

Lippincott Williams & Wilkins

Journal of Developmental and Behavioral Pediatrics

Journal Code: DBP

Jobname: dbp99632

Page: 409 to 417

Date: 10/27/2001

Time: 12:34

The Behavioral Phenotype in Fragile X: Symptoms of Autism in Very Young Children with Fragile X Syndrome, Idiopathic Autism, and Other Developmental Disorders

SALLY J. ROGERS, Ph.D.
ELIZABETH A. WEHNER, Ph.D.

Department of Psychiatry, University of Colorado Health Sciences Center, Denver, Colorado

RANDI HAGERMAN, M.D.

M.I.N.D. Institute and Department of Pediatrics, University of California Davis, Davis, California

ABSTRACT. This study was designed to explore the behavioral phenotype of autism in a group of young children with fragile X syndrome (FXS). Twenty-four children with FXS, ages 21 to 48 months, were compared with two well-matched groups: 27 children with autism (AD) and 23 children with other developmental delays (DD), on two standardized autism instruments, as well as on measures of development and adaptive behavior. Two FXS subgroups emerged. One subgroup (n = 16) did not meet study criteria for autism. Their profiles on the autism instruments and the developmental instruments were virtually identical to the other DD group. The other FXS subgroup (n = 8, or 33% of the total FXS group) met study criteria for autism. Their profiles on the autism instruments were virtually identical to the group with autism. The finding of two FXS subgroups raises a hypothesis of additional genetic influences in the FXS autism group, warranting further genetic studies. *J Dev Behav Pediatr* 22:409-417, 2001. Index terms: *fragile X syndrome, autism, behavioral phenotype, development, mental retardation.*

Fragile X syndrome (FXS) is a genetic mental retardation syndrome caused by a mutation on the X chromosome in the fragile X mental retardation 1 gene (FMR1). This mutation is associated with an extended number of CGG repeats, leading to a deficit in production of the FMR1 protein. The behavioral phenotype associated with FXS has long been recognized as involving cognitive and language problems, social anxiety, avoidance of eye contact, and hand stereotypies as reviewed by Hagerman.¹ In addition, many persons with FXS demonstrate some or all of the symptoms of autism.

Autism is a pervasive developmental disorder characterized by a lack of social reciprocity or responsiveness, abnormal use of language and communication, and a restricted, repetitive repertoire of activities and interests. Autism is currently considered to be a multifactorial disorder that involves a strong genetic influence.^{2,3} It is over-represented as part of the behavioral phenotype in several genetic disorders, including FXS, tuberous sclerosis, phenylketonuria (PKU), Rett syndrome, and duplications in chromosome 15 in the q arm.^{4,5} Summary reports of multiple studies demonstrate that between 2.5%⁶ and approximately 5% to 6% of autistic individuals have fragile

X syndrome^{4,7,8} and that individuals with autism should be routinely studied with FMR1 DNA testing to rule out fragile X. Current estimates of the prevalence of autism in FXS are approximately 15% to 25%.^{4,9-11} An additional 50% to 90% of individuals with FXS have been reported to have some symptoms of autism, including poor eye contact, hand flapping, hand biting, perseveration in speech, and tactile defensiveness.^{9,10,12,13}

Although some behavioral symptoms seen in FXS appear similar to autism, some have argued that there are distinct differences between persons with FXS with autistic symptomatology and persons with autism.¹³ The ability of males with FXS to recognize facial and emotional expressions is equivalent to IQ-matched controls.^{14,15} A study by Mazzocco, Pennington, and Hagerman¹⁶ of affected women with FXS revealed their ability to demonstrate appropriate performance for their IQ on emotion-perception and perspective taking on theory-of-mind tasks, key deficit areas in autism. However, none of these studies has compared persons with FXS to those with autism.

It has been hypothesized that the autistic features seen in FXS are secondary to a generalized hyperarousal that leads to avoidance or withdrawal from social stimuli.^{8,17} An overarousal hypothesis has also been used to explain autism for many years.¹⁸ Several empirical studies have supported the FXS hyperarousal hypothesis. Cohen and colleagues examined gaze avoidance in children with FXS and in children with autism.^{19,20} They demonstrated that children

Address for reprints: Sally J. Rogers, Ph.D., JFK Partners, University of Colorado Health Sciences Center, 4200 East 9th Avenue/Campus Box C234, Denver, CO 80262; e-mail: Sally.Rogers@uchsc.edu.

with FXS were sensitive to parental gaze and were avoidant of direct eye contact, whereas children with autism were not sensitive to others' gazes and did not appear to avoid them. Cohen et al¹⁹ suggested that social anxiety led to gaze avoidance in FXS, whereas a more general social deficit was responsible for the gaze problems in autism. Sudhalter and colleagues²¹ compared the pragmatics of speech in boys with FXS, matched on language level and age, with those with Down syndrome (DS) and those with autism without FXS on measures of conversational pragmatics. They found that, although the DS group did not produce deviant conversational patterns, the group with autism and the group with FXS differed in pragmatics problems: the FXS group produced more perseverative speech and the autism group produced more echolalia and greater levels of pragmatic impairment. The authors suggested that the perseveration of the FXS group reflected word-retrieval problems and social anxiety. Recent studies using electrodermal measures in FXS show evidence of hyperarousal by demonstrating enhanced electrodermal responses to stimuli and poor habituation compared with typically developing controls.^{22,23} Heart rate research has also demonstrated decreased vagal tone in young boys with FXS²⁴ compared with typically developing control subjects. Thus, several types of studies have demonstrated increased arousal levels in FXS compared with typically developing controls.

