CBCL subscales with highest *T*-scores (n = 38). CBCLw was the only significant predictor of ASD diagnosis, in particular of the No ASD status. **b**: Summary of similar analyses conducted for ABC-C, including all five subscales (n = 53). ABCsw was the only significant correlate of ASD diagnosis, and also a better predictor of the No ASD status. Both sets of analyses displayed here correspond to the basic logistic regression models, without co-variates. CBCLw, Child Behavior Checklist-Withdrawn subscale; ABC-C, Aberrant Behavior Checklist-Community; ABCsw-Aberrant Behavior Checklist-Lethargy/Social Withdrawal subscale.

ABCsw items). Figure 3a illustrates the basic regression model for the five ABCsw items, which correctly classified 68.2% of subjects with Yes ASD status in comparison to 45.5% for total ABCsw scores (Fig. 2b). As for the total subscale scores, the five ABCsw items were better predictors of the No than the Yes ASD status; nonetheless, there was no substantial difference in predictive value between total or selected items ABCsw scores. Analyses of the CBCLw showed a similar profile, with a relatively greater representation of items linked to SA (i.e., CBCLw75-SA, CBCLw88-SA, CBCLw-111-SA, CBCLw42-SA + SI, CBCLw65-SA + SI) than those reflecting mainly SI (i.e., CBCLw80-SI, CBCLw102-SI).

Given the dynamic nature of the neurobehavioral phenotype of boys with FraX [Hagerman, 2002; Fisch et al., 2002], we examined in detail the role of age in the relationship between social withdrawal and ASD by dividing the *main* FraX cohort into two age groups (younger or <5 years, older or ≥5 years), and by analyzing a *longitudinal* subset (n = 29) of the *main* cohort that included up to three observations per subject. The 5 years cut-off was selected on the basis of several criteria, including stability of the ASD

TABLE II. Skill and Problem Behavior Profiles of the Fragile X Cohort*

	Main cohort				Longitudinal cohort			
	FraX + ASD		FraX-only		FraX + ASD		FraX-only	
Cohorts	mean (SD)	N	mean (SD)	N	mean (SD)	N	mean (SD)	N
Age (months)	57.0 (15.4)	24	57.1 (12.9)	32	56.2 (16.3)	11	59.7 (10.9)	19
FSIQ	46.9 (15.7)	24	63.6 (14.1)	32	45.5 (15.5)	11	65.0 (10.5)	19
PLSrec	56.9 (11.9)	21	66.4 (14.4)	31	56.2 (10.6)	10	67.3 (13.0)	18
PLSexp	56.0 (12.3)	21	60.8 (13.2)	31	54.8 (8.3)	10	61.0 (13.1)	18
VABSsoc	63.5 (10.6)	24	80.9 (11.8)	32	62.5 (11.3)	11	81.9 (12.2)	19
CBCLw	61.6 (7.2)	22	55.1 (6.1)	31	61.0 (6.2)	11	53.3 (3.1)	18
ABCsw	8.0 (6.6)	22	3.0 (3.7)	31	6.3 (4.0)	10	2.3 (2.1)	19

FSIQ, full scale IQ; PLSrec, Preschool Language Scale-3-receptive; PLSexp, Preschool Language Scale-3-expressive; VABSsoc, Vineland Adaptive Behavior Scale-socialization; CBCLw, Child Behavior Checklist-withdrawal; ABCsw, Abberant Behavior Checklist-social withdrawal.
*Scores listed include *T*-scores for CBCLw, raw scores for ABCsw, and standard scores for the VABSsoc.

