Rates and predictors of co-occurring autism spectrum disorder in boys with fragile X syndrome

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Abstract
Background and aims: Males with fragile X syndrome display many behavioral features of autism spectrum disorder. Despite this overlap, our understanding of autism spectrum disorder symptoms and severity in fragile X syndrome is limited due to variation in assessment methods in the literature. Furthermore, the relationship between autism spectrum disorder symptoms and child characteristics, like age, language, and cognitive abilities, are not well understood in individuals with fragile X syndrome. Therefore, the first research aim was to compare the rates of autism spectrum disorder classifications from three commonly reported autism spectrum disorder assessments in the literature. Our second research aim was to examine the relationship between autism spectrum disorder characteristics and other child characteristics.

Methods: The present study compared autism spectrum disorder classifications and symptoms using the Autism Diagnostic Observation Schedule, Autism Diagnostic Interview, Revised, and Childhood Autism Rating Scale, second edition in a sample of 33 school-age and adolescent boys with fragile X syndrome. In addition, the participants completed nonverbal IQ testing, expressive vocabulary and grammar tests, and a conversation language sample.

Results: The majority of the participants met criteria for autism spectrum disorder on the Autism Diagnostic Observation Schedule (96.97%) and Autism Diagnostic Interview, Revised (90.91%), while only half met criteria for autism spectrum disorder on the Childhood Autism Rating Scale, second edition. Sixteen boys (48.48%) met criteria for autism spectrum disorder on all three measures, and all participants met criteria for autism spectrum disorder on at least one measure. Expressive vocabulary accounted for a unique amount of variance in Childhood Autism Rating Scale, second edition and Autism Diagnostic Observation Schedule scores. Additionally, grammatical complexity accounted for a unique amount of variance in Childhood Autism Rating Scale, second edition scores. None of the child variables accounted for the variance found in Autism Diagnostic Interview, Revised scores. Although nonverbal IQ scores did not account for a significant amount of variance on the Autism Diagnostic Observation Schedule, Autism Diagnostic Interview, Revised, and Childhood Autism Rating Scale, Second Edition, boys who met criteria for autism spectrum disorder on all three measures had lower nonverbal IQ compared to the boys who did not. Additionally, mean length of
utterance and expressive vocabulary scores were lower in the boys who met criteria for autism spectrum disorder on all three measures than those who did not.

**Conclusions:** Our findings identify areas of overlap and difference in the Autism Diagnostic Observation Schedule, Autism Diagnostic Interview, Revised, and Childhood Autism Rating Scale, second edition when used with males with fragile X syndrome. Variation in assessments may differentially identify the phenotypic behaviors of boys with fragile X syndrome that lead to a co-diagnosis of autism spectrum disorder, which contributes to the variation in reported co-morbidity of fragile X syndrome and autism spectrum disorder. Also, expressive language abilities, especially expressive vocabulary, are associated with autism spectrum disorder symptomatology.

**Implications:** When interpreting comorbid fragile X syndrome and autism spectrum disorder rates in the literature, it is important to consider the assessment tool that was used. Although the assessments that we used in the present study yielded scores that were highly correlated (i.e. Autism Diagnostic Observation Schedule and Childhood Autism Rating Scale, second edition), their categorical classifications did not align perfectly. Our findings also highlight the importance of considering language skills when assessing autism spectrum disorder severity in fragile X syndrome.

**Keywords**
Autism spectrum disorders, intellectual disability, assessment, fragile X syndrome

**Introduction**
Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability (Crawford, Acuña, & Sherman, 2001). FXS is caused by a CGG trinucleotide expansion in the FMR1 gene on the X chromosome (Oostra & Willemsen, 2003; Verkerk et al., 1991). This trinucleotide repeat inhibits the production of the FMR1 protein, which is important for cognitive development (Bassell & Warren, 2008; Loesch et al., 2002; Tassone et al., 1999). Although the exact prevalence of FXS is unknown, it is estimated that 1.4 per 10,000 males have FXS (Hunter et al., 2014).

The phenotypic characteristics associated with FXS are more pronounced in males, relative to females who typically have an unaffected X chromosome (Loesch, Huggins, & Hagerman, 2004). Males typically present with nonverbal IQs that fall in the range of intellectual disability (Kover, Pierpont, Kim, Brown, & Abbeduto, 2013; Skinner et al., 2005). In addition, males with FXS have significant deficits in executive function, language, and social skills, and present with notable anxiety (Haebig, Sterling, & Hoover, 2016; Hooper et al., 2018; Klusek, Martin, & Losh, 2014a; Matherly et al., 2018; Roberts, Mirrett, & Burchinal, 2001; Sterling & Warren, 2008).