A second theory suggests that anxiety leading to shyness and social interaction problems may underlie the association between FXS and autism. In females with FXS, both the severity of anxiety and the degree of autistic features are independent of IQ.²⁵ In 30 girls with FXS, the severity of autistic features correlated inversely with the size of the posterior cerebellar vermis as obtained by magnetic resonance imaging (MRI).²⁵ Although the anxiety composite score did not correlate with the size of the posterior cerebellar vermis, it did correlate with the number of autistic features.²⁵ That is, the more severe the anxiety score, the greater the number of autistic features. However, the question of whether the autistic-like symptoms seen in FXS differ from those of idiopathic autism and are due to anxiety and/or overarousal has not been answered.

Those who have compared the autism symptom profiles of FXS to idiopathic autism have reported conflicting findings. Bailey and colleagues¹⁰ compared autistic symptoms in 57 boys with FXS, ages 2 to 11 years, to a referral group of 391 individuals with autism using the Childhood Autism Rating Scale (CARS).²⁶ In the FXS group, 25% met the CARS cutoff for autism, and the symptom profile of the FXS group was very similar to that of the idiopathic autism group, although virtually all the children with FXS and autism had symptoms in the mild-to-moderate, rather than the severe range. Thus, this young group had a higher rate of autism than that found in older groups,^{9,12,27} but not a unique behavioral profile. In contrast, Turk et al²⁸ compared behavioral profiles of boys with FXS, DS, and mental retardation. They did not find elevated rates of autism in the FXS group, but rather a unique behavioral profile that differentiated FXS from both comparison groups (however, a comparison group with autism was not used).

The relationship of severity of retardation to the presence of autism in FXS has also been examined. Bailey et al²⁹ recently found that individuals with FXS and autism had a lower IQ than nonautistic individuals with FXS alone, which has been also been found by others.^{17,25}

To summarize, current literature suggests that the full syndrome of autism occurs in a minority of persons with FXS, may be more prominent earlier in life, and may occur more often among those with more severe mental retardation. However, the age groups used in past studies have been so broad that the effects of chronological age may have been somewhat masked. It is also not clear to what extent those persons with FXS and autism have a somewhat different profile of symptoms than persons with idiopathic autism. Finally, it is possible that different approaches to diagnosing autism have created inconsistent past findings. Most studies have used only DSM diagnoses or simple screening checklists. Only the Turk et al²⁸ and Bailey et al^{10,29,30} studies have used a detailed, directly administered autism rating system. Considerable research on the diagnosis of autism has been accomplished in the past few years, and it has resulted in diagnostic tools now considered the gold standard, the Autism Diagnostic Interview-Revised (ADI-R)³¹ and the Autism Diagnostic Observation Schedule-Generic (ADOS-G).³²

The purpose of the present study was to examine symptoms of autism and relationships between autism symptoms and developmental variables, using state-of-the-art measures, in very young children with FXS compared with groups of children with idiopathic autism and children with other kinds of developmental disorders. The study, part of a larger longitudinal study, was carried out to test several hypotheses:

1. Children with FXS will have elevated scores on autism instruments compared with developmentally delayed (DD) control subjects.
2. Children with FXS will demonstrate a unique profile of autistic symptoms compared with children with idiopathic autism.
3. Autistic symptoms will be related to the severity of developmental delays in children with FXS.

METHOD

Participants

Recruitment. The participants included 27 children with autistic disorder (AD), 24 children with fragile X syndrome (FXS), and 23 children with other developmental delays (DD). There were no siblings included in any group. The participants ranged in age from 21 to 48 months. Subjects were recruited for the larger, longitudinal study from centers that specialized in the diagnosis and treatment of young children with autism, other developmental delay, and FXS. Recruiting was done by mailing advertisements about the study to past clients and by mentioning it directly to current clients. The study was also advertised through parent newsletters. Parents volunteered for the longitudinal study, knowing that it entailed several visits and would be conducted across 2 years. All families who volunteered and whose children qualified were enrolled. All children in

the AD and DD groups came from the Denver area, primarily from two tertiary medical centers. Because of the low incidence of identified 2 and 3 year olds with FXS, children with FXS were also recruited from FXS tertiary care centers in Chapel Hill, NC, and San Francisco, CA, as well as from the Fragile X Treatment and Research Center at the Children's Hospital in Denver. The majority of parents volunteered for the study; they received minimal payments and information about their child's test scores. Testing for the study was conducted during both home visits and lab visits for most children. Occasionally, all testing was conducted in the home or the lab (for children from locations other than Colorado). All participants were biological or adopted children of their parents. Medical information concerning the presence of other conditions was gathered from all parents and from primary care physicians for most subjects, including DNA testing for all children with FXS. Further, all participants had normal vision and hearing, or vision and hearing that was corrected to within the normal range, had unimpaired hand use, and were mobile.

Autism Diagnosis. A set of diagnostic criteria was established to insure that each subject was accurately diagnosed. To be considered as having autism, a child had to have been previously diagnosed as having autistic disorder (AD). In addition, the child had to meet criteria for AD on two of three diagnostic systems: the Autism Diagnostic Interview-Revised (ADI-R),³¹ the Autism Diagnostic Observation Schedule-Generic (ADOS-G),³² and AD criteria as defined by the American Psychiatric Association Diagnostic and Statistical Manual-4th Edition (DSM-IV). Given the young age of the subjects, we followed guidelines regarding the presence of restricted and stereotyped behaviors used by Cox et al.³³ That is, children 36 months or younger were considered as meeting ADI-R criteria for AD even if they did not meet the cutoff score on the Repetitive Behaviors and Stereotyped Patterns scale. Finally, each subject also needed to have the current AD diagnosis confirmed by a clinical psychologist on the project. In contrast, those included in the DD group (1) failed to meet criteria for AD on both the ADI-R and (2) the ADOS-G; (3) did not meet DSM-IV criteria for AD; (4) were previously diagnosed with a developmental delay, but not AD; and (5) evidenced current cognitive or language delays on developmental testing, but no evidence of an autistic spectrum disorder.