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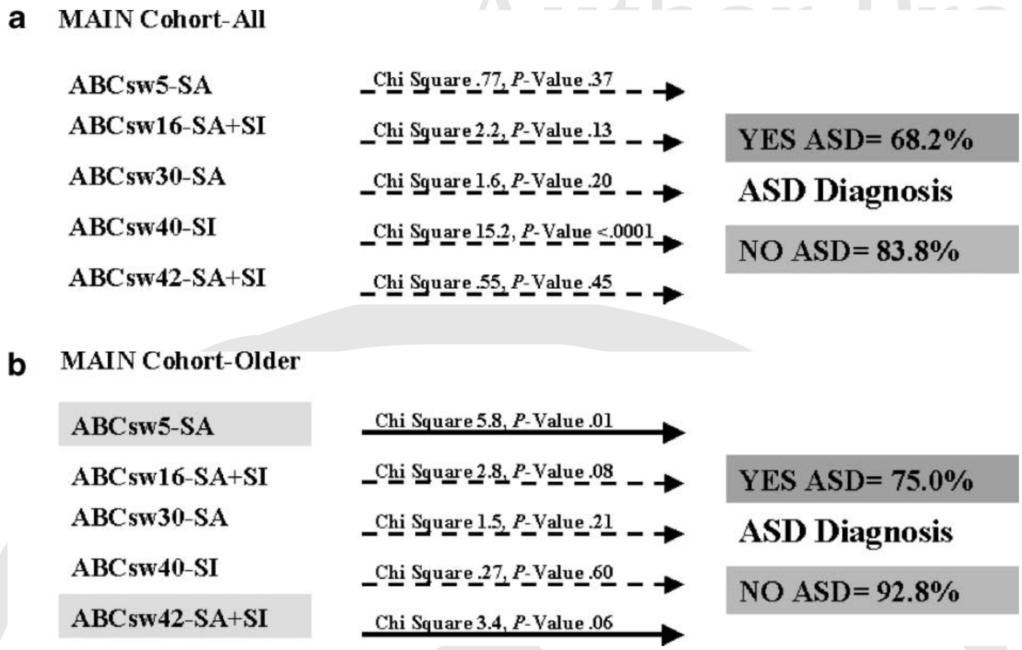

Fig. 3. Analyses of the relationship between items in the ABCsw subscale and ASD diagnosis. **a**: Logistic regression model, for the entire *main* cohort (mean age, 57.1 months; n = 53), including five selected or "informative" ABCsw items: ABCsw5-SA, ABCsw16-SA + SI, ABCsw30-SA, ABCsw40-SI, and ABCsw42-SA + SI. This combined model, without introducing co-variates, had a high predictive value for ASD diagnostic status (~68% Yes ASD, ~84% No ASD). Among individual ABCsw items, although the majority was associated with avoidant behavior (SA), the most important single correlate of ASD was a social indifference (SI)-related item (ABCsw40-SI). **b**: Similar logistic regression model for the older subgroup within the *main* cohort (≥5 years; mean age, 70.5 months; n = 22), showing an overall higher diagnostic predictive value. In addition to a greater correlation with No ASD status, individual items representing SA made a more significant contribution to ASD diagnosis.

diagnosis in the idiopathic autistic population [Lord et al., 1994] and prominence of hyperarousal and other related features that can influence avoidant behavior in FraX [Hagerman, 2002]. The CBCLw/ABCsw-ASD relationship was stronger in the older boys (n = 22, mean age 70.5 months) than in the entire *main* cohort (n = 53, mean age 57.1 months). As depicted in Figure 3, using the five informative ABCsw items, the correlation with both Yes ASD and No ASD status was higher in the older subjects (Yes ASD: 68.2% in entire cohort vs. 75.0% in older group; No ASD: 83.8% in entire cohort vs. 92.8% in older group). Furthermore, in the older subgroup (Fig. 3b), the influence of SI (i.e., ABCsw40-SI item) was substantially lower than in the entire *main* cohort (Fig. 3a), emphasizing the relationship between SA and ASD diagnosis in boys with FraX. As for other analyses, similar results were obtained for the CBCLw data. Analyses of the *longitudinal* cohort were consistent with our cross-sectional data. While the five informative ABCsw items were responsible for ~19% of the overall predictive value's variance at baseline (mean age, 58.7 months), this contribution increased to ~38% 2 years later (mean age, 83.9 months). This resulted in a higher individual item correlation with ASD diagnosis, with an even higher influence of SA-linked items, and a greater predictive value for the Yes ASD status as well (at baseline: 50.0%; 2 years later: 77.8%). All the regression models reported in this section were not substantially affected by the introduction of age and/or IQ measures as co-variates, although in general terms co-varying for VIQ led to more significant correlations.