Furthermore, it is estimated that as many as 90% of males with FXS demonstrate characteristics of autism spectrum disorder (ASD), such as hand biting, hand flapping, poor eye contact, repetitive behaviors, and restricted interests (Bailey, Hatton, Skinner, & Mesibov, 2001; Hartley et al., 2015; Rogers, Wehner, & Hagerman, 2001; Thurman, McDuffie, Kover, Hagerman, & Abbeduto, 2015). Although there is a debate in the field as to whether the characteristics noted in individuals with FXS are a reflection of the same underlying impairments in idiopathic ASD, there is consensus that the behaviors associated with the core deficits of ASD are occurring to some degree in many, if not all males with FXS. Therefore, the present study aims to further examine ASD characteristics in school-age and adolescent males with FXS and to assess the relationship between ASD symptom severity and other child characteristics.

Although almost all males with FXS present with some ASD characteristics, the rates of comorbid ASD and FXS diagnoses greatly differ across the literature. Research has documented that 20–75% of males with FXS meet criteria for a co-diagnosis of ASD (e.g. Bailey, Raspu, Olmsted, & Holiday, 2008; Caravella & Roberts, 2017; Harris et al., 2008; Hartley et al., 2015; Klusek, Martin, & Losh, 2014b; Roberts et al., 2018). This large difference in rates is partially related to differences between research methods and clinical or educational methods used to identify ASD in FXS (Abbeduto, McDuffie, & Thurman, 2014; Klusek et al., 2014b). Klusek et al. (2014b) compared clinical versus research ASD diagnostic classifications in children with FXS. Specifically, they examined parent-reported clinical diagnoses and compared them to a classification determined by a research-administered assessment of ASD severity (i.e. the Autism Diagnostic Observation Schedule (ADOS), Lord, Rutter, DiLavore, & Risi, 1999, or the Autism Diagnostic Interview, Revised (ADI-R), Rutter, LeCouteur, & Lord, 2003). The boys with FXS were not identified as having a clinical ASD diagnosis either because: (a) the child had been evaluated for ASD, but
did not meet diagnostic criteria, or (b) the child had never been evaluated for ASD. Males with FXS were overwhelmingly more likely to be classified as having co-morbid ASD by the ADI-R or ADOS (74.5% and 78%, respectively) than to have had received a clinical ASD diagnosis (25.5%). Although this difference in clinical versus research ASD classification is substantial, it is important to note that even within the research literature, different diagnostic tools have been used and varying rates of comorbid FXS and ASD have been reported.

Although several of the ASD diagnostic tools have assessed sensitivity and specificity to identify individuals with ASD by including children who do not have ASD (e.g. global developmental delay, conduct disorder), the currently available ASD diagnostic tools were created to identify idiopathic ASD (also referred to as nonsyndromic ASD; Abbeduto et al., 2019, 2014). Researchers have therefore questioned the precision of these measures when used with individuals with FXS, who may also have syndromic ASD (Abbeduto et al., 2019, 2014). Accurate diagnoses are critically necessary in clinical and research settings, not only in terms of selecting appropriate interventions but also understanding the developmental pathways that lead to the diagnosis (Abbeduto et al., 2014).

It also is important to understand how measures identify ASD in FXS because other variables, such as intellectual disability, could overly influence the measures when used outside of idiopathic ASD (Abbeduto et al., 2014). Research has begun to explore how child characteristics like nonverbal IQ, chronological age, and language skills relate to ASD characteristics in FXS (Abbeduto et al., 2019; Klusek et al., 2014b; Lee, Martin, Berry-Kravis, & Losh, 2016; Thurman et al., 2015). In the present study, we also assessed whether nonverbal IQ, age, and expressive language (grammar and vocabulary production) predicted ASD symptom severity in boys with FXS and whether these associations differed across the three ASD assessments.

Assessment of ASD characteristics in individuals with FXS

Three of the most commonly reported assessment tools used to measure ASD characteristics in the FXS literature include the ADOS (ADOS-2 and ADOS; Lord et al., 2012, 1999), the ADI-R (Rutter et al., 2003), and the Childhood Autism Rating Scale (CARS and Childhood Autism Rating Scale, second edition (CARS-2); Schopler, Reichler, & Renner, 1988; Schopler, Van Bourgondien, Wellman, & Love, 2010). The ADOS is a semi-structured assessment designed to identify and measure the core characteristics associated with ASD. The ADOS has four different modules; modules are selected based on the individual’s language production abilities. It was updated in 2012 and includes algorithm items that are specifically in line with the Diagnostic Statistical Manual (DSM-V) definition of ASD (American Psychiatric Association, 2013). This direct assessment tool is often used in conjunction with the ADI-R.

The ADI-R is a semi-structured interview designed for caregivers of individuals with ASD. Questions target communication and social development, play, and restricted and repetitive behaviors and interests. Caregivers are asked to report their child’s behaviors across the child’s lifespan, with an emphasis on behaviors within the fourth and fifth year of life (Rutter et al., 2003). Therefore, the ADI-R reflects ASD behaviors that have been demonstrated at earlier points in development while allowing for current information to be gathered as well. The ADI-R Diagnostic Algorithm score has been found to appropriately reflect ASD characteristics (Hus & Lord, 2013).