Autism Group. The AD group originally consisted of 29 children. Two children were dropped because they did not have ADOS-G data. None of the children had any significant medical condition. For the remaining 27 children, all had outside and current clinical diagnoses of autism or pervasive developmental disorder not otherwise specified (PDDNOS). Twenty-four children met criteria for AD in all three diagnostic systems. The remaining three children met criteria in two of the three systems and were included in the autism group (two missed the ADI-R criteria and one missed the ADOS-G criteria).

Developmentally Delayed Group. The DD group originally consisted of 24 children. No child had a previous diagnosis of autism. One child was eliminated from the study

completely because he met ADOS-G criteria for AD and current clinical diagnosis confirmed autism. One child met ADI-R criteria for AD but not DSM-IV nor ADOS-G criteria. However, both previous and current clinical diagnoses excluded autism, and there were concerns about the validity of the parent report on the ADI-R interview. Across four lab visits, this child did not demonstrate any symptoms of autism, and it was decided to continue to include him in the DD group. Any bias that might occur because of this decision would be a conservative bias. Of the children included in the DD group, 10 had Down syndrome, 1 had an abnormality of chromosome 18, and 12 had delays of unknown etiology. The children with Down syndrome were compared with the other children with developmental delays on all variables of interest in this study. There were no significant differences between the two DD groups; therefore, the two groups were combined for all subsequent analyses.

Fragile X Group. The FXS group consisted of 24 children. No child was premature or had significant injuries or illnesses. Three children had additional medical conditions: one had mild asthma, one had urinary track problems, and one had chronic sinusitis and visual perception problems.

The children with fragile X recruited in Colorado represented consecutive referrals of all children ages 24 to 48 months seen in the fragile X clinic at The Children's Hospital during the years 1997 to 1999. Approximately 80% of the referrals participated in this study; the 20% who declined did so because they had traveled to Denver and were facing time constraints or child illness. The children who were recruited from San Francisco and North Carolina represented all children between ages 24 and 48 months known to the fragile X centers in those sites during 1997. All of these children participated except one who was not

Table 1. Demographic Characteristics of Subjects

Variables	Groups (n)	Mean	SD
Age	AD (27)	33.70	3.53
	FXS (24)	35.08	7.11
	FXS AD (8)	34.50	7.52
	FX NAD (16)	35.33	7.38
	DD (23)	34.26	6.66
Mental Age	AD (27)	20.06	5.82
	FXS (23)	19.13	7.03
	FXS AD (8)	15.00	5.04
	FXS NAD (16)	21.34	7.08
Gender	DD (23)	22.42	5.49
	AD (27)	22:5 (Male:Female)	
	FXS (24)	23:1	
	FXS AD (8)	8:0	
	FXS NAD (16)	15:1	
Ethnicity	DD (23)	12:11	
	AD (27)	23:4 (Majority:Minority)	
	FXS (24)	20:4	
	FXS AD (8)	7:1	
	FXS NAD (16)	13:3	
	DD (23)	19:4	

AD, autistic disorder; FXS, fragile X syndrome; FXS AD, fragile X with autism; FXS NAD, fragile X without autism; DD, other developmental disabilities.

yet ambulatory and thus did not meet inclusion criteria. Thus, the sample consisted of a large majority of all children identified with fragile X in this age group in three different regions of the country. The prevalence of fragile X is currently considered to be 1 in 3500 to 4000. With a birthrate of 30,000 children in the Denver metro region in 1997, we would expect fewer than 10 children with fragile X born per year. Furthermore, fragile X syndrome is not typically identified in children as young as 2 years unless there are identified older siblings. It thus seems reasonable to assume that no selection bias was operating and that the group is representative of identified 2 and 3 year olds with fragile X syndrome.

The three diagnostic groups did not differ significantly on chronological age, overall mental age, or socioeconomic status. The means and standard deviations for these variables are presented in Table 1, along with gender and ethnicity breakdowns by group.

Measures

Autism Diagnostic Interview-Revised. The ADI-R³¹ is a semistructured, standardized parent interview developed to assess the presence and severity of symptoms of autism in early childhood across the three main symptom areas involved in autism: social relatedness, communication, and repetitive, restrictive behaviors. The ADI-R elicits elaborate parent descriptions of the child's behavior in response to over 90 questions concerning a variety of social and communicative situations. Each question is scored separately, and then scores for the items found to be most discriminating of autism are combined into an algorithm involving three summary scores: (1) social reciprocity, (2) communication, and (3) restricted, repetitive behaviors. Classification of autism requires meeting a cutoff for each of these three scores. The ADI-R has been carefully validated across a wide range of ages and severity levels in autism.

An algorithm has been established that differentiates autism from other developmental disorders at high levels of sensitivity and specificity (over 90% for both) for subjects with mental ages (MA) of 18 months and older. For subjects with younger MAs, it has been found that the algorithm needs to be adapted slightly, by reducing cutoffs on the third set of behaviors.³³

Dr. Catherine Lord, an author of the ADI-R, trained one author (S.J.R.) to reliability on use of this measure. S.J.R. then trained other raters in her lab to reliability of 85% or better item agreement on the full range of scores (0–3). Trained raters administered the ADI-R to parents of all subjects in the study.

Autism Diagnostic Observation Schedule-Generic. The ADOS-G³² is a semi-structured standardized interview administered directly for the purposes of diagnosing autism. The ADOS-G uses developmentally appropriate social and toy-based interactions in a 30- to 45-minute interview to elicit symptoms of autism in four areas: social interaction, communication, play, and repetitive, restrictive behaviors. The ADOS-G consists of four different modules, each directed at a particular level of language ability. In the present study, all subjects received Module 1, designed for

preverbal children or those just beginning to speak. Scores for the items found to be most discriminating of autism are combined into an algorithm involving four summary scores: (1) social, (2) communicative, (3) play, and (4) restricted, repetitive behaviors. Classification of autism requires meeting a cutoff on the first two summary scores. The ADOS-G has been carefully validated across a wide range of ages and severity levels in autism.