Delay in Acquisition of Selective Adaptive Socialization Skills Is Correlated With ASD in FraX

We extended the strategy for identifying specific social withdrawal-related behaviors associated with ASD to the study of the relationship between delayed adaptive socialization skills and ASD diagnosis. We reported that, among communication and socialization skills, delayed adaptive socialization measured by the VABS was the strongest correlate of ASD diagnosis [Kaufmann et al., 2004]. For the *main* FraX cohort, a model including receptive and expressive language skills (i.e., PLS-3), VABS Communication, and VABS Socialization (VABSsoc) correctly classified 79% of subjects with ASD and 77% of those without ASD. The prediction of the No ASD status increased up to 93% when the model introduced IQ measures (mainly VIQ) as co-variates. Consequently, applying hierarchical regression analyses to the 50-item VABSsoc, we found that approximately 20 items (items 22–42) accounted for most of the variability in adaptive socialization in the *main* FraX cohort and that six of them were responsible for the correlation between VABSsoc and ASD diagnosis.

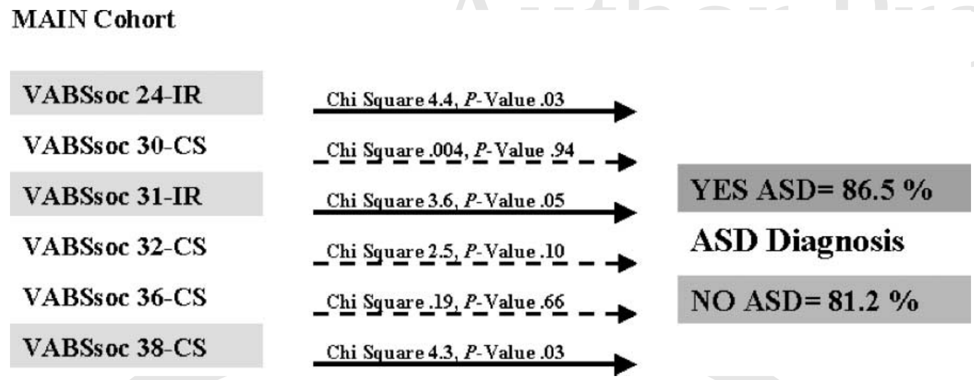

FIG. 4. Analysis of the relationship between items in VABS Socialization and ASD diagnosis. The illustrated logistic regression model incorporated the entire *main* FraX cohort (mean age, 57.1 months; n = 56), and included six selected or "informative" VABSsoc items: VABSsoc24-IR, VABSsoc30-CS, VABSsoc31-IR, VABSsoc32-CS, VABSsoc36-CS, and VABSsoc38-CS. No co-variates were introduced to the model. Note that, in addition to the overall high predictive value of the model (52% variance), three VABSsoc items covering the developmental spectrum of skills had a significant association with ASD status (colored rectangles and solid-lined arrows). VABSsoc, vineland socialization; IR, interpersonal relationships; CS, coping skills.

These items corresponded to skills normally acquired between 42 and 84 months; in sequence of skill acquisition: VABSsoc24, VABSsoc30, VABSsoc31, VABSsoc32, VABSsoc36, and VABSsoc38. According to VABS' guidelines, VABSsoc24 and VABSsoc31 are considered Interpersonal Relations-related skills (IR) while the rest are categorized as Coping Skills (CS). Analogous to the analyses of social withdrawal scales, the six informative VABSsoc items had higher overall predictive value for ASD diagnosis than VABSsoc total scores (~52% vs. ~35% variance, respectively). Figure 4 displays the basic logistic model for the relationship between VABSsoc items and ASD status, which was not substantially influenced by the introduction of age and/or VIQ/NVIQ as co-variates. The contribution of individual VABSsoc items, however, was affected by VIQ or NVIQ, with VIQ increasing the accuracy of No ASD classification.

Analyses of the *main* cohort, categorized as younger and older groups, and of the *longitudinal* cohort did not reveal age-dependence in the relationship between VABSsoc and ASD. No particular pattern of VABSsoc item contribution to the model was revealed either, leading to the conclusion that developmental delay is not a likely explanation for the inverse correlation between VABSsoc and ASD diagnosis. For instance, in our *longitudinal* cohort, VABSsoc32 was a strong and consistent correlate of ASD over time. This contrasted with VABSsoc24 (a 3–4-years old skill) and VABSsoc38 (a 6–7-years old skill), which showed a slight decline in their predictive value in the 2-year interval between the baseline and third assessments.