Reliability of the ADOS and ADI-R diagnostic classifications are high, with 75% agreement (Zander et al., 2016). Administration and coding of the ADOS and ADI-R, for research purposes, require a high level of training, which are strength of the instruments. Studies in FXS have used the ADOS to measure ASD characteristics, and reported ranges from 49–95% of male participants meeting ASD criteria (e.g. Estigarribia, Roberts, Sideris, & Price, 2011; García-Nonell et al., 2008; Hernandez et al., 2009; Klusek et al., 2014a; Thurman et al., 2015). With the ADOS and ADI-R together, rates vary from 13–83% in males with FXS (e.g. Clifford et al., 2007; Harris et al., 2008; Klusek et al., 2014b; Thurman et al., 2015).

The CARS and CARS-2 (Schopler et al., 1988, 2010) are another commonly used measure to assess ASD characteristics in FXS. The CARS was developed to identify children with idiopathic ASD and distinguish ASD from intellectual disability or developmental delays without ASD. The CARS has been used in numerous studies in FXS (e.g. Bailey, Hatton, & Skinner, 1998; Brady, Warren, Fleming, Keller, & Sterling, 2014; Froli, Piscopo, & Conson, 2015; Hatton et al., 2006; Tonnisen, Malone, Hatton, & Roberts, 2013; Warren, Brady, Sterling, Fleming, & Marquis, 2010). The CARS is composed of a number of subscales that focus on behaviors associated with the classic definition of the core impairments in ASD (Creak, 1961; Kanner, 1943). Although the CARS has been found to measure the core features of ASD as identified by different versions of the DSM, it is important to note that the CARS items and scoring have not been updated since the original publication in 1988. However, a recent study found the CARS-2
had high sensitivity and specificity in identifying ASD using DSM-V criteria in individuals with idiopathic ASD (Dawkins, Meyer, & van Bourgondien, 2016). Furthermore, a recent factor analysis of the CARS-2 found that the scores across the 15 items loaded onto three factors: social communication, stereotyped behavior and sensory sensitivity, and emotional reactivity, and they noted that the first two factors closely aligned with symptom domains in the DSM-V (Moulton, Bradbury, Barton, & Fein, 2019).

Unlike the ADOS-2, the CARS and CARS-2 have minimal training guidelines. The CARS-2 manual states that before using the CARS-2 rating form, clinicians should thoroughly read the manual, which contains explanations of each item, the scoring system, and a discussion of sample cases. Importantly, the CARS-2 has high internal consistency ratings of .93 and correlates with clinical ratings of other ASD assessments that have been used with individuals with idiopathic ASD (classification agreement range = 70–100%; Schopler et al., 2010). Notably, although some researchers have found high or even perfect agreement with the DSM-IV and CARS ASD classifications (scores 30 and above; Rellini, Tortolani, Trillo, Carbone, & Montecchi, 2004), others have suggested that the CARS lacks sensitivity and suggest lower cut-off scores ranging between 25.5 and 28 (Chlebowski, Green, Barton, & Fein, 2010; Mayes et al., 2011; Mesibov, Schopler, Schaffer, & Michal, 1989; Tachimori, Osada, & Kurita, 2003). The CARS manual states that a cut-off of 28 is suggested for adolescents (13-years-old or older) and adults with IQs below 80 to differentiate children with ASD from children with cognitive deficits without ASD. In addition, Tachimori et al. (2003) found that a cut-off score of 25.5 resulted in the best sensitivity and specificity (.86 and .83, respectively) for differentiating children with pervasive developmental disorder - not otherwise specified (PDD-NOS) from children with intellectual disability without ASD (non-PDD-NOS). Chlebowski and colleagues (2010) also found that a 25.5 CARS cut-off yielded the optimal sensitivity and specificity for preschool children with idiopathic ASD (.82 and .95, respectively, for the four-year-old participants). To our knowledge, the alternative CARS cut-off scores have not been examined with boys with FXS.

Relative to studies using the ADOS, studies using the CARS with children with FXS have reported the full range of possible scores (Brady et al., 2014; Hahn, Brady, Warren, & Fleming, 2015; Sterling, Rice, & Warren, 2012; Tonnsen et al., 2013; Warren et al., 2010), with average scores elevated relative to typically developing children, but slightly below the cut-off for ASD (26.6–27.75). Instead of using the CARS to create subcategories of FXS (i.e. FXS-Only and FXS+ASD), some studies have used the CARS as a continuous measure to allow for a better understanding of the continuum and potential profiles of ASD features in FXS (Abbeduto et al., 2019; Adlof, Klusek, Shinkareva, Robinson, & Roberts, 2015; Hooper et al., 2018).

Table 1 presents an outline of studies that have used either the ADOS, ADI-R, or the CARS to classify males with FXS as having FXS-Only or FXS+ASD. Although we have striven to create a thorough list of relevant publications, we also attempted to avoid citing multiple publications that present data using overlapping samples; therefore, Table 1 does not present a comprehensive list of every publication including a measure of ASD used with individuals with FXS. As can be seen, there is significant variability in comorbid ASD classifications within individuals with FXS across each assessment tool, but overall, the CARS tended to have lower rates of comorbid ASD within individuals with FXS, with an average of 30.19% of the males categorized as having FXS+ASD. This contrasts with higher averages when studies used the ADOS or ADI-R (69.83% and 65.73%, respectively). Despite the frequency of use of these assessments in the literature, it is difficult to compare rates of ASD in individuals with FXS from studies using the CARS to studies using the ADOS and/or ADI-R, given that these measures have not been compared within the same sample of individuals with FXS. The primary aim of the current study addressed this gap in the literature.