An algorithm has been established that differentiates autism from other developmental disorders at high levels of sensitivity and specificity for subjects with MAs of 18 months and older, with only 4 of 60 children (2 of 42 with autism, 2 of 18 with other developmental disorders) being misclassified. In the present study, Dr. Catherine Lord, an author of ADOS-G, trained two of the authors to reliability on this measure at the University of Chicago. One author (E.A.W.) then trained other raters in the lab to reliability of 85% or better item agreement on the full range of scores (0–3). Trained raters administered the ADOS-G to all subjects in the study.

Mullen Scales of Early Learning. The Mullen Scales of Early Learning (MSEL)³⁴ is a standardized developmental test for children ages 3 months to 60 months. The MSEL consists of five subscales: Gross Motor, Fine Motor, Visual Reception, Expressive Language, and Receptive Language. The MSEL allows for separate standard verbal and nonverbal summary scores to be constructed. The MSEL was standardized on a nationally representative sample. The MSEL was administered to all subjects according to standard instructions. Reinforcers were used at times to reward cooperation and attention. One child with FXS received the Bayley Scales of Infant Development, 2nd Edition, instead of the MSEL.

The Vineland Adaptive Behavior Scales, Interview Edition. The Vineland³⁵ assesses adaptive behavior via parent interview in both personal and social areas, across the lifespan. The Vineland consists of four domains of adaptive behavior: Communication, Daily Living Skills, Socialization, and Motor Skills. A fifth scale, Maladaptive Behaviors, was not used. The scale is norm referenced, standardized on a representative national sample including both typical samples and those with developmental delays. Scores for each subscale include both standard scores and developmental equivalents; developmental equivalent scores were used throughout this study. The Vineland was administered by interview to subjects' mothers, usually during a home visit, according to manual instructions.

Procedures

These data were gathered as part of a larger longitudinal study funded by National Institute of Child Health and Human Development (NICHD), National Institutes of Health, Bethesda, MD. The entire study was carried out under Institutional Research Review Board approval. Families were enrolled as they volunteered for the study. Consent forms were read to each family, risks and benefits explained, and all questions answered before consent was obtained and before any measures were gathered. Each child's mother was interviewed about her child's develop-

ment and behavior using the ADI-R and the Vineland, generally during a home visit. The parents also completed a demographics questionnaire. The developmental assessment and the ADOS-G were completed either in a lab or during a home visit, along with other measures not being reported here.

RESULTS

The first two hypotheses of the study concern the presence and pattern of symptoms of autism in children with fragile X syndrome (FXS), compared with children with autism and those with nonautistic developmental disorders. As a first step in addressing these hypotheses, the same diagnostic criteria developed to define the autistic disorder (AD) group were applied to the children with FXS. There were 7 children who met all study criteria for AD. These 7 children were positive for AD on all three diagnostic systems, and current clinical judgment was that they had autism. One child did not receive an Autism Diagnostic Interview-Revised (ADI-R) interview but was positive for AD on all remaining measures, and his clinical diagnosis was also AD. Therefore, 8 children with FXS (33% of the FXS group) were considered to have AD. Sixteen children with FXS failed to meet study criteria for AD. Of these, 12 did not meet criteria for AD on any of the three diagnostic systems in use, nor did clinicians consider them to have AD. The remaining 4 children met ADI-R criteria for AD, met pervasive developmental disorder (PDD) but not autism cutoffs on the Autism Diagnostic Observation Schedule-Generic (ADOS-G), did not meet Diagnostic and Statistical Manual-4th Edition (DSM-IV) criteria, nor did clinicians diagnose the children with AD. In these four cases, the clinical diagnosis was taken as the child's diagnosis in this study. Two FXS subgroups were thus identified: a FXS group without autism (FXS-NAD), and a FXS group with autism (FXS-AD).

The second step was to determine whether these two groups were truly distinct groups within FXS or simply two ends of an autism-symptom continuum arbitrarily split into two groups. To examine this, the frequency distributions for the two groups were plotted on the same axes for (1) each of the ADI-R algorithm scale scores and (2) each of the ADOS-G algorithm scores. A representative example of these distributions is presented in Figure 1. As can be seen, there is little overlap of the two distributions, suggesting that the two groups are distinct in terms of their autism symptoms. This pattern held across the various ADI-R and ADOS-G algorithm scales.

To examine the autism symptom pattern within FXS in more detail, profile analyses were completed, comparing the profiles of the autistic and nonautistic FXS groups to the developmentally delayed (DD) and AD groups on the three algorithm scales of the ADOS-G and the three algorithm scales of the ADI-R. Before this, however, the variables were examined to determine how well they met the statistical assumptions underlying a multivariate analysis of variance (MANOVA). The assumptions were adequately met across groups and variables. MANOVAs were used to determine whether the profiles of these two sets of variables differed across groups. The omnibus test for the ADOS-G variables was significant (Wilks' lambda = .244; $F = 23.53$; $df = 6,138$; $p < .001$). Comparisons across the profiles indicate that the profiles of the DD and FXS-NAD groups did not differ significantly from one another nor did the profiles of the AD and FXS-AD groups. However, the profiles of the DD and FXS-NAD groups differed significantly from those of both the AD and the FXS-AD groups. The F values ranged from 54.33 to 129.37, all of which were significant at $p < .001$.

