Symptoms of Autism in Males with Fragile X Syndrome: A Comparison to Nonsyndromic ASD using Current ADI-R Scores

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Abstract

Symptoms of autism are frequent in males with fragile X syndrome (FXS), but it is not clear whether symptom profiles differ from those of nonsyndromic ASD. Using individual item scores from the Autism Diagnostic Inventory-Revised (ADI-R), we examined which current symptoms of autism differed in boys with FXS relative to same-aged boys diagnosed with nonsyndromic ASD. In addition, different subsamples of participants were matched on autism diagnostic status and severity of autism symptoms. Between-group comparisons revealed that boys with FXS showed significantly less impairment in Social Smiling than did age-, diagnostic, and severity-matched boys with nonsyndromic ASD. Severity-matched boys with FXS showed more impairment in Complex Mannerisms than did boys with nonsyndromic ASD. Behavioral differences between FXS and nonsyndromic ASD may be of theoretical importance in understanding the causes and correlates of ASD in FXS and in developing and implementing appropriate treatments.

Considerable variability is observed in the behavioral phenotype of males with fragile X syndrome (FXS) (Hessl et al., 2001; Hagerman, 2002), the leading inherited cause of intellectual impairment. One dimension of this variability is the presence and degree of autism symptoms. Autistic-like behaviors observed in FXS are secondary to an identified genetic etiology. By comparison, nonsyndromic (or idiopathic) ASD is a behaviorally defined disorder for which genetic causes are suspected but not clearly established (Caglayan, 2010). The goal of the present study was to identify similarities and differences in symptoms of autism reported for boys with FXS relative to same-aged boys with nonsyndromic ASD by comparing current item scores obtained from the Autism Diagnostic Interview-Revised (Rutter, LeCouteur, & Lord, 2008). Results of such a comparison can have important implications for the cross-syndrome application of behavioral and pharmacological intervention approaches.

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The Behavioral Phenotype of Fragile X Syndrome

FXS results from expansion of a repeated CGG nucleotide sequence in the FMR1 gene, located on the X chromosome (Kaufmann & Reiss, 1999). This expansion causes methylation and transcriptional silencing of the FMR1 gene, resulting in a reduction or absence of its protein product, FMRP (Verkerk et al., 1991). FMRP is critical for the regulation of biochemical processes involved in synaptic maturation and experience-dependent learning (Bhakar, Dolen, & Bear, 2012). As is the case for most X-linked disorders, males with FXS are, on average, more impaired than females, who have one X chromosome that carries a healthy FMR1 allele (Gallagher & Hallahan, 2012).

Although variable in its presentation across individuals, the behavioral phenotype of males with FXS is typically characterized by moderate to severe cognitive delays. Virtually all males with the FXS full mutation have IQ scores in the range of intellectual disability (IQ<70; Hessl et al., 2009). Additionally, FXS is associated with many behavioral challenges, including hyperactivity, impulsivity and inattention (Cornish, Scerif, & Karmiloff-Smith, 2007; Munir, Cornish, & Wilder, 2000; Turk, 1998), aggression and self-injury (Symons, Clark, Hatton, Skinner, & Bailey, 2003), social anxiety (Cordeiro, Ballinger, Hagerman, & Hessl, 2011; Merenstein, Sobesky, Taylor, Riddle, Tran, & Hagerman, 1996), unusual speech patterns and language delay (Abbeduto, Kover, & Brady, 2007; Roberts, Martin, Moskowitz, Harris, Foreman, Nelson, 2007a), and symptoms of autism (Clifford, Dissanayake, Bui, Huggins, Taylor, & Loesch, 2007; Kau, Reider, Payne, Meyer, & Fruend, 2000).

Symptoms of Autism in FXS

More than 90% of males with FXS display behaviors that are similar to those observed in individuals with nonsyndromic ASD (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000; Feinstein & Reiss, 1998). Autistic-like behaviors in FXS include perseverative and noncontingent speech (Martin, Roberts, Helm-Estabrooks, Sideris, Vanderbilt, & Moskowitz, 2012), motor stereotypies such as hand flapping, and poor eye contact (Hagerman, 1999; Merenstein et al., 1996; Roberts, Weisenfeld, Hatton, Heath, 2007). When using gold standard diagnostic instruments, as many as 60% of males with FXS display behaviors that are frequent and severe enough to warrant a comorbid diagnosis of an ASD (Clifford et al., 2007; Harris et al., 2008; McDuffie et al., 2010)

There is not yet consensus, however, as to whether comorbid ASD in FXS represents a categorically distinct and qualitatively different disorder within the FXS behavioral phenotype or whether this diagnosis represents the more severe end of a continuum of impairment that covaries with nonverbal cognition and other behavioral characteristics, such as hyperarousal, and social anxiety (Clifford et al., 2007; Hall, Lightbody, Hirt, Rezvani, Reiss, 2010; Moss & Howlin, 2009; Wolff, Bodfish, Hazlett, Lightbody, Reiss, & Piven, 2012). Those who would support the former conceptualization are likely to view symptoms of autism in FXS as the final common pathway of the same underlying neurological and psychological dysfunctions as in nonsyndromic ASD. Those who would support the latter conceptualization are likely to question whether overt behavioral symptoms of autism are qualitatively the same in individuals with FXS relative to individuals with nonsyndromic ASD and whether similar behavioral symptoms may have arisen from different neural substrates or reflect different underlying psychological impairments (Hall et al., 2010; Harris et al., 2008). In support of the latter view, recent neuroimaging studies suggest that potentially important structural and functional differences exist between individuals with FXS and those with nonsyndromic ASD (for review, see Gallagher & Hallahan, 2012). Findings of differences in neural topography and circuitry (Dalton, Holsen, Abbeduto, &

Davidson, 2008; Hazelett et al., 2009, Hoeft et al., 2010) highlight the importance of exploring potential differences in behavior which may exist.