Predictors of ASD characteristics in individuals with FXS

To better understand ASD characteristics in individuals with FXS, previous studies have examined the relationship between ASD features with other child characteristics, including chronological age, nonverbal IQ, anxiety, and language variables. Many of the variables that have been examined have yielded somewhat mixed findings that could potentially be related to the assessment used to measure ASD characteristics. In the current study, we examined the associations between the most frequently assessed child characteristics: chronological age, nonverbal IQ, and language (e.g. Abbeduto et al., 2019; Brady et al., 2014; Kaufmann et al., 2004; Klusek et al., 2014b; Thurman et al., 2015).
| Authors (year)         | Sample characteristics | Diagnostic procedures | Detected rates of autism                                      |
|-----------------------|------------------------|-----------------------|-------------------------------------------------------------|
| Hernandez et al. (2009) | Time 1: 56 males       | ADI-R, ADOS, and DSM-IV | Time 1: 95.5% ADOS (sub-sample: n = 21)                    |
|                       | Mean age: 4.78 years ($\text{SD} = 1.16$) |                        | Time 1: 42.9% ADI-R and DSM-IV                              |
|                       | Time 2: 44 males       |                        | Time 2: 43.9% ADI-R and DSM-IV                              |
|                       | Mean age: 5.76 years ($\text{SD} = 1.10$) |                        | Time 3: 35.3% ADI-R and DSM-IV                              |
|                       | Time 3: 34 males       |                        |                                                             |
|                       | Mean age: 6.86 years ($\text{SD} = 1.10$) |                        |                                                             |
| Rogers et al. (2001)  | 22 males               | ADI-R, ADOS, and DSM-IV | 50% on ADI-R                                                |
|                       | Mean age: 2.92 years ($\text{SD} = 0.59$) |                        | 31.8% on ADI-R                                              |
| Clifford et al. (2007)| 33 males               | ADOS                   | 36.4% on ADI-R                                              |
|                       | Mean age: 23.15 years (range: 5.8–60.7) | ADI-R and DSM-IV      | 57.6% on ADOS                                              |
|                       |                        |                        | 18.2% on ADI-R and ADOS                                    |
|                       |                        | ADI-R, ADOS, and DSM-IV | 67% on ADI-R and/or ADOS                                    |
| Harris et al. (2008)  | 63 males               | ADI-R, ADOS and DSM-IV | 49% on ADI-R                                                |
|                       | Mean age: 7.9 years ($\text{SD} = 4.3$, range: 2.8–19.5) | ADI-R and DSM-IV | 59% on ADOS                                                |
|                       |                        | ADI-R                  | 27% on ADI-R and DSM-IV                                     |
|                       |                        | ADOS                   | 27% on ADI-R and DSM-IV                                     |
|                       |                        | ADI-R and DSM-IV      | 74.5% on ADI-R                                              |
|                       |                        | ADOS                   | 78.0% on ADOS                                              |
| Klusek et al. (2014b)| 51 males               | ADI-R and ADOS         | 60.0% on ADI-R                                              |
|                       | Mean age: 11.76 years ($\text{SD} = 3.11$, range: 6.47–17.90) | ADI-R and ADOS | 91.8% (45/49) on ADI-R                                     |
|                       |                        | ADI-R and ADOS         | 88.7% (47/53) on ADOS                                      |
| Thurman et al. (2015)| 53 males               | ADI-R and ADOS         | 83.7% (41/49) on ADI-R                                      |
|                       | Mean age: 7.51 years ($\text{SD} = 1.98$, range: 4.06–10.62) | ADI-R and ADOS | 96.2% (51/53) on ADI-R                                     |
|                       |                        | ADI-R and DSM-IV      | 75% on ADI-R and ADOS                                       |
| Abbeduto et al. (2019)| 44 males               | ADI-R and ADOS         | 75% on ADI-R and ADOS                                       |
|                       | Mean age: 18.31 years ($\text{SD} = 2.31$, range: 15.03–22.92) | ADI-R and ADOS | 63.3%                                                       |
|                        |                        | ADOS and DSM-IV       | 63.3%                                                       |
| Garcia-Nonell et al.  | 90 males               | ADOS and DSM-IV        | 63.3%                                                       |
| (2008)                | Mean age: approximately 9 years ($\text{SD} = 4.8$, range: 3–25) | ADOS and DSM-IV | 63.3%                                                       |
| Hall, Lightbody, &    | 31 males               | ADOS                   | 74.2% (autism or ASD)                                       |
| Reiss (2008)          | Mean age: 13.21 years ($\text{SD} = 3.16$, range: 5–20) | ADOS               | 74.2% (autism or ASD)                                       |
| Price et al. (2008)   | 71 males               | ADOS                   | 49.3% (autism or ASD)                                       |
|                       | Mean age: approximately 9.15 years ($\text{SD} = 2.5$, range: 2.9–14.4) | ADOS | 49.3% (autism or ASD)                                       |
| Bailey et al. (1998)  | 57 males               | CARS                   | 24.6%                                                       |
|                       | Mean age: 5.56 years (range: 2.08–11.08) | CARS | 24.6%                                                       |
| Demark et al. (2003)  | 12 males               | CARS                   | 58.3%                                                       |
|                       | Mean age: 11.8 years ($\text{SD} = 2.6$) | CARS | 58.3%                                                       |
| Hatton et al. (2006)  | 147 males              | CARS                   | 25.9%                                                       |
|                       | Mean age: 4.71 years ($\text{SD} = 2.77$, range: 1.5–14.7) | CARS | 25.9%                                                       |
| Klusek et al. (2015)  | 51 males               | CARS                   | 19.6%                                                       |
|                       | Mean age: 10.2 years ($\text{SD} = 1.7$, range: 7.9–13.2) | CARS | 19.6%                                                       |
| Komesidou et al. (2017)| 31 males              | CARS                   | 22.6%                                                       |
|                       | Mean age: 4.26 years ($\text{SD} = 0.54$, range: 2.92–5.67) | CARS | 22.6%                                                       |