Two children, one with AD and one with FXS-AD, did not have ADI-R data. Therefore, the numbers of subjects in the groups for the profile analysis of the ADI-R variables are 23, 26, 16, and 7 for the DD, AD, FXS-NAD, and FXS-AD groups, respectively. The omnibus test of the

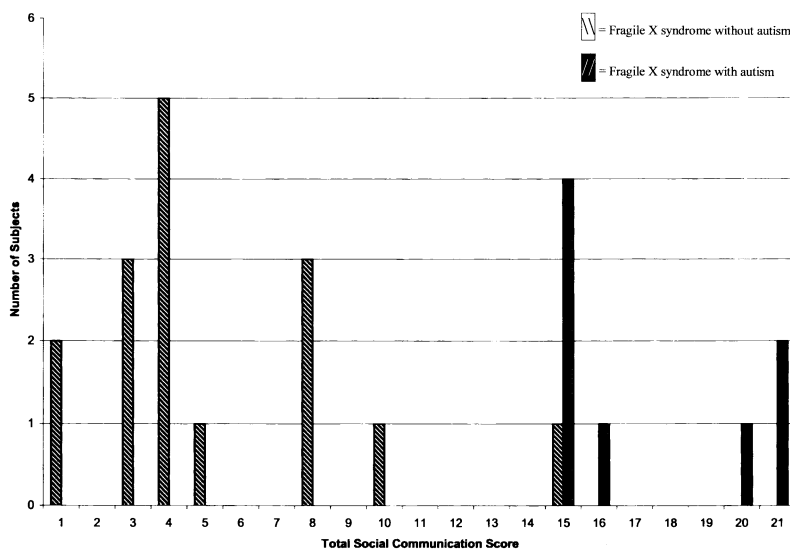


FIGURE 1. Autism Diagnostic Observation Schedule-Generic: Combined Communication and Social Scale scores for individuals with fragile X.

three ADI-R scales was significant (Wilks' lambda = .336, $F = 10.09$, $df = 9,161$, $p < .001$). Contrasts across the profiles indicate that the profile of the DD group differs significantly from those of the other three groups, (F values ranging from 10.57 to 107.18; p values all less than or equal to .002), that the FXS-NAD group also differed significantly from the AD and FXS-AD groups (F values of 35.95 and 12.40, respectively; both p values $< .001$) which did not differ from one another.

Follow-up analyses of variance (ANOVAs) for each of the six ADI-R and ADOS-G variables were all significant. The F values ranged from 7.84 to 66.64 ($df = 3,70$, for the ADOS-G variables; $df = 3,68$ for the ADI-R variables). They were all significant at $p < .001$.

Single degree-of-freedom contrasts were completed to pinpoint the source of the differences between the groups on the six variables. For each variable, a family-wise error rate of $\alpha = .05$ was set, and a Bonferroni correction was used to maintain the family-wise error rate for each set of contrasts at .05. The AD and FXS-AD groups did not differ significantly on any of the six variables. In contrast, the AD and nonautistic FXS groups differed on five of the six variables (only the Repetitive Behaviors and Stereotyped Patterns scale of the ADI-R was nonsignificant), and the AD and DD groups differed on all six variables. Similarly, the autistic FXS group differed significantly from the nonautistic FXS group on five of the six variables (again, only the Repetitive Behaviors and Stereotyped Patterns scale of the ADI-R was nonsignificant) and from the DD group on all six variables. Finally, the nonautistic FXS group did not differ significantly from the DD group on any of the six variables. However, the F value for the Repetitive Behaviors and Stereotyped Patterns scale of the ADI-R approached significance ($p < .06$). A plot of the profiles of each of the four groups is presented in Figure 2. This figure illustrates clearly the similarity of the AD and FXS-AD groups on the two autism measures. Further, despite somewhat higher scores on the ADI-R, the FXS-NAD

group shows an autism symptom pattern that is similar to that of the DD group.

The next hypothesis concerned the possible relationship between developmental level and the presence of symptoms of autism. To test this hypothesis, two additional profile analyses were completed. The dependent measures for the first included the age equivalents on the five scales of the Mullen Scales of Early Learning (MSEL). Several children did not have complete data on the MSEL and, therefore, were not included in the analysis. The numbers of subjects in the groups were 22, 27, 14, and 6 for the DD, AD, FXS-NAD, and FXS-AD groups, respectively. The dependent variables for the second profile analysis included the age equivalents on the four scales of the Vineland. Again, a number of children did not have complete data on this measure and were excluded from the analysis. The numbers of subjects sampled in the groups were 23, 26, 13, and 6 for the DD, AD, FXS-NAD, and FXS-AD groups, respectively. All developmental variables were examined to determine if they met the statistical assumptions underlying a MANOVA. The assumptions were adequately met across groups and variables.

A MANOVA was used to determine whether the profiles of the MSEL variables differed across groups. The omnibus test was significant (Wilks lambda = .345; $F = 5.21$; $df = 15,166$; $p < .001$). Contrasts of the four profiles indicated that the profiles of the DD, AD, and FXS-NAD groups did not differ significantly from one another. However, the profile of the FXS-AD group differed significantly from each of the other three groups. The F values ranged from 6.22 to 10.01; all were significant at $p < .007$.

One-way ANOVAs on each of the MSEL variables, followed by single degree-of-freedom contrasts of the significant variables, were completed to pinpoint which areas distinguished the FXS-AD group from the other three groups. All scales except the Visual Reception scale were significant (F values ranged from 4.22 to 6.69; $df = 3,64$;

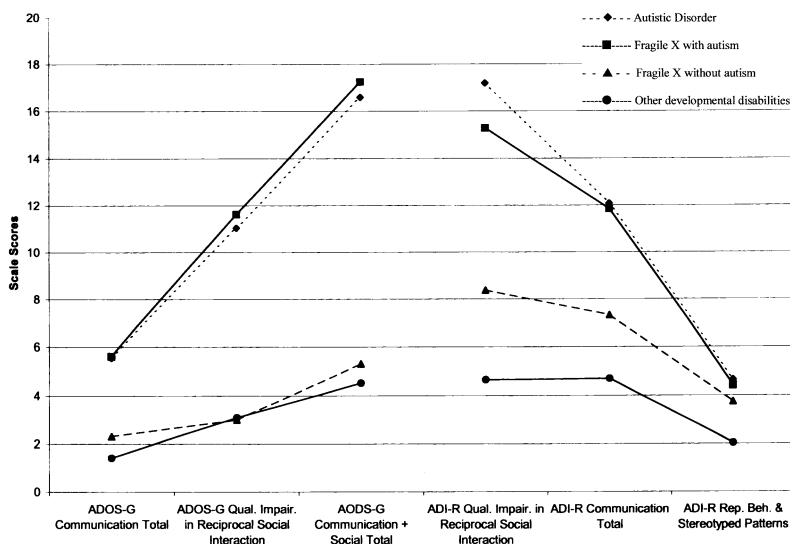


FIGURE 2. Profiles of scale scores from the Autism Diagnostic Observation Schedule-Generic (ADOS-G) and the Autism Diagnostic Interview-Revised (ADI-R) for the four diagnostic groups. Qual. Impair., Qualitative Impairment; Rep. Beh., Repetitive Behavior.