Delay in Socialization Skills Is Linked to Verbal Reasoning in FraX + ASD

Informal characterization of the six VABSsoc informative items revealed that they represent skills such as recognition and application of rules of social behavior (VABSsoc30-CS, VABSsoc32-CS, VABSsoc36-CS, VABSsoc38-CS), recognition of emotions (VABSsoc24-IR, VABSsoc31-IR), and/or verbal labeling of emotions (VABSsoc24-IR, VABSsoc31-IR, VABSsoc32-CS). In order to further delineate the cognitive processes underlying this set of adaptive behavior skills in FraX, the subgroup of boys in the *main* cohort (n = 34/56) who was tested by the Stanford-Binet (SB-IV) was subjected to regression analyses between VABSsoc and the four Composite scores of the SB-IV. We found a significant positive correlation between VABSsoc total scores and SB-IV's Verbal Reasoning, which was driven by the Vocabulary and Absurdities components, and a trend-level relationship between VABSsoc and SB-IV's Comprehension and Quantitative Reasoning (Table III). A model combining the six VABSsoc items confirmed the aforementioned association between VABSsoc and Verbal Reasoning. Furthermore, analyses using individual items demonstrated that VABSsoc24-IR was the only skill with a selective association, specifically with Vocabulary, though at a trend level ($P = 0.009$; significance after Bonferroni correction: $P = 0.008$). Although these analyses involved an older (mean age 62.7 vs. 57.1 months

TABLE III. Relationship Between Adaptive Socialization Skills and Cognitive Composities

SB-IV composites	*t*-value	*P*-value
Verbal reasoning	4.23	0.0002
-Vocabulary	3.32	0.0025
-Comprehension	2.45	0.02
-Absurdities	2.94	0.007
Abstract/visual reasoning	1.49	0.15
Quantitative reasoning	2.12	0.04
Short-term memory	1.67	0.1

Analyses used VABSsoc total standard scores and age as a co-variate. *P*-value set at 0.0125 (0.0125–0.025 for trends) after Bonferroni corrections. Significant results are highlighted in gray.

TABLE IV. FraX + ASD Subgroups According to Social Behavior Predictors

	Only vabssoc (n = 5)	VABSsoc & ABCsw (n = 13)	No vabssoc (n = 4)	No ASD (n = 32)
Variable	mean (SD)	mean (SD)	mean (SD)	mean (SD)
Age (months)	58.8 (10.1)	55.2 (16.2)	64.5 (19.7)	57.1 (12.9)
FSIQ	43.3 (18.5)	44.5 (12.3)[b]	58.9 (20.5)[b]	63.6 (14.1)
PLSrec	55.2 (7.2)	56.4 (10.5)	66.7 (23.9)	66.5 (14.4)
PLSexp	52.0 (3.5)	55.0 (8.0)	70.3 (27.8)	60.8 (13.2)
VABSsoc	65.8 (11.5)	59.5 (5.7)	76.8 (14.9)	80.9 (11.8)
ABCsw	4.0 (3.2)	10.2 (7.1)	6.0 (6.1)	3.0 (3.7)
ADI-R Recs-1	2.2 (1.6)	3.2 (1.6[a]	2.0 (1.4[a]	1.2 (1.3)
ADI-R Recs-3	1.4 (1.5)[b]	3.9 (1.8[b]	2.3 (2.9)	1.2 (1.2)

[a]Statistical trend.
[b]Statistically significant; ADI-R Recs-1 and ADI-R Recs-3, Autism Diagnostic Interview-Revised total scores for sections B1 and B3, respectively, of the Reciprocal Social Interaction domain.

for the *main* cohort) and relatively higher function (mean IQ: 63.7 vs. 55.8 for the *main* cohort) group of boys with FraX, these preliminary data may shed some light on the nature of the cognitive processes underlying ASD in FraX.

Adaptive Socialization and Social Withdrawal Are Independent Predictors of ASD in FraX

Following the study of their individual relationship with ASD diagnosis, we explored the relative contribution of adaptive socialization and social withdrawal to the diagnostic classification of ASD in boys with FraX. In integrated models applied to the *main* cohort, we determined that adaptive socialization was a stronger correlate of ASD diagnosis than social withdrawal. In an age-adjusted model that correctly classified 77% of subjects with ASD and 90% of those without ASD, VABSsoc was significantly correlated with ASD classification (Chi square: 14; $P < 0.0001$) while the relationship for ABCsw was only at a trend level (Chi square: 3.2; $P < 0.08$). This pattern was also reflected in VABSsoc's and ABCsw's contribution to variance in regression models: ~37% for VABSsoc & ABCsw, ~32% for VABSsoc alone, and ~17% for ABCsw alone.