ADI-R: Autism Diagnostic Interview, Revised; ADOS: Autism Diagnostic Observation Schedule; DSM: Diagnostic Statistical Manual; CARS: Childhood Autism Rating Scale; ASD: autism spectrum disorder; SCQ: Social Communication Questionnaire.

Note: ASD classifications were based on autism and autism spectrum categorizations. Studies are listed first by diagnostic procedure and then by alphabetical order.
between these child characteristics and ASD severity have included different ASD assessments; therefore, it is unclear whether these same relationships are present depending on the ASD assessment used.

**Chronological age.** Chronological age has been found to be significantly associated with ASD severity in males with FXS in the preschool to school-age years when ASD characteristics are measured using the ADOS and CARS (Hatton et al., 2006; Lee et al., 2016; Thurman et al., 2015). However, ADOS severity scores have not been found to be associated with chronological age in adolescent males and young adult males with FXS (Abbeduto et al., 2019). Additionally, ASD characteristics have sometimes been found to improve (lessen) with increased age when characteristics are measured using the ADI-R (Hernandez et al., 2009; McDuffie et al., 2010).

**Nonverbal IQ.** In contrast to chronological age, nonverbal IQ has consistently had a negative relationship with ASD characteristics in males with FXS. As IQ scores increase, ASD symptom severity decreases (Bailey et al., 2001; Hatton et al., 2006; Kau et al., 2004; Rogers et al., 2001; Wolff et al., 2012). This relationship has been documented in both the ADOS social affect scores (Thurman et al., 2015) and the restricted and repetitive behaviors scores (Abbeduto et al., 2019; Lee et al., 2016; Thurman et al., 2015). Furthermore, the same relationship has been documented when ASD characteristics are measured using the ADI-R (Kau et al., 2004; Kaufmann et al., 2004) and the CARS (Bailey et al., 2001; Brady et al., 2014; Demark et al., 2003).

**Language.** Associations between child language abilities and ASD symptomatology also have been examined in males with FXS. Most of these studies have examined this association between ADOS severity scores and vocabulary skills. Thurman and colleagues (2015) found that expressive vocabulary, measured using the Expressive Vocabulary Test, second edition (EVT-2; Williams, 2007), was negatively associated with the ADOS severity score for the restricted and repetitive behaviors domain. Similar findings were reported by Thurman and colleagues, with ADOS restricted and repetitive behaviors severity scores being significantly and negatively associated with receptive vocabulary (Peabody Picture Vocabulary Test, fourth edition (PPVT-4); Dunn & Dunn, 2007; Thurman, McDuffie, Hagerman, Josol, & Abbeduto, 2017). In addition, Thurman et al. (2017) found that as EVT-2 scores increased, the severity of restrictive and repetitive behaviors decreased; however, this relationship only reached marginal significance. In addition, Klusek and colleagues (2014b) found that receptive and expressive vocabulary scores were negatively associated with qualifying for a co-morbid ASD classification according to the ADOS or ADI-R in a group of 50 males and 35 females with FXS; however, the associations were not significant in the male-only subgroup with FXS. Furthermore, Lee et al. (2016) failed to identify associations between ADOS severity scores and receptive and expressive vocabulary scores that were measured using the PPVT-4 and EVT-2.