Table 2. Means and Standard Deviations for the Adaptive and Developmental Measures

Variables	Groups (n)	Mean	SD
Mullen Gross Motor Age Equivalent	AD (27)	24.15	4.59
	FXS AD (6)	17.17	2.14
	FXS NAD (15)	20.08	5.50
Mullen Visual Reception Age Equivalent	DD (22)	20.55	5.42
	AD (27)	24.85	8.62
	FXS AD (6)	16.83	4.92
Mullen Fine Motor Age Equivalent	FXS NAD (13)	23.38	6.81
	DD (22)	23.64	7.20
	AD (27)	22.85	4.69
Mullen Expressive Language Age Equivalent	FXS AD (6)	16.50	5.09
	FXS NAD (13)	19.85	5.70
	DD (22)	23.14	4.64
Mullen Receptive Language Age Equivalent	AD (27)	16.30	6.69
	FXS AD (6)	7.67	3.67
	FXS NAD (13)	16.15	9.19
Vineland Communication Age Equivalent	DD (22)	19.27	6.68
	AD (27)	16.19	7.19
	FXS AD (6)	12.67	3.27
Vineland Daily Living Age Equivalent	FXS NAD (13)	24.08	9.22
	DD (22)	22.95	7.32
	AD (26)	12.81	4.65
Vineland Socialization Age Equivalent	FXS AD (6)	10.67	2.58
	FXS NAD (13)	17.62	5.95
	DD (23)	18.26	4.35
Vineland Motor Skills Age Equivalent	AD (26)	16.73	3.09
	FXS AD (6)	15.00	3.79
	FXS NAD (13)	18.54	6.29
Vineland Communication Age Equivalent	DD (23)	18.74	4.11
	AD (26)	11.50	3.57
	FXS AD(6)	12.50	2.74
Vineland Daily Living Age Equivalent	FXS NAD (13)	15.46	7.32
	DD (23)	18.61	4.65
	AD (26)	22.62	3.86
Vineland Socialization Age Equivalent	FXS AD (6)	18.17	3.19
	FXS NAD (13)	19.46	4.50
	DD (23)	21.78	5.17

AD, autistic disorder; FXS NAD, fragile X syndrome without autism; FXS AD, fragile X syndrome with autism; DD, other developmental disabilities.

p values ranged from .001 to .009). For the single degree-of-freedom contrasts, a family-wise error rate of $\alpha = .05$ was set for each variable, and a Bonferroni correction was used to maintain the family-wise error rate for each set of contrasts at .05. The single degree-of-freedom contrasts indicated (1) that the DD and FXS-AD groups differed on the Fine Motor, Receptive Language, and Expressive Language scales, (2) that the AD and FXS-AD groups differed significantly on the Gross Motor, Fine Motor, and Expressive Language scales, and (3) that the FXS-NAD and FXS-AD groups differed on the Receptive Language scale.

Finally, a MANOVA of the Vineland variables was completed. The omnibus test was significant (Wilks lambda = .457; $F = 4.64$; $df = 12,162$; $p < .001$). Profile contrasts indicate that the DD group differed significantly from both the AD and FXS-AD groups ($F_s = 10.41$ and 9.54 , respectively; $df = 1,64$; $ps < .003$) but not from the FXS-NAD group. The profile of the AD group did not differ significantly from either FXS group. However, the profiles

of the two FXS groups did differ significantly from one another ($F = 4.03$; $df = 1,64$; $p < .05$).

One-way ANOVAs on each of the Vineland variables, followed by single degree-of-freedom contrasts of the significant variables, were completed to pinpoint developmental areas that distinguish the various groups from one another. The Communication and Social scales were significant ($F_s = 8.46$ and 9.46 , respectively; $df = 3,64$; $ps < .001$), and the Motor scale approached significance ($F = 2.60$, $p < .06$). For the single degree-of-freedom contrasts, a family-wise error rate of $\alpha = .05$ was set for each variable, and a Bonferroni correction was used to maintain the family-wise error rate for each set of contrasts at .05. The single degree-of-freedom contrasts indicated (1) that the DD and two autistic groups differed on both Communication and Social scales, (2) the FXS-NAD group differed significantly from the two autistic groups on the Communication scale, and (3) the AD and FXS-AD groups did not differ significantly on either scale. The means and standard deviations for all the developmental variables are presented in Table 2. It should be noted that the standard deviations on these variables are large in some cases, making the interpretation of these results less straightforward. However, despite this, it is clear that the FXS-AD group is, developmentally, the lowest functioning group.