There is some evidence, albeit limited, that individuals with comorbid FXS and ASD differ in their symptom profiles from those with FXS only, with the former being similar in many respects to individuals with nonsyndromic autism (Bailey et al., 1998; Demark et. al., 2003; Rogers, Wehner, & Hagerman, 2001). For example, Rogers et al. (2001) found no significant differences in ADI-R and ADOS total scores or domain scores between a group of 21- to 48-month olds with comorbid FXS and ASD and a comparison group of similarly aged children with nonsyndromic ASD. Moreover, with the exception of the Restricted and Repetitive Behavior domain of the ADI-R, Rogers et al. found that both groups of participants with ASD differed significantly from a FXS-only group on all ADI-R and ADOS domains examined. Because the between-group analyses compared only overall domain scores from the ADOS and ADI-R, however, the Rogers study does not address the question of whether more specific behavioral symptoms are the same across the two disorders. In the current study, we examined individual ADI-R items to provide a more nuanced descriptions of the specific behavioral symptoms that may differentiate the groups.

More recent studies have delved more deeply into the autism symptom profile associated with FXS; however, these studies have not typically included a comparison group of individuals with nonsyndromic ASD. In many of these studies, participants with FXS have been dichotomized into groups based upon comorbid autism status. Kaufmann et al. (2004) used the ADI-R to characterize the behavioral profiles of 56 mostly nonverbal young boys with FXS, ages 3 to 8 years. Although these authors characterized the distribution of ADI-R scores as a continuum of impairment within this sample of participants, they also identified performance on the Reciprocal Social Interaction domain as the primary difference between individuals with FXS + ASD and individuals with FXS only. The subdomains representing Failure to Develop Peer Relationships and Lack of Social Emotional Reciprocity were more impaired for individuals with comorbid diagnoses and accounted for almost 75% of the variance in predicting total ADI-R scores. In a subsequent three-year longitudinal examination of the same sample of boys, Hernandez, Feinberg, Vaurio, Passanante, Thompson, and Kaufmann (2009) reported that the boys with comorbid FXS and ASD had significantly lower nonverbal IQ scores than the boys with FXS, but only at the first two yearly time points. Additionally, Hernandez et al. reported general improvement in the behavioral profile of boys with comorbid FXS and ASD over time, and general worsening of the profile of boys with only FXS, leading to less differentiation between the two groups at the final time point.

McDuffie and colleagues (2010) examined lifetime and current ADI-R profiles for a group of 50 older children and adolescents (35 boys, 15 girls) with FXS. This sample of participants differed from that of Kaufmann and colleagues (2004, 2007) in that the McDuffie et al. sample included older and verbal participants of both genders. In contrast to the findings of Kaufmann et al. (2004) and Hernandez et al. (2009), McDuffie et al. (2010) found that diagnostic algorithm scores for Reciprocal Social Interaction did not differ between participants with FXS only and those with comorbid FXS + ASD after controlling for nonverbal IQ. There were significant between-group differences, however, for diagnostic algorithm items in the Communication domain (i.e., pointing, nodding, head shaking, imitation of actions, imitative social play, and stereotyped utterances) and the Restricted Interests and Repetitive Behaviors domain (compulsions and rituals, repetitive object use, and circumscribed interests). For current ADI-R items, McDuffie et al. found significant differences in Communication (i.e., stereotyped utterances, reciprocal conversation) and Restricted Interests and Repetitive Behaviors (i.e., circumscribed interests, compulsions and rituals, unusual sensory interests). The lack of significant between-group differences for

behaviors representing social reciprocity raises the possibility that an autism diagnosis in FXS represents a fundamentally different set of core impairments than is found in nonsyndromic ASD.

To further explore the issue of how individuals with FXS differ from individuals with nonsyndromic ASD, Dissanayake, Bui, Bulhak-Paterson, Huggins, & Loesch, (2009) conducted a between-group comparison of ADOS raw scores for the Reciprocal Social Interaction and Communication domains. All participants with nonsyndromic ASD as well as those with FXS met criterion for the more stringent classification of Autistic Disorder (AD) on the ADOS, rather than the more inclusive category of ASD. Participants of both genders were included in the study sample and spanned a wide age range (5 – 51 years). Participants with FXS had significantly higher raw scores on the ADOS Reciprocal Social Interaction domain before controlling for full scale IQ and significantly higher raw scores on the ADOS Communication domain after controlling for full scale IQ. These results, which suggest that communication challenges may contribute to exceeding diagnostic cutoffs for autism in individuals with FXS, must be interpreted cautiously because of the broad range of participant ages and the failure to include participants who had milder symptoms of autism (i.e., ASD) in either diagnostic group. Moreover, raw scores from the ADOS are known to be strongly associated with participant characteristics such as IQ and expressive language level (Gotham, Pickles, & Lord, 2009), making the comparison of raw scores, rather than calibrated severity scores, a less than optimal analysis approach. Finally, failure to examine ADOS item-level differences makes it difficult to determine whether similar domain scores masked more nuanced between-group differences in performance on individual ADOS items.

Hall and colleagues (2010) also examined profiles of autistic symptoms on the ADOS for a group of 37 males with FXS ranging in age from 5 to 25 years; ADOS item scores for participants with FXS were compared with scores from a reference sample of individuals with autism included in the ADOS manual. Youth with FXS received significantly lower (i.e., less impaired) scores than the reference sample for 8 of 13 algorithm items in the Reciprocal Social Interaction domain and for 4 of 9 algorithm items in the Communication domain. No significant differences were observed for repetitive behaviors. Results from the Hall et al. study, are difficult to interpret, however, because the sample was not matched to the ADOS reference sample on important characteristics, such as age and IQ and the examiner, of course, differed for the participants with FXS and those in the reference sample.