Beyond vocabulary, Rogers and colleagues (2001) documented that young males with FXS performed more poorly on an omnibus measure of receptive language (Mullen Scales of Early Learning—Receptive Language subtest; Mullen, 1995) if they qualified for a co-morbid ASD diagnosis, according to the ADOS. In addition, Kaufmann et al. (2004) found that expressive language, measured using the Preschool Language Scales, third edition (Zimmerman, Stein, & Pond, 1997), was inversely related to ADI-R scores such that higher expressive language scores in young males with FXS were associated with higher ASD characteristics on the ADI-R. In contrast to the ADOS restricted and repetitive behaviors severity score findings noted above, Thurman and colleagues (2017) found that social affective ADOS severity scores were positively associated with receptive and expressive vocabulary scores and receptive grammar scores in school-age males with FXS. Thurman and colleagues suggested that this unexpected relationship may exist because deficits in social affect may be more easily detected in children with more advanced language.

Like vocabulary skill, syntactic abilities are an area of significant difficulty in males with FXS (Estigarribia et al., 2011; Haebig et al., 2016; Roberts et al., 2001; Sterling, 2018). In fact, syntactic abilities are thought to be impacted to a greater extent than vocabulary skills (Thurman et al., 2017). There have been relatively fewer studies that have examined the association between syntactic language skills and ASD characteristics, and the extant literature has yielded somewhat mixed findings. For instance, McDuffie and colleagues reported that ASD characteristics, measured using the ADOS, were not associated with expressive syntax in school-age and adolescent males with FXS (measured using the Syntax Construction subtest of the Comprehensive Assessment of Spoken Language (CASL); Carrow-Woolfolk, 1999; McDuffie, Kover, Abbeduto, Lewis, & Brown, 2012; Thurman et al., 2017). Also, ADOS severity scores were not found to be related to expressive syntax when measured using language samples (i.e. mean length of utterance (MLU); Kover, McDuffie, Abbeduto, & Brown, 2012). Finally, Komesidou and colleagues found that, while CARS scores were negatively associated with expressive syntax scores derived from a language
sample in a sample of male and female preschool-age children with FXS, the association was no longer significant when limiting the analyses to males with FXS (Komesidou et al., 2017).

In contrast, Abbeduto and colleagues (2019) found a significant association between ASD characteristics and expressive syntax in adolescent and young adult males with FXS. Males with FXS with more severe ASD features (for both the ADOS Comparison score and the ADOS social affect calibrated severity score) had lower CASL syntax construction scores. Abbeduto et al. (2019) noted that expressive syntactic skills are particularly important to examine when studying ASD symptomatology because the ability to effectively communicate affords individuals opportunities to engage with peers and to develop social skills. Furthermore, syntactic abilities have been associated with social cognition within the broader language literature (de Villiers, 2007; Rakhlin et al., 2011; Spanoudis, 2016).

Current study

Although there is a debate in the field as to whether the ASD symptoms noted in individuals with FXS are a reflection of the same underlying impairments in idiopathic ASD (Clifford et al., 2007; Loesch et al., 2007; Thurman et al., 2015; Wolff et al., 2012), the research literature consistently demonstrates that the behaviors associated with the core deficits of ASD are occurring to some extent in many, if not all, boys with FXS. However, as outlined above, the agreement between the CARS, the ADOS, and ADI-R is unknown given that no previous study has compared ASD classifications with these three measures within the same sample of participants. Therefore, our first research question asked whether the rates of ASD classifications differed according to the three most commonly reported ASD assessments in the literature on FXS (i.e. ADOS, ADI-R, and CARS-2). Additionally, because different CARS-2 cut-off scores have been suggested in the literature on idiopathic ASD, our second research question asked whether the cut-off scores of 25.5 and 28 influence the rates of comorbid ASD and FXS in our sample. Finally, given the variation in reported findings of associations between ASD symptomatology and other child characteristics, our third research question asked whether there were significant associations between ASD characteristics measured using the ADOS, ADI-R, and CARS-2 and other child characteristics, including: chronological age, nonverbal IQ, expressive vocabulary, and expressive syntax. We addressed this aim using a categorical approach (i.e. FXS + ASD or FXS-Only) as well as a continuous approach (i.e. examining ASD scores along the continuum). Furthermore, we included vocabulary and syntactic scores that were derived from standardized assessments and language samples to examine whether expressive language scores have different associative relationships with ASD symptomatology depending on the type of language assessment. Furthermore, measures of ASD symptomology use tasks that provide opportunity for expressive language as a response format. Because of this task demand, and our interest in the relationship between language ability and ASD scores by the type of language measure used (standardized assessment versus language sample), we limited our study to examining expressive language.

We hypothesized that rates of ASD would be higher on the ADOS and ADI-R compared to the CARS-2, given the rates reported in previous studies. The different CARS-2 scores were expected to influence ASD classifications; however, we did not expect to see drastically different rates according to the specific cut-off score. Additionally, we predicted that nonverbal IQ and expressive language skills would account for a significant amount of variance in ASD severity on the ADOS, ADI-R, and CARS-2. Although the ADOS algorithm (Gotham, Pickles, & Lord, 2009) was created to limit the influence of language abilities on ASD symptom severity scores in idiopathic ASD, given the findings from the FXS literature, we anticipated a negative association between ASD characteristics measured using the ADOS and expressive language scores. Finally, we expected lexical and syntactic scores from language samples would be more strongly associated with ASD scores given that language sample elicitation techniques are relatively more social in nature compared to standardized assessments.