DISCUSSION

The purpose of this study was to compare the symptoms of autism in very young children with fragile X syndrome (FXS) to those with idiopathic autism and with other developmental disorders. The hypotheses were that the children with FXS would demonstrate a unique pattern of behavior compared with the other two groups and would display more symptoms of autism than the developmentally delayed (DD) group, but with a unique pattern compared to the group with idiopathic autism. These hypotheses were partially supported by the findings. As a group, the children with FXS shared an increased rate of autism symptoms compared to the DD group. However, they did not demonstrate a unique pattern of behavior across the measures used in this study, compared with the children with idiopathic autism. Instead, two subgroups emerged, in the FXS group. One FXS subgroup performed virtually identically to the nonautistic DD group on all autism measures and similarly on the developmental measures. These children did not have the social/communicative characteristics of autism. The second FXS group performed virtually identically to the group with idiopathic autism on all autism measures. However, this group was developmentally lower functioning than the autistic disorder (AD) group, particularly in terms of their motor functioning, both fine and gross motor.

The third hypothesis concerned the relationship of the severity of mental retardation to symptoms of autism in FXS. This hypothesis was more difficult to test, in part because of the uneven cognitive profile of children with AD. Profile analyses of a number of developmental variables did reveal a developmentally more immature pattern associated with autism in the FXS group. Although lower functioning, the fragile X syndrome with autistic

disorder (FXS-AD) group showed a similar pattern of developmental scores to the AD group, with relatively higher functioning in the motor and daily living domains compared with their social and communication skills. In contrast, the DD and fragile X syndrome without autistic disorder (FXS-NAD) groups showed more even developmental profiles. Thus, this hypothesis was supported.

The children with FXS in this study represent the youngest group yet examined for symptoms of autism. The young age of the FXS group raises the question of whether the increased rate of autism in that group is specifically related to their ages and early developmental levels, and whether, as they mature, they will demonstrate a reduction in autism symptoms. If that is the case, then follow-up assessment should reveal decreases in symptoms of autism in the FXS-AD group. However, if the issue is more related to IQ deficits (as a marker of more severe central nervous system involvement) than to developmental levels, we would not expect to see changes in autism symptoms with development. The data collected when these subjects are followed up in 2 years will, we hope, provide some answers to these questions.

The subgroup of children with both FXS and autism is of significant size (33%) and represents a larger percentage of children with autism in the population with FXS than had previous studies, which assessed older children with FXS.^{10,12,27,28} However, the recruitment of those children from only a few FXS centers may have affected findings. Because of the relatively small number of children with fragile X seen in this study and the very young ages involved, we do not know whether these results are representative of the population of preschoolers with fragile X. Thus, these findings should be interpreted with considerable caution until replicated. However, the findings that some children with fragile X have full-blown autism and that some have very few symptoms of autism have previously been reported in other studies of other age groups. It is the proportion with autism in this age range that needs replication. In addition, the FXS sample contained only a single female. The current results may not hold for both genders. The large standard deviations for a number of the variables also reduce the interpretability of the results. An additional weakness involved DNA testing. Although all of the children with FXS had DNA testing to diagnose their condition, it was not clear from the medical records of the other two groups whether physicians ruled out FXS based on clinical features or on DNA testing. Thus, the current findings should be considered preliminary and the study replicated with a larger sample and with DNA testing carried out on all children in all groups. Based on these preliminary studies, we expect that the percentage of

these preschool FXS children with autism will decrease with age and intensive intervention.

A possible explanation for the similarity between the AD group and the FXS-AD group can be drawn from recent findings concerning the genetics of autism. The strong similarities between these two groups suggest the hypothesis that those with FXS and autism have additional background alleles that may have a synergistic effect with the fragile X mental retardation 1 gene (FMR1) mutation to create autism. This hypothesis is supported by the work of Bailey et al²⁹ that followed 13 children with FXS, 13 children with autism, and 13 children with FXS and autism. The children with FXS and autism had the lowest IQ, and in a subsequent study³⁰ the FMR1 protein levels (FMRP) did not correlate with autism features and accounted for less variance in the developmental level than did autistic behavior. Bailey et al³⁰ also suggested that autism in FXS may come from a second hit or background genes, which predispose to autism, as was also suggested by Feinstein and Reiss.¹¹ Further genetic testing in research protocols of these two subgroups, FXS with and without autism, is warranted and might be helpful for identifying alleles associated with autism. It may be easier to identify a few of these alleles when they are combined with the FMR1 mutation, because FXS may dramatically predispose a patient to autism and only one to three additional alleles may be needed to create autism, compared with an idiopathic autism, in which there may be greater than 10 alleles predisposing to autism.²

In summary, there was little evidence for a unique behavioral phenotype related to FXS on these autism-related measures. This may, in part, be due to the immaturity of this group of children, who were chronologically only 2 to 3 years of age and developmentally only 12 to 24 months of age. With more developmental maturity, behavior may well become more differentiated and more unique. However, it may also be that uniqueness is associated with other behavioral indices than those measured here. Given the descriptions and theorizing that has been done concerning the sensory, arousal, and temperamental characteristics of fragile X syndrome, those may be the most appropriate behavioral arenas in which to try to map out a unique FXS behavioral phenotype.

Acknowledgments. This work was partially supported by National Institute of Child Health and Human Development (NICHD) Grant PO1 HD35468. Dr. Rogers was also supported by Maternal and Child Health Bureau Grant (MCHB) 2T73MC00011-04 and by the Administration on Developmental Disabilities Grant 90DD0414. Dr. Hagerman was also supported by NICHD Grant HD36071 and by MCHB Grant MCJ-089413. The ongoing help of the Developmental Psychobiology Research Group is gratefully acknowledged, as is the assistance of Ms. Teneke Warren and Ms. Cynthia Uhlhorn with manuscript preparation.