Wolff et al (2012) compared profiles of social communication in 38 young boys with FXS to 28 age-matched boys with nonsyndromic ASD using items from module 1 of the ADOS, which is appropriate for children who range from producing no spoken words to those who produce 1–2 word combinations. ADOS Social Communication items were categorized according to whether they pressed for initiations or responses. Within the category representing social initiations, boys with comorbid FXS and ASD were significantly less impaired in gaze integration and quality of social interactions than were age-matched boys with nonsyndromic ASD. Within the category representing social responses, boys with comorbid FXS and ASD were significantly less impaired in social smiling, facial expressions, and response to joint attention than age-matched comparison boys.

Given that cognitive level was significantly lower in the group of boys with FXS, Wolff et al. extended their original analyses to a subset of children with nonsyndromic ASD who were matched to 23 participants with comorbid FXS and ASD on the basis of chronological age and IQ scores. For this matched sample, all of the social response items from the previous analyses continued to be significantly less impaired for boys with comorbid FXS

and ASD than for those with nonsyndromic ASD. Based upon these findings, Wolff et al. suggested that some degree of responsiveness to the social bids of others seems to differentiate children with comorbid FXS and ASD from those with nonsyndromic ASD.

In summary, the behavioral symptoms that may distinguish individuals with FXS from individuals with nonsyndromic ASD have yet to be thoroughly investigated. Previous studies have used domain-level scores that mask the identification of specific behavioral symptoms, have included participants with a wide range of ages and developmental levels, have not controlled for differences in cognitive functioning, and, with the exception of Wolff et al. (2012), have not directly compared individuals with FXS to those diagnosed with nonsyndromic ASD (e.g., Brock & Hatton, 2010; Hernandez et al., 2009; Kaufmann et al., 2004; McDuffie et al., 2010; Roberts, et al., 2009). The question of how the behavioral symptoms of autism are expressed in FXS relative to nonsyndromic ASD is particularly pressing given that behavioral and pharmacological interventions are currently being developed to target specific behavioral profiles and neurological mechanisms in individuals with intellectual disabilities.

Research Question

As a step toward clarifying the conceptualization of ASD in FXS, the present study was designed to examine current symptoms of autism in boys with FXS relative to same-aged boys diagnosed with an ASD for whom a genetic diagnosis of FXS had been ruled out. To overcome some of the limitations of previous studies, the current study included participants who were within a narrow range of chronological ages, employed a comparison group of boys with nonsyndromic ASD, utilized individual ADI-R item scores as the metric of current autism symptoms, and controlled for diagnostic classification and autism symptom severity through the use of group-wise matching of participants. We hypothesized that, relative to boys with nonsyndromic ASD, boys with FXS would be significantly less impaired in specific behaviors that represent the core social impairments of ASD. The following research question was addressed: Are significant between-group differences in current symptoms of ASD observed in boys with FXS relative to boys with nonsyndromic ASD when participants are matched on chronological age, autism diagnostic status, and severity of autism affectedness?

Method

Participants

Participants were drawn from a larger longitudinal study examining language learning in males with FXS (n=57) and males with nonsyndromic ASD (n=61). Participants were recruited nationally and were tested at one of two university sites (one in the Midwest and one on the West coast). The study was approved by the IRBs of the respective universities. Inclusion in the larger study required participants in both diagnostic groups to meet the following criteria according to parent report: (a) between 4 and 10 years of age; (b) English was the primary language spoken at home; (c) the child could comply with simple instructions (e.g., “Give me the ball”); (d) spoken language was the child’s primary means of communication; (e) the child had used approximately 10 different spoken words spontaneously within the month preceding the Time 1 visit; and (f) the child had no uncorrected motor or sensory impairments that would preclude participation. In addition, participants with FXS had a confirmed *FMR1* full mutation (i.e., >200 CGG repeats, with or without mosaicism). Participants with nonsyndromic ASD had (a) a community diagnosis of an ASD and (b) negative results on a genetic test for FXS. The measures reported in the current study were collected at Time 1 for the larger study.

Measures

Nonverbal cognition—The Brief IQ Screener of the Leiter International Performance Scales - Revised (Leiter; Roid & Miller, 1997) was administered to provide a measure of nonverbal cognitive ability. The Brief IQ subtests are: Figure Ground, Form Completion, Sequential Order, and Repeated Patterns. These subtests provide metrics of fluid reasoning, visualization, visual-spatial memory, and attention. Mean IQ for the Leiter standardization sample is 100, with a standard deviation of 15.

Autism status—The Autism Diagnostic Interview-Revised (ADI-R; Rutter, LeCouteur, & Lord, 2008) and the Autism Diagnostic Observation Schedule (ADOS, Lord, Rutter, DiLavore, & Risi, 2007) were administered and used to assign research classifications of ASD as described below. Both instruments were administered by project staff trained to research reliability.

The ADI-R is a standardized interview, which elicits information relevant to the domains of Reciprocal Social Interaction (RSI), Communication (Comm), and Restricted Interests and Stereotyped Behaviors (RBS). For the current study, the biological mother was interviewed. The ADI-R algorithm scores provided one of the metrics used to determine group membership. Additionally, current scores for 28 individual ADI-R items (10 in RSI, 10 in Comm, 8 in RBS) were used as the dependent measures in the between-group analyses. Scores of 0, 1, 2, and 3 were retained (i.e., scores of 3 were not converted to 2) to obtain maximum variability in the range of scores. ADI-R current items included in the analyses were those items administered to all participants regardless of chronological age as well as those items administered to participants who are considered to be verbal for purposes of the ADI-R.

The ADOS is comprised of a series of activities and materials used to systematically elicit a sample of an individual's social and communication behaviors. Participants in the current study received ADOS modules 1, 2 or 3, depending on their expressive language level. Calibrated severity scores were computed from ADOS algorithm totals using the conventions described by Gotham and colleagues (2009).