Methods

Participants

Thirty-three school-age and adolescent boys with FXS participated in the current study (mean age: 12.27 years, range: 9.0–16.4 years). The participants were from a larger study examining language abilities in boys with FXS and ASD (Haebig & Sterling, 2017; Sterling, 2018). FXS was confirmed via molecular genetic report. The participants had word-level to sentence-level language skills and were monolingual English speakers. Parents provided written informed consent and children provided verbal or written assent. Study procedures were approved by the institutional review board at (The University of Wisconsin-Madison). Participants were included in the current study if they had the full mutation of FXS and if data were successfully collected for all three ASD assessments of interest. The race of the 33 boys was reported to be as follows: 27 white, four African
American, one “other”, and one white and American Indian. Additionally, 27 boys were reported to be non-Hispanic, three boys were reported to be Hispanic, and ethnicity was not reported for three of the boys. See Table 2 for participant characteristics.

Predictors of ASD severity

Nonverbal IQ. Nonverbal IQ was assessed using the Leiter International Performance Scale, Revised (Leiter-R; Roid & Miller, 1997). Participants completed the Brief-IQ battery: Figure Ground, Form Completion, Sequential Order, and Repeated Patterns. The Leiter-R yields norm-referenced scores for individuals between 2 and 20 years of age. Standard scores were calculated based on a mean of 100 and a standard deviation of 15. The Leiter-R has been found to have high reliability and validity relative to other cognitive assessments (Roid, Nellis, & McLellan, 2003). Furthermore, it has been found to effectively estimate nonverbal cognitive abilities in individuals with FXS (e.g. Haebig et al., 2016; Sterling, 2018). In the current study, one participant did not complete the TEGI Screener and two participants did not complete the Past Tense Probe.

We also measured expressive language using language sample analysis. The participants completed a 12-minute conversation language sample with an experienced clinician. The examiner followed a semi-structured list of topics while using language elicitation strategies such as expectant pauses, requests for details, and avoidance of yes/no questions (Berry-Kravis et al., 2013). The Berry-Kravis et al. (2013) protocol was created for individuals with FXS and has continued to be an informative protocol for measuring expressive language (Spaulding, Plante, & Farinella, 2006) and has high test–retest reliability, ranging from .82 to .95 (Rice & Wexler, 2001). Additionally, the TEGI has been successfully used to measure expressive grammar in boys with FXS (Haebig et al., 2016; Sterling, 2018). In the current study, one participant did not complete the TEGI Screener score, which is derived from the Third Person Singular Probe and the Past Tense Probe. For each probe, the child views a colored image and the examiner provides a verbal prompt to create an obligatory context for specific linguistic forms (e.g. “This is a teacher. Tell me what a teacher does.”, “Here the boy is painting. Now he is done. Tell me what he did”; Rice & Wexler, 2001, p. 17 and 21). Examples of a target child response would include “She teaches” and “He painted”; however, if necessary, the examiner may provide the subject for the child and the child may produce the just the verb (e.g. examiner: “She…”, child: “teaches”). The percentage of accuracy was calculated for each probe and averaged to create the screening score; norm-referenced criterion scores are available. The TEGI has been found to be a reliable assessment to identify language impairments in children with acceptable sensitivity and specificity (Spaulding, Plante, & Farinella, 2006) and has high test–retest reliability, ranging from .82 to .95 (Rice & Wexler, 2001). Additionally, the TEGI has been successfully used to measure expressive grammar in boys with FXS (Haebig et al., 2016; Sterling, 2018). In the current study, one participant did not complete the TEGI Screener score, which is derived from the Third Person Singular Probe and the Past Tense Probe.

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Table 2. Participant characteristics.

|                      | Mean (SD) | Range    |
|----------------------|-----------|----------|
| Chronological age (in years) | 12.27 (1.96) | 9.00–16.42 |
| CARS-2 total score   | 29.52 (5.15) | 18.5–38  |
| ADI-R algorithm total| 36.82 (9.80) | 15–52    |
| ADOS algorithm total | 15.58 (5.38) | 3–28     |
| ADOS severity score  | 7.03 (1.76)  | 2–10     |
| Nonverbal IQ         | 45.55 (8.44) | 36–65    |
| Expressive vocabulary, EVT-2a | 57.45 (17.30) | 20–84    |
| Expressive grammar, EVT-2a | 63.60 (29.18) | 0–97.22  |
| TEGI Screenerb       |           |          |
| Expressive vocabulary, TEGI Screenerb | 148.31 (48.90) | 20–250   |
| Expressive grammar, MLUc | 3.35 (1.20)  | 1.12–6.38 |

CARS-2: Childhood Autism Rating Scale, second edition; ADI-R: Autism Diagnostic Interview, Revised; ADOS: Autism Diagnostic Observation Schedule; EVT-2: Expressive Vocabulary Test, second edition; TEGI: Test of Early Grammatical Impairment; NDW: number of different words; MLU: mean length of utterance.

aStandard scores.
bThree children did not complete both of the TEGI Screener subtests.
cOne child did not complete the language sample.