REFERENCES

1. Hagerman R: Fragile X syndrome, in Hagerman R (ed): *Neurodevelopmental Disorders: Diagnosis and Treatment*. New York, NY, Oxford University Press, 1999, pp 61–132
2. Le Couteur A, Bailey A, Goode S, et al: A broader phenotype of autism: The clinical spectrum of twins. *J Child Psychol Psychiatry* 37:785–802, 1996

3. Cook EH: Genetics of autism. *MRDD Res Rev* 4:113–120, 1998
4. Dykens E, Volkmar F: Medical conditions associated with autism, in Cohen D, Volkmar F (ed): *Handbook of Autism and Pervasive Developmental Disorders*. New York, NY, Wiley, 1997, pp 388–410
5. Gillberg C: Chromosomal disorders and autism. *J Autism Dev Disord* 28:415–425, 1998
6. Bailey A, Phillips W, Rutter M: Autism: Towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. *J Child Psychol Psychiatry* 37:89–126, 1996
7. Brown WT, Jenkins EC, Cohen IL, et al: Fragile X and autism: A multicenter survey. *Am J Med Genet* 23:341–352, 1986
8. Hagerman R: Physical and behavioral phenotype, in Hagerman R, Cronister A (ed): *Fragile X Syndrome: Diagnosis, Treatment, and Research*, 2nd ed. New York, NY, Oxford University Press, 1996, pp 3–87
9. Hagerman RJ, Jackson AW 3rd, Levitas A, Rimland B, Braden M: An analysis of autism in fifty males with the fragile X syndrome. *Am J Med Genet* 23:359–374, 1986
10. Bailey DB Jr, Mesibov GB, Hatton DD, Clark RD, Roberts JE, Mayhew L: Autistic behavior in young boys with Fragile X syndrome. *J Autism Dev Disord* 28:499–508, 1998
11. Feinstein C, Reiss AL: Autism: The point of view from fragile X studies. *J Autism Dev Disord* 28:393–405, 1998
12. Baumgardner TL, Reiss AL, Freund LS, Abrams MT: Specification of the neurobehavioral phenotype in males with fragile X syndrome. *Pediatrics* 95:744–752, 1995
13. Kerby DS, Dawson BL: Autistic features, personality, and adaptive behavior in males with the fragile X syndrome and no autism. *Am J Ment Retard* 98:455–462, 1994
14. Simon EW, Finucane BM: Facial emotion identification in males with fragile X syndrome. *Am J Med Genet* 67:77–80, 1996
15. Turk J, Cornish KM: Face recognition and emotion perception in boys with fragile-X syndrome. *J Intellect Disabil Res* 42:490–499, 1998
16. Mazzocco MM, Pennington BF, Hagerman R: Social cognition skills among females with fragile X. *J Autism Dev Disord* 24:473–485, 1994
17. Cohen IL: Behavioral profiles of autistic and nonautistic fragile X males. *Dev Brain Dysfunct* 12:303–308, 1995
18. Dawson G, Lewy A: Arousal, attention, and the socioemotional impairments of individuals with autism, in Dawson G (ed): *Autism: Nature, Diagnosis, and Treatment*. New York, NY, Guilford Press, 1989, pp 49–74
19. Cohen IL, Vietze PM, Sudhalter V, Jenkins EC, Brown WT: Parent-child dyadic gaze patterns in fragile X males and in non-fragile X males with autistic disorder. *J Child Psychol Psychiatry* 30:845–856, 1989
20. Cohen IL, Vietze PM, Sudhalter V, Jenkins EC, Brown WT: Effects of age and communication level on eye contact in fragile X males and non-fragile X autistic males. *Am J Med Genet* 38:498–502, 1991
21. Sudhalter V, Cohen IL, Silverman W, Wolf-Schein EG: Conversational analyses of males with fragile X, Down syndrome, and autism: Comparison of the emergence of deviant language. *Am J Ment Retard* 94:431–441, 1990
22. Belser RC, Sudhalter V: Arousal difficulties in males with fragile X syndrome: A preliminary report. *Dev Brain Dysfunct* 8:270–279, 1995
23. Miller LJ, McIntosh DN, McGrath J, et al: Electrodermal responses to sensory stimuli in individuals with fragile X syndrome: A preliminary report. *Am J Med Genet* 83:268–279, 1999
24. Roberts JE: Bio-behavioral regulations in boys with fragile X. Presented at the 31st annual Gatlinberg Conference on Research and Theory in Mental Retardation and Developmental Disabilities, Charleston, SC, March 12, 1998
25. Mazzocco MM, Kates WR, Baumgardner TL, Freund LS, Reiss AL: Autistic behaviors among girls with fragile X syndrome. *J Autism Dev Disord* 27:415–435, 1997
26. Schopler E, Reichler RJ, Renner BR: *The Childhood Autism Rating Scale*. Los Angeles, CA, Western Psychological Services, 1988
27. Reiss AL, Freund L: Behavioral phenotype of fragile X syndrome: DSM-III-R autistic behavior in male children. *Am J Med Genet* 43:35–46, 1992
28. Turk J, Graham P: Fragile X syndrome, autism and autistic features. *Autism* 1:175–197, 1997
29. Bailey DB Jr, Hatton DD, Mesibov G, Ament N, Skinner M: Early development, temperament, and functional impairment in autism and fragile X syndrome. *J Autism Dev Disord* 30:49–59, 2000
30. Bailey DB, Hatton DD, Skinner M, Mesibov G: Autistic behavior, FMR1 protein, and developmental trajectories in young males with fragile X syndrome. *J Autism Dev Disord* 31:165–174, 2001
31. Lord C, Rutter M, Le Couteur A: Autism Diagnostic Interview-Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 24:659–685, 1994
32. Lord C, Rutter M, DiLavore P, et al: *Autism Diagnostic Observation Schedule-WPS Edition*. Los Angeles, CA, Western Psychological Services, 1999
33. Cox A, Klein K, Charman T, et al: Autism spectrum disorders at 20 and 42 months of age: Stability of clinical and ADI-R diagnosis. *J Child Psychol Psychiatry* 40:719–732, 1999
34. Mullen E: *Mullen Scales of Early Learning*. Cranston, RI, T.O.T.A.L. Child, Inc., 1989
35. Sparrow SS, Balla DA, Cicchetti D: *Vineland Adaptive Behavior Scales*. Circle Pines, MN, American Guidance Service, 1984