Diagnostic classification of ASD—A categorical classification of ASD versus no ASD was assigned to each participant based on the combined use of the ADOS and ADI-R as proposed by Risi and colleagues (2006). A diagnostic classification of ASD was assigned in one of three ways: (1) receiving a calibrated severity score of 4 or above on the ADOS and meeting the autism cutoff for the ADI-R Social Reciprocity Domain and *either* the Communication or Repetitive Behavior domains; (2) receiving a calibrated severity score of 4 or above on the ADOS and coming within one point of the cutoffs for both the Social Reciprocity and Communication domains; or (3) receiving a calibrated severity score of 4 or above on the ADOS, and meeting the autism cutoff on one ADI-R domain and coming within two points on the other.

Participant Grouping Strategies

For each matched sample, group equivalence on the variable(s) of interest was evaluated using Mann Whitney U Tests. Nonparametric tests were used because autism symptom severity and nonverbal IQ were not normally distributed. Descriptive characteristics, significance levels, and corresponding effect sizes for all matched samples are presented in Table 1.

Chronological Age-Matched Sample—All participants in the larger study (FXS, n=57; nonsyndromic ASD, n=61). were considered for inclusion in the current study if they: (a)

completed at least two subtests of the Leiter Brief IQ such that an IQ standard score could be calculated; (b) had nonverbal IQ scores under 85, as this cutoff is inclusive of essentially all males with FXS (Hessl, et al., 2009); and, (c) an ADOS and an ADI-R had been administered by project staff. Use of these criteria resulted in the exclusion of 17 children: 2 children (both FXS) did not complete the required Leiter subtests, 18 children (1 FXS, 17 nonsyndromic ASD) had nonverbal IQ scores over 85, 3 children (1 FXS, 2 nonsyndromic ASD) did not complete the ADOS, and 4 (all FXS) did not have a complete ADI-R. Participants with nonsyndromic ASD also needed a research classification of an ASD, which required (a) a calibrated autism severity score of at least 4 on the ADOS (Gotham et al., 2009) and (b) a classification of ASD according to the revised diagnostic criteria for the ADI-R (Risi et al., 2006). Applying these two criteria resulted in the elimination of 3 additional participants with nonsydromic ASD. The final CA-Matched sample (CA-MATCH) consisted of 88 individuals (FXS=49, nonsyndromic ASD=39) matched on chronological age ($p = .66$, $r = .05$). In this final sample, 11, 31, and 7 participants with FXS had received ADOS modules 1, 2, and 3, respectively. Similarly, 15, 12, and 12 participants with nonsyndromic ASD had received ADOS modules 1, 2, and 3, respectively.

Diagnosis-Matched Sample—Additional matched samples were created to enable a direct comparison between participants with FXS who had research classifications of ASD and those participants with nonsyndromic ASD. Nine participants with FXS in the CA-Matched sample were excluded from this subsample because they did not meet criteria for a research classification of ASD described previously. The final Diagnosis-Matched subsample (DX-MATCH) consisted of 79 individuals (FXS=40, ASD=39) matched group-wise on both diagnostic classification and chronological age ($p = .61$; $r = .06$).

Severity-Matched Sample—Participants with FXS in the Diagnosis-Matched sample had significantly lower ADOS severity scores ($Mean_{\text{Severity}} = 6.55$, $SD = 1.68$) than did participants with nonsyndromic ASD ($Mean_{\text{Severity}} = 8.21$, $SD = 1.53$), $p = .001$, $r = .48$; therefore, we created a subsample of participants who were matched group-wise on calibrated severity scores derived from the ADOS (Gotham et al., 2009). The Severity-Matched subsample (Severity-MATCH) consisted of 42 individuals (FXS=21, nonsyndromic ASD=21) matched on ADOS severity scores ($p = .74$, $r = .05$), diagnostic classification, and chronological age ($p = .59$, $r = .08$).

Verbal Participants—Matched participant samples also were created, using the logic described above, to allow group-wise comparisons of current ADI-R items administered only to those participants who were judged to produce non-prompted, functional three-word phrases on a daily basis (ADI-R Question 30).

Analysis Plan

Nonparametric Mann-Whitney *U* Tests were used to evaluate between-group differences in current ADI-R scores between participants with FXS and those with nonsyndromic ASD because the ADI-R uses an ordinal scoring metric. The Mann-Whitney *U* Test evaluates whether the distribution of scores from two independent samples comes from the same population. Conceptually, this metric is obtained by ranking each score for a given item without regard to group membership and then computing the average rank obtained by each group. Thus, the mean rank for a given group of participants only has meaning in comparison to the mean rank obtained for the same item in the matched participant sample (i.e., mean ranks should not be compared across items). ADI-R items were grouped for analyses based upon the domain (Social, Communication, Restricted Repetitive Behavior) to which the item belonged. Two-tailed *p*-values were used and adjusted for family-wise error based on the number of individual ADI-R items within a single domain that were included

each analysis. Tables 2, 3, and 4 display the mean ranks for each ADI-R current item for each of the matched participant samples.

Results

ADI-R Reciprocal Social Interaction Items: All Participants

The first series of analyses examined the 10 items from the ADI-R Reciprocal Social Interaction domain that were queried for the entire sample of participants. For all significant comparisons in this domain, participants with FXS demonstrated less impairment than did participants with ASD regardless of the matching strategy used. After controlling for multiple comparisons (α_{fw} =.005), three current items emerged as significantly different in the CA-MATCH sample: Social Smiling [U(88)=1381.00, Z=3.83, p=.001, r = .41], Showing and Directing Attention [U(88)=1315.00, Z=3.19, p =.001, r=.34], and Offering to Share, U(88)=1278.00, Z=2.89, p =.002, r = .31]. For the DX-MATCH sample, Social Smiling [U(79)=1074.50, Z=3.10, p=.002, r = .35] as well as Showing and Directing Attention [U(79)=1044.00, Z=2.73, p=.006, r = .31] remained significantly different after controlling for multiple comparisons. Finally, for the Severity-MATCH sample, Social Smiling [U(42)=327.00, Z=2.92, p=.003, r = .45] remained significantly different after controlling for multiple comparisons.