Analyses of the *longitudinal* FraX cohort further emphasized the role of adaptive socialization as primary determinant of ASD diagnosis. An integrated VABSsoc & ABCsw model, at baseline, contributed ~15% of the overall variance and resulted in correct classification of 72% subjects at a follow-up evaluation 2 years later, again with better prediction of the No ASD status (87.5% No ASD vs. 44.4% Yes ASD). VABSsoc scores alone, at baseline, significantly predicted ASD status 2 years later in ~68% of the sample (44.4% for the Yes ASD status and 81.3% for the No ASD status; model's variance: 15%). On the contrary, although baseline ABCsw scores (alone) were correlated with contemporary ASD status (mainly No ASD), they had minimal influence on overall diagnostic classification 2 years later (model's variance: 4.3%).

The logistic classification models presented above were extended to the impact of each VABSsoc and ABCsw to the correct diagnosis of every FraX subject. The analyses revealed three distinctive profiles of boys with FraX + ASD: with primary delayed socialization (only VABSsoc influence, n = 5), with delayed socialization and social withdrawal (combined VABSsoc & ABCsw influence, n = 13), and without delayed socialization or prominent social withdrawal (No VABSsoc influence, n = 4). The first two groups were characterized by relatively lower cognitive performance (FSIQ, language skills) and a greater delay in expressive language skills than either the No VABSsoc FraX + ASD group or the FraX group without ASD (Table IV). In terms of autistic behavior profiles, the larger VABSsoc & ABCsw subgroup had more severe fundamental deficits in Reciprocal Social Interaction (ADI-R's Reciprocal Social Interaction's subdomains 1 and 3). Among the FraX + ASD subgroups, we interpreted the lack of differences in ADI-R's Reciprocal Social Interaction's subdomains 2 and 4 as evidence that impairment in these ADI-R components is a fundamental disturbance in FraX + ASD, as previously reported by us [Kaufmann et al., 2004]. Distinguished by its relatively higher cognitive skills, the No VABSsoc subgroup of boys with FraX + ASD resembled in several aspects cohorts of boys with idiopathic ASD [Kau et al., 2004]. While we recognize their preliminary nature, these analyses suggest that ASD in FraX may have distinct behavioral patterns and neurobiological substrates.

DISCUSSION

Most young males with FraX display behavioral abnormalities that can be interpreted as atypical social interaction or autism spectrum behaviors [Hatton et al., 1999; Hagerman, 2002]; however, only a subset of boys with this genetic disorder fulfills

DSM-IV criteria for the diagnoses of autism or ASD [Bailey et al., 1998; Kau et al., 2004]. The importance of precisely delineating the behavioral profile of boys with FraX + ASD is underscored by the fact that these children display other severe cognitive and behavioral abnormalities that have considerable medical and educational consequences. This study intended to expand our previous work on the relationship between different abnormal social behaviors and the diagnosis of ASD in boys with FraX [Kau et al., 2004; Kaufmann et al., 2004]. In line with current views on the spectrum of social behavior abnormalities in idiopathic ASD [Eigsti and Shapiro, 2003; Pelphrey et al., 2004], we have focused on two aspects linked to ASD in FraX: delayed adaptive socialization and social withdrawal. We demonstrated that, as delayed socialization, social withdrawal is also a correlate of ASD in FraX. Moreover, the association between these social behavior variables and ASD is driven by specific behaviors identified by CBCLw, ABCsw, and VABSsoc items. True social avoidance, but not social indifference, appears to be linked to ASD in FraX particularly in older boys. In terms of adaptive socialization skills, items representing recognition and application of rules of social behavior, recognition of emotions, and verbal labeling of emotions were correlated with verbal reasoning and selectively associated with ASD status in FraX. Statistical models integrating socialization skills and social withdrawal demonstrated that delayed adaptive socialization is the primary determinant over time, particularly, of the No ASD status in boys with FraX. These models also allowed the identification of distinctive subgroups of males with FraX + ASD, depending on the relative influence of impaired socialization skills and social withdrawal. Altogether, our findings suggest that two distinct but interrelated social behavior abnormalities, one linked to impaired cognitive processes and the other to exaggerated emotional responses, play a role in the development of ASD in boys with FraX.