ADI-R Nonverbal Communication Items: All Participants

Analyses were then conducted to examine performance on the four current items from the ADI-R Communication Domain that were queried for all participants, regardless of verbal status. For the CA- MATCH sample, the between-group difference in the use of Conventional and Instrumental Gestures [U(88)=1231.50, Z=2.61, p=.009, r = .28] remained significant after adjusting for multiple comparison (α_{fw} = .0125). Participants with FXS in the CA- MATCH sample demonstrated significantly less impairment in gesture use than did participants with ASD. This same item, however, was not significantly different for either the DX- MATCH or Severity- MATCH samples after controlling for multiple comparisons.

ADI-R Restricted Interests and Repetitive Behavior Items: All Participants

A final analysis compared scores for seven current items from the ADI-R Restricted Interests and Repetitive Behaviors Domain that were queried for all participants. After controlling for multiple comparisons (α_{fw} = .007), two items remained significantly different for participants in the CA-MATCH sample: Unusual Preoccupations [U(88)=1320.00, Z=3.42, p=.001, r = .36] and Compulsions and Rituals [U(88)=1289.00, Z=3.18, p=.001, r = .34]. These same items were significantly different in the DX- MATCH samples after adjusting for multiple comparisons: Unusual Preoccupations, [U(79)=1079.50, Z=3.24, p=.001, r = .36], and Compulsions and Rituals [U(79)=1053.50, Z=3.00, p=.003, r = .34]. Participants with FXS in both the CA- and DX- MATCH samples were significantly less impaired for these items than were their matched counterparts with nonsyndromic ASD. Additionally, between group differences in Complex Mannerisms, [U(42)=95.50, Z=−3.34, p<.001, r = .52] were observed for the Severity-MATCH sample with participants with FXS being more impaired than participants with nonsyndromic ASD.

ADI-R Items Administered to Verbal Participants

There are six items in the Communication Domain and one item in the Restricted Interests and Repetitive Behavior Domain of the ADI-R that are administered only to participants who are reported to use spontaneous (i.e., nonprompted) three-word phrases on a daily basis. For the CA-MATCH sample of verbal participants, two of these items differed significantly after controlling for multiple comparisons (α_{fw} = .007): Social Verbalization/Chat

[U(67)=728.50, Z= 2.73, p<.003, r = .33] and Pronominal Reversal [U(67)=818.50, Z= 3.97, p<.001, r = .49]. For both items, participants with FXS were less impaired than were matched participants with nonsyndromic ASD. Pronominal Reversal [U(58)=634.00, Z= 3.63, p<.001, r = .48] continued to differ significantly for participants in the DX-Match sample, even after controlling for multiple significance tests. No significant differences emerged for the Severity-MATCH sample of verbal participants.

Discussion

The current study was designed to examine whether symptoms of autism, as measured by current items of the ADI-R, were comparable in FXS and nonsyndromic ASD. Addressing the issue of how autism is expressed in FXS is gaining theoretical, as well as clinical, importance. Form a theoretical perspective, it has been argued (e.g., Belmonte & Bourgeron, 2006) that fragile X syndrome provides a window into the etiology of nonsyndromic ASD. This is based on the assumption that symptom overlap between the two conditions represents shared neural or cognitive mechanisms. To the extent that in-depth analyses at the individual symptom level do uncover important differences in the autism symptom profiles of FXS and nonsyndromic autism, different underlying etiologies and neuropathologies would be suggested. Thus, extrapolation from FXS to ASD might be less straightforward than originally thought. From a clinical perspective, shared symptoms between FXS and ASD have led many (e.g., Berry-Kravis et al., 2012; Hagerman, Lauterborn, Au, & Berry-Kravis, 2012), to claim that targeted pharmaceutical interventions shown to be efficacious for core symptoms of FXS, especially those in the social realm, also are likely to be useful therapeutics for individuals with nonsyndromic ASD. To the extent that an autism diagnosis in those with FXS differs from that in nonsyndromic cases, however, the potential for common treatments may decrease accordingly. Relatedly, understanding the nuanced differences in symptom profiles between the two conditions is important for selecting clinical endpoints for any treatment trial involving individuals affected with one or both of the disorders (Berry-Kravis et al., 2013).

Utilizing a chronological age matching strategy, boys with FXS were found to be less impaired, on average, than boys with nonsyndromic ASD on several ADI-R social reciprocity items (Social Smiling, Showing and Directing Attention, and Offering to Share), one nonverbal communication item (Conventional and Instrumental Gestures), and two repetitive behavior items (Unusual Preoccupations, Compulsions and Rituals). For chronological age-matched participants who were verbal, significant between group differences emerged for two verbal communication items (Social Verbalization/Chat and Pronominal Reversal). Thus, boys with FXS demonstrate more shared affect with social partners as evidenced by social smiling; more motivation to engage in triadic interactions as evidenced by sharing, showing, and directing attention; and more nonverbal gestures to communicate than do age-matched peers with nonsyndromic ASD. In addition, verbal boys with FXS are more likely to engage in social chat and less likely to reverse pronouns relative to age-matched boys with nonsyndromic ASD.

One might argue that matching boys with FXS to those with nonsyndromic ASD on the basis of chronological age is not ideal given that some of the boys with FXS in the age-matched group do not have a comorbid diagnosis of ASD. To address this issue, we implemented an additional matching strategy by including only those boys with FXS who met the behavioral criteria for an ASD classification. This comparison yielded a similar pattern of significant between-group differences; that is, participants with FXS and comorbid ASD were significantly less impaired in social smiling, showing and directing attention, unusual preoccupations, and compulsions and rituals than were age-matched participants with nonsyndromic ASD. Verbal participants with FXS and comorbid ASD also

were less likely to reverse pronouns when talking. If one considers the ability to correctly use pronouns (as in answering a question with "I" instead of "you") to be a reflection of perspective taking, than this ability seems to be less affected in verbal individuals with FXS who have a comorbid diagnosis of an ASD than it is for same aged participants with nonsyndromic ASD.

The use of a matching strategy based on meeting the combined ADOS/ADI-R criteria for a categorical research classification of an ASD is most similar to that utilized in studies of autism symptoms in FXS that have included a comparison group of individuals with nonsyndromic ASD (Bailey et al., 1998; Dissanayake et al., 2009; Smith et al., 2012; Rogers et al., 2001; Wolff et al., 2012). One potential source of difference between the current study and previous studies is the criteria used for autism classification. In the current study, we utilized the broader autism spectrum caseness criteria proposed by Risi et al. (2006) in determining which participants were classified as having an ASD. In contrast, some previous studies have applied the ADOS or ADI-R cutoffs for autistic disorder. We chose to utilize broader (i.e., more inclusive) eligibility criteria to achieve closer correspondence with the current conceptualization of ASD as outlined by the DSM-5. Nevertheless, our results agree with the previously published findings of Wolff and colleagues (2012), who found that boys with FXS and comorbid ASD were less impaired than age- and IQ-matched boys with nonsyndromic ASD in social smiling, quality of social overtures, and facial expressions as measured by the ADOS as well as in the presence of compulsions and rituals as measured by the Repetitive Behavior Scales (Bodfish, Symons, Parker, Lewis, 2000). Compulsions and rituals, whether measured with the RBS, the ADOS, or the ADI-R, can be conceptualized as representing the construct of insistence on sameness (Bishop et al., 2013) in contrast to behaviors representing repetitive sensory motor mannerisms. Thus, results of the current study support the proposal that boys with comorbid FXS and ASD are less impaired than are boys with nonsyndromic ASD in behaviors reflecting insistence on sameness, although they may not be less impaired for sensorimotor-based repetitive behaviors such as hand flapping.

When boys with FXS who met criteria for a comorbid diagnosis of ASD in the current study were compared group-wise to boys with nonsyndromic ASD, a difference in autism severity emerged. That is, boys with FXS and comorbid ASD had lower severity scores, on average, than did age-matched boys with nonsyndromic ASD. Thus, it might be argued that any group differences observed between participants matched on diagnostic status could be attributed to lower levels of autism severity in boys with FXS. Our final matching strategy, therefore, was to match boys with FXS to those with nonsyndromic ASD based on severity of autism affectedness. We used ADOS calibrated severity scores for this comparison as we considered it important to utilize a matching variable that would be independent of the dependent measures of autism symptoms (i.e., domain scores for the ADI-R).

For the severity-matched analyses, we found that social smiling and complex mannerisms remained significantly different after controlling for multiple significance tests, with the former being less impaired and the latter being more impaired in participants with FXS than in those with nonsyndromic ASD. There were no significant between group differences for verbal participants who were matched on autism severity. These results suggest that, even when matched on overall level of autism symptoms, differences do still remain between individuals with FXS and those with nonsyndromic ASD.

As defined by the ADI-R protocol, social smiling can be used to spontaneously initiate a social interaction or to respond to the initiation of a social partner. Social smiling functions to indicate interest in or affective sharing with another individual and can be used independently of verbal language to mediate the expression of social reciprocity. It seems particularly telling that social smiling is less impaired in individuals with FXS relative to

individuals with ASD when compared at the same levels of chronological age and autism severity and despite the high levels of social anxiety that are characteristic of the FXS behavioral phenotype (Cordeiro, Ballinger, Hagerman, Hessl, 2011). Smiling at another individual, whether as a form of social initiation or social response, demonstrates some degree of affective sharing. Additionally, social reciprocity can be established through a shared smile, regardless of the limitations that may be imposed by impairments in cognition or verbal language ability. In this regard, boys with FXS seem to be less socially impaired than are boys with nonsyndromic ASD, regardless of autism diagnostic status, or autism severity. At present, however, no studies have directly compared levels of social anxiety between individuals with FXS and those with nonsyndromic ASD.

In terms of complex mannerisms, Bishop, Richler and Lord (2006) have reported a negative association between nonverbal IQ and the presence and severity of complex mannerisms as measured by the ADI-R. For children with nonsyndromic ASD between 6 and 12 years of age, Bishop et al. reported the presence of complex mannerisms in 75% of individuals with nonverbal IQ scores under 50 and 62% of individuals with nonverbal IQ scores between 51 and 70. In the current analysis, participants with FXS displayed higher (i.e., more severe) levels of complex mannerisms that participants with nonsyndromic ASD who were matched on autism symptoms severity. This was, in fact, the only current symptom of autism that emerged as more severely impaired in individuals with FXS and only for the comparison of individuals who were matched on autism severity.

It should be noted that, on average, participants with FXS in the current analyses had nonverbal IQ scores that were lower than their peers with nonsyndromic ASD regardless of the matching strategy used (i.e., chronological-age, diagnostic classification, or autism severity). These differences in nonverbal IQ, although not always reaching two-tailed significance, were accompanied by moderate effect sizes (*r*'s ranging from .20 to .33). Thus, participants with FXS had lower levels of cognitive ability than participants with nonsyndromic ASD, but were nonetheless less impaired for those symptoms of autism that differed significantly between the groups (with the exception of complex mannerisms). Findings of the current study support the contention of other authors (e.g., Clifford et al., 2007; Loesch et al., 2007) that autism in FXS represents the lower end of a continuum of impairment that covaries with cognitive ability rather than a qualitatively different and comorbid disorder within FXS.