Social avoidant behavior has been recognized as a frequent and characteristic abnormality in individuals with FraX [Hagerman, 2002]. Whether hyperarousal, social anxiety, or a different process leading to withdrawal, we [Kau et al., 2004; Kaufmann et al., 2004] and others [Hatton et al., 2002] have shown that rating scales of problem/aberrant behavior demonstrate markedly high scores on their corresponding social withdrawal subscales in boys with FraX + ASD. Considering that the continuum of CBCLw and ABCsw scores paralleled the spectrum of severity in autistic behavior [Kaufmann et al., 2004], and that delayed adaptive socialization skills is a selective predictor of ASD in FraX, we examined the possibility that severity of social withdrawal was also a correlate of FraX + ASD. Indeed, despite high scores on several other problem behavior subscales (e.g., attentional difficulties, stereotypic behavior), in this study, CBCLw and ABCsw were selective predictors of ASD status in FraX. Interestingly, social withdrawal scores were better predictors of the No ASD status. In other words, less severe withdrawn behavior correlates with lower likelihood of a positive ASD diagnosis in FraX. Using a combined cross-sectional and longitudinal approach, we found that the relationship between social withdrawal and ASD diagnosis is stronger in boys older than 5 years. This finding is in agreement with previous observations of higher prevalence of social anxiety [Hagerman, 2002] and better-delineated ASD profiles [Bailey et al., 1998] in older boys with FraX. Regarding the nature of the behaviors measured by social withdrawal scales, our "informal" classification of CBCLw and ABCsw items as either social avoidance- or social indifference-like behaviors led to the conclusion that avoidance is the main correlate of ASD in FraX. Since similar studies have not been reported in other developmental disorders associated with ASD, it is not possible at this point to determine the specificity of the social avoidance–ASD correlation in FraX. Nevertheless, our recent work in Down syndrome indicates that social withdrawal scales are useful for distinguishing subjects with ASD from individuals with other behavioral conditions associated with this genetic disorder [Capone et al., 2005]. On the other hand, data on other behavioral measures suggest that in idiopathic ASD, there is a major link between social indifference or aloofness, but not avoidance, and the diagnosis of ASD [Eaves et al., 1994; Amaral et al., 2003]. Presently, it is not possible to identify the nature of the process underlying severe social withdrawal in FraX + ASD. Nonetheless, the age-dependence of the association between social withdrawal and ASD in FraX indicates that social anxiety, which is more prominent in late childhood, may be a main contributor. Therefore, studies directly evaluating the relationship between social anxiety and other aspects of social behavior in FraX, including ASD diagnosis, are needed in order to further characterize the spectrum of behavioral abnormalities in FraX + ASD.

There seems to be a unique profile of adaptive socialization skills correlated with ASD in FraX. The VABSsoc six-items group that led to a correct ASD status classification of boys with FraX represented behaviors dealing with rules of social interaction, recognition of emotions, and verbal labeling of emotions. This VABSsoc profile was not influenced by age, at least in the 3–8 years range, because the individual contribution of each VABSsoc item to the classification model over time was unrelated to the skill's expected emergence. Our preliminary attempt to identify cognitive processes underlying delayed adaptive socialization in FraX + ASD, through correlative analyses with Stanford-Binet's Composite scores, revealed Verbal Reasoning as a major link.

Among the Verbal Reasoning components, Vocabulary was the primary correlate of the VABSsoc informative items. The latter is not surprising considering that this aspect of communication has a major role in understanding and implementing rules of social behavior and verbal labeling of emotions, all of them selectively impaired in FraX + ASD. The complexity of the cognitive processes affected in FraX + ASD is underscored by the positive correlation between VABSsoc and the Absurdities component of Verbal Reasoning. The association between delayed adaptive socialization and ASD in FraX is relatively well established; in addition to our recent study [Kaufmann et al., 2004], Rogers et al. [2001] reported on VABS profiles in young boys with FraX with and without autism. Studies of individuals with idiopathic ASD have also demonstrated the relative selectivity of the association between delayed adaptive behavior and ASD in the context of moderate cognitive impairment [Kraijer, 2000; Fenton et al., 2003]. The analyses reported here provide initial insight into the nature of impaired adaptive socialization in FraX + ASD; among a wide range of skills recorded between 3–8 years by VABSsoc, only a subset appears to be relevant to ASD in FraX. For instance, Play & Leisure Time-labeled items, such as spontaneously sharing toys, were not contributory to ASD status. The identified VABSsoc items are linked to complex verbal skills and executive function, domains known to be selectively affected in FraX [Reiss et al., 1995; Cornish et al., 2004]. Thus, the more severe delay in adaptive socialization in boys with FraX + ASD seems to reflect a greater involvement of cognitive domains that are selectively vulnerable in this genetic disorder.