Limitations

The current study had several limitations, most of which center around questions of group comparability. All of the males with FXS included in the current sample had nonverbal IQ scores below 85, with a mean IQ of 57.9 (*SD* 13.9). This cognitive level is in line with the levels of nonverbal cognition that have been reported for young boys with FXS in other studies (Hessl et al., 2009; Skinner et al., 2004) In contrast, Lord et al. (2012) recently reported a mean nonverbal IQ score of 86.1 (SD=25.3) for a group of over 2100 nonsyndromic individuals, ranging in age from 4–18 years, who met criteria for ASD on both the ADOS and ADI-R. In order to reduce the between-group discrepancy in nonverbal IQ, we chose to exclude from the present study those boys with nonsyndromic ASD who had nonverbal IQ scores above 85. Thus, although we considered our sample to be representative of the range of nonverbal cognitive abilities that is characteristic of males with FXS, the current sample was lower functioning and not representative, on average, of 6- to 11-year old boys with nonsyndromic ASD.

It is important to emphasize that, even after excluding participants with nonsyndromic ASD who had nonverbal IQ scores above 85, participants with FXS continued to have lower levels of nonverbal IQ. Thus, none of the subsamples used in the current study would be

considered to be well-matched for nonverbal IQ according to the matching criterion (i.e., $p > .50$) proposed by Mervis and Robinson (1999). Attempting to match on nonverbal IQ would have dramatically limited our sample sizes. We reasoned, however, that finding significant between-group differences generally demonstrating lower levels of autism affectedness in FXS, regardless of their lower levels of nonverbal IQ, provides strong evidence that the behavioral phenotype of males with FXS differs in important ways from the behavioral phenotype of males with nonsyndromic ASD.

It should also be noted that several of the exclusion criteria for the larger study (i.e., that the child could comply with simple instructions, used spoken language as the primary means of communication, and had spontaneously produced at least 10 different words during the month prior to the study visit) may have resulted in a sample of boys that was higher functioning and more verbal than a random sampling of 6- to 10-year old boys with FXS. Thus, the findings of the current study may be limited in generalizability. Fewer between-group differences might have been observed if the sample had been more representative of boys with FXS in general.

Finally, the findings reported for the current study are based on parent report. Mothers of boys with FXS are themselves pre- or full-mutation carriers of the FRM1gene and are known to have challenges to psychological well-being (Bailey, Sideris, Roberts, & Hatton, 2008) that may have influenced their responses to the ADI-R. Using a direct observational measure, such as the ADOS, may have yielded more unbiased results. However, given the larger number of individual symptoms that are queried, the ADI-R might yield a more nuanced comparison of behaviors.

Future Directions

In the current analyses, only two significant differences in current symptoms of autism emerged between boys with FXS and those with nonsyndromic ASD after matching on chronological age, diagnostic classification, and autism symptom severity, and controlling for family-wise error. Social Smiling was less impaired and Complex Mannerisms were more impaired in boys with FXS relative to boys with nonsyndromic ASD. However, we additionally found that all three matched samples of boys with FXS were significantly less impaired in Offering to Share (in the Reciprocal Social Interaction Domain) and Use of Conventional and Instrumental Gestures (in the Communication Domain) although these differences did not continue to be statistically significant following correction for multiple comparisons. Given the small size of our sample, we feel that these additional findings are suggestive of important between-group differences that should be considered in future studies.

The findings of the current study should not be interpreted to mean that these are the only behavioral differences that distinguish between these two populations of boys. One might question whether gold standard instruments for diagnosing ASD can adequately capture the qualitative differences that may exist between these FXS and nonsyndromic ASD (Tranfaglia, 2011). The deliberate inclusion in the ADOS and ADI-R of items (and associated code descriptions) that have particularly high sensitivity and specificity for discriminating between ASD and other non-spectrum developmental disabilities (Kim & Lord, 2012) may make it even more difficult for a clinician to use these instruments to evaluate and interpret the autistic-like behaviors of males with FXS. Additionally, there may be other behaviors which differ between individuals with FXS and nonsyndromic ASD that are not included in these instruments. Additionally, Hus, Gotham, and Lord (2013) have pointed out that individuals matched on autism severity scores cannot be assumed to have similar profiles of autism symptoms. An individual with a severity score of 10, the highest severity score, may have very severe social communication deficits and less severe

repetitive behaviors or vice versa. Thus, although severity scores may quantify an individual's overall level of autism symptomatology, these scores do not necessarily reflect the extent of impairment in social communication relative to repetitive behaviors. An important extension of the current study would be to examine both within- and between-group differences utilizing separate metrics of severity for the Social Affective domain and Restricted and Repetitive Behavior domain of the ADOS. Such separate metrics were recently introduced by Hus and colleagues (2013). Use of these dual severity scales may facilitate a more fine-grained analysis of the similarities and differences between FXS and nonsyndromic ASD.

It is also important to emphasize that similar behavioral endpoints may have different developmental or concurrent correlates. Thus, the brain or neurocognitive mechanisms that underlie behaviors queried in the ADI-R may not be the same across these two neurodevelopmental disorders. Recent studies have begun to examine other psychological constructs (e.g., anxiety) that may differentially predict behaviors in FXS and nonsyndromic ASD. For example, Gotham et al. (2013) suggest that anxiety is not related to core symptoms of autism for individuals with nonsyndromic ASD, whereas Thurman et al. (under review) have reported a significantly stronger association between anxiety and social withdrawal for boys with FXS than for boys with nonsyndromic ASD. Taken together, these findings suggest that anxiety may be a key factor influencing social affective functioning in boys with FXS relative to boys with nonsyndromic ASD. Examining other aspects of behavioral functioning in addition to symptoms of autism may lead to a more complete picture of the behavioral phenotypes of FXS and nonsyndromic ASD.

Future studies will allow us to further develop and test more nuanced models for describing the symptoms of ASD across different behavioral phenotypes as well as identifying the underlying mechanisms that lead to the emergence of symptoms of ASD in these disorders. Such research is vitally important for the development of effective pharmacological and behavioral treatments; that is, it may not be the case that treatments designed to address the core deficits observed in children with nonsyndromic ASD are equally efficacious for children with FXS if underlying neurophysiological symptoms differ between the two disorders (Hall et al., 2010).

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Table 1

Participant Characteristics: Mean (and Standard Deviations), Between-Group Differences and Effect Sizes[1]

	CA Match						Diagnostic Group Match						Autism Severity Match					
	All Participants						All Participants						All Participants					
	FXS (n=49) *M* *SD*		ASD (n=39) *M* *SD*		*p*	*r*	FXS (n=40) *M* *SD*		ASD (n=39) *M* *SD*		*p*	*r*	FXS (n=21) *M* *SD*		ASD (n=21) *M* *SD*		*p*	*r*
CA	7.50	2.03	7.27	1.90	.66	.05	7.54	2.05	7.27	1.90	.61	.06	7.54	1.95	7.19	1.99	.60	.08
NVIQ	57.9	13.9	63.6	13.9	.06	.20	55.9	13.4	63.6	13.9	.02	.27	57.1	12.5	64.8	13.0	.06	.29
CSS	6.14	2.11	8.21	1.53	.00	.49	6.55	1.68	8.21	1.53	.00	.48	7.38	1.72	7.57	1.66	.75	.05
Verbal	41		26				32		26									
	Verbal Participants						Verbal Participants						Verbal Participants					
	FXS (n=41) *M* *SD*		ASD (n=26) *M* *SD*		*p*	*r*	FXS (n=32) *M* *SD*		ASD (n=26) *M* *SD*		*p*	*r*	FXS (n=14) *M* *SD*		ASD (n=14) *M* *SD*		*p*	*r*
CA	7.72	2.00	7.47	1.85	.65	.06	7.84	2.02	7.47	1.85	.50	.09	7.54	2.06	7.21	1.52	.54	.10
NVIQ	58.1	14.2	65.6	14.7	.05	.24	55.5	13.6	65.6	14.7	.01	.33	56.2	13.8	68.0	16.3	.09	.33
CSS	6.07	2.15	8.50	1.48	.00	.54	6.56	1.63	8.50	1.48	.00	.53	7.50	1.74	7.71	1.49	.80	.15
Verbal	41		26				32		26				14		14			

[1] p-values and effect sizes are based on Mann-Whitney U Tests

Table 2

Mean Rank Score for ADI-R Current Items by Domain[1]

Item #	ADI-R Social Domain	FXS			ASD		
		CA Match	DX Match	Severity Match	CA Match	DX Match	Severity Match
51	Social Smile	35.82+*	32.64+*	16.43+*	55.41	47.55	26.57
57	Range of Facial Expression Used to Communicate	41.14	36.80	17.95+	48.72	43.28	25.05
52	Showing/Directing Attention	37.16+*	33.40+*	18.50	53.72	46.77	24.50
53	Offering to Share	37.92+*	35.08+	18.07+	52.77	45.06	24.93
54	Shared Enjoyment	38.79+	35.10+	20.12	51.68	45.03	22.88
31	Use of Other's Body	39.75+	35.19	19.50	48.24	42.91	22.43
55	Offering Comfort	39.74+	36.42	18.93	50.47	43.67	24.07
56	Social Overtures	41.12	36.99	18.48	48.74	43.09	24.52
58	Inappropriate Facial Expression	41.44	37.38	21.00	48.35	42.69	22.00
59	Appropriateness of Social Response	39.29+	36.11	20.19	51.05	43.99	22.81

+Between-group difference p<.05

*Significantly different after controlling for multiple comparisons (adjusted *p*-value = .005)

Table 3

Item #	ADI-R Communication Domain	FXS			ASD		
		CA Match	Dx Match	Severity Match	CA Match	Dx Match	Severity Match
42	Pointing to Express Interest	41.40	38.20	19.60	48.40	41.75	23.40
43	Nodding	43.40	39.90	20.92	45.78	40.13	22.10
44	Head shaking	46.50	43.08	20.71	41.86	36.85	22.29
45	Conventional/Instrumental Gestures	38.87+*	34.91+	17.71+	51.58	45.22	25.29
34	Social Verbalization/Chat^{V}	29.23+*	25.78+	13.75	41.52	34.08	15.25
35	Reciprocal Conversation^{V}	31.27	28.22	15.50	38.31	31.08	13.50
33	Stereotyped Utterances/Echolalia^{V}	33.30	29.81	14.50	35.10	29.12	14.50
36	Inappropriate Questions^{V}	33.52	29.05	13.82	34.75	30.06	15.18
37	Pronominal Reversal^{V}	27.05+*	22.69+*	11.85	44.95	37.88	17.14
38	Neologisms/Idiosyncratic Language^{V}	32.63	28.02	13.46	36.15	31.33	15.54

^{V}Items administered only for participants who are considered Verbal (score of "0" on Item 30)

$^{+}$Between-group difference $p<.05$

*Significantly different after controlling for multiple comparisons (adjusted $p<.0125$ for nonverbal items; adjusted $p<.007$ for verbal items)

Table 4

Item #	ADI-R Repetitive Behavior Domain	FXS			ASD		
		CA Match	Dx Match	Severity Match	CA Match	Dx Match	Severity Match
67	Unusual Preoccupations	37.06+*	32.51+*	17.74+	53.85	47.68	25.26
68	Circumscribed Interests	46.22	41.81	25.48+	42.33	38.14	17.52
39	Verbal Rituals V	31.93	21.20	13.95	37.27	32.33	15.04
70	Compulsions/Rituals	37.68+*	33.16+*	18.19+	53.06	47.01	24.81
77	Hand/Finger Mannerisms	45.00	40.99	24.83	43.87	38.99	21.17
78	Complex Mannerisms	47.98	44.61	27.45+*	40.15	35.27	15.55
69	Repetitive Object Use	38.66+	35.99	19.36	50.58	44.12	23.64
71	Unusual Sensory Interests	39.00+	37.92	18.96	50.17	43.05	24.14

+Between-group difference $p<.05$

*Significantly different after controlling for multiple comparisons (adjusted $p< .007$)