Further refinement of the relationship between social withdrawal or adaptive socialization and ASD in FraX allowed the study of the differential role of these two aspects of social behavior in establishing the diagnosis of FraX + ASD. Logistic models showed that delayed adaptive socialization is the primary determinant of ASD status, with approximately equal accuracy in classifying subjects with and without ASD diagnosis. Controlling for variance in verbal abilities (i.e., VIQ as co-variate) increased the ability of predicting the No ASD status, which is the main correlate of social withdrawal in corresponding models. In line with these data, we were able to identify two subgroups of boys with FraX + ASD in whom delayed adaptive socialization is the main diagnostic predictor. The larger of these two subgroups was also characterized by social withdrawal. A third and smallest subgroup appeared to be unrelated to either delayed socialization or severe withdrawal. As expected from the link between adaptive socialization and cognitive function, the two subgroups with low VABSsoc scores had lower IQs. The subgroup with both delayed socialization and social withdrawal showed the most severe ADI-R profiles, particularly in the ADI-R subdomains that are more variably impaired in FraX + ASD (i.e., fundamental nonverbal socialization and shared enjoyment) [Kaufmann et al., 2004]. Although these analyses should be considered preliminary in nature, because of the relatively small number of subjects ($n = 22$), our findings are significant in that they represent one of the first attempts at delineating types and behavioral mechanisms of ASD in FraX. The high correspondence between cross-sectional and longitudinal findings, and the adequacy of the models for predictions over time, are supportive of our analytical strategy. We postulate that delay in the development of certain complex communication and attentional processes, most likely representing abnormal cortical development [Reiss et al., 1995; Kates et al., 2002], is a risk factor for ASD in FraX. If in addition to the latter, there is a limbic dysfunction, leading to severe avoidant behavior [Amaral et al., 2003; Pelphrey et al., 2004], the likelihood of manifesting the ASD behavioral syndrome in FraX increases considerably. Individuals with combined cortical and limbic dysfunction would obviously be more severely affected, almost certainly not only in terms of social behavior but also in other neurological domains. This hypothesis, exclusively based on our clinically-oriented behavioral rating data, requires formal testing using other approaches. Experimental behavioral paradigms that allow a better characterization of the social avoidance phenomenon, such as the Social Approach Scales that we are currently analyzing in a cohort of males with FraX [Roberts et al., 2005b], and neuroimaging methods that we have applied for the evaluation of cerebellar abnormalities in FraX + ASD [Kaufmann et al., 2003b] are examples of complementary strategies to the present study.

In summary, ASD in boys with FraX is linked to two distinct but interrelated abnormalities in social behavior: delayed adaptive socialization (primary factor), which is present in most boys with FraX + ASD, and severe social withdrawal or avoidance (secondary contributor), which is observed in a high proportion of these children. Taking into consideration that our study included a relatively small sample and was based on instruments designed for clinical assessments that use predominantly parental information, these findings need to be replicated in a larger group of boys with FraX and with an expanded behavioral battery. However, the fact that data on our cross-sectional and longitudinal cohorts were highly concordant further supports our study design, suggesting as well that serial observations are extremely valuable in the evaluation of FraX + ASD [Sabaratnam et al., 2003; Cortell et al., 2004]. The severity of the cognitive and behavioral abnormalities present in boys with FraX + ASD emphasizes the importance of characterizing this subphenotype of FraX. Behavioral analyses, such as those reported in this study, could have implications for early diagnosis and for more specific pharmacologic and non-pharmacologic interventions that target selective behavioral aspects and subtypes of FraX + ASD (e.g., SSRIs only for individuals with FraX + ASD and severe social withdrawal). Future studies in our multidisciplinary research program on ASD in FraX will examine the relationship between delayed adaptive socialization and social withdrawal and other measures of social behavior, as well as they will also evaluate the relationship between the behavioral parameters and biomarkers relevant to FraX + ASD.

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