Our standardized language assessments included the EVT-2 (Williams, 2007) and the Test of Early Grammatical Impairment (TEGI; Rice & Wexler, 2001). The EVT-2 assesses expressive vocabulary skills. A child is shown a colored image and asked to name the image or respond to a question related to the image. Norm-referenced scores are derived from each participant’s raw scores (mean of 100, standard deviation of 15). The test–retest reliability of the EVT-2 is high, ranging from .94 to .97 (Williams, 2007). Furthermore, the EVT has been found to be an appropriate assessment to use with individuals with ASD and individuals with FXS who have a wide range of expressive vocabulary abilities (e.g. Kjelgaard & Tager-Flusberg, 2001; McDuffie et al., 2012). The TEGI was used as a measure of expressive syntax. In the current study, we used the TEGI Screener score, which is derived from the Third Person Singular Probe and the Past Tense Probe. For each probe, the child views a colored image and the examiner provides a verbal prompt to create an obligatory context for specific linguistic forms (e.g. “This is a teacher. Tell me what a teacher does.”, “Here the boy is painting. Now he is done. Tell me what he did”; Rice & Wexler, 2001, p. 17 and 21). Examples of a target child response would include “She teaches” and “He painted”; however, if necessary, the examiner may provide the subject for the child and the child may produce the just the verb (e.g. examiner: “She…”, child: “teaches”). The percentage of accuracy was calculated for each probe and averaged to create the screening score; norm-referenced criterion scores are available. The TEGI has been found to be a reliable assessment to identify language impairments in children with acceptable sensitivity and specificity (Spaulding, Plante, & Farinella, 2006) and has high test–retest reliability, ranging from .82 to .95 (Rice & Wexler, 2001). Additionally, the TEGI has been successfully used to measure expressive grammar in boys with FXS (Haebig et al., 2016; Sterling, 2018). In the current study, one participant did not complete the TEGI Screener score, which is derived from the Third Person Singular Probe and the Past Tense Probe.
ASD assessments

Observational measures. The ADOS-1 and ADOS-2 (Lord et al., 2012, 1999) is a semi-structured observational assessment. An examiner who was research-reliable or training to be research-reliable (with a research-reliable examiner present for live scoring and coding) administered the appropriate ADOS module according to the child’s language level. Four research-reliable ADOS examiners participated in the administration and coding. The boys in this study received either a Module 1 (word-level language), Module 2 (phrase-level language), or Module 3 (verbally fluent). Two participants received the Module 1, 18 received the Module 2, and 12 received the Module 3. The ADOS was completed at the end of the visit to ensure consistency across all participants in the study. We used the Gotham et al. (2009) autism severity scoring algorithm as our measure of ASD severity. The revised algorithm items have yielded high sensitivity and specificity (range: 82–100%; Gotham et al., 2008); however, the sensitivity and specificity of identifying ASD in individuals with intellectual disability is lower than the overall sensitivity and specificity findings (Lord et al., 2012).

The CARS-2, Standard Version (Schopler et al., 2010) consists of 15 items that quantify ASD characteristics. An examiner rates the child’s ASD characteristics based on observations and interactions with the child. As previously noted, the CARS-2 has high internal consistency ratings of .93 and correlates with clinical ratings of other ASD assessments that have been used with individuals with idiopathic ASD (classification agreement range = 70–100%; Schopler et al., 2010). At the end of the visit, the primary examiner (who had completed the language sample, TEGI, EVT-2, and Leiter-R), with input from the ADOS examiner, completed the CARS-2 based on observations from the entire visit. Therefore, CARS-2 scores included observations from the ADOS assessment as well as all other assessments and breaks. The primary examiner who scored the CARS-2 had received at least the clinical training for the ADOS, if not the research reliability ADOS training as well.

Parent report. Parent-reported ASD characteristics were measured using the ADI-R (Rutter et al., 2003). The ADI-R has been found to effectively measure ASD characteristics even in individuals with intellectual disability and to have high agreement with the ADOS (Bildt et al., 2004; Sappok et al., 2013). Two research-reliable ADI-R examiners completed the interviews. A parent was asked to describe current and past child behaviors to evaluate lifetime and current symptoms. Categorical classifications followed the guidelines specified by the revised diagnostic criteria for the ADI-R (Risi et al., 2006). The ADI-R total scores were calculated by summing the ADI-R A total score, B total score, C total score, and D total score. The total scores for sections A–D consisted of scores from items that were identified as the diagnostic algorithm items on the ADI-R forms (Rutter et al., 2003).

Analysis plan

To address our first question, we compared rates of ASD on the ADOS, ADI-R, and CARS-2 using a categorical approach. We identified participants who met classification cut-offs based on the norming information for each assessment. To address our second question, we assessed the different cut-off scores that have been identified in the previous literature for children with idiopathic ASD when using the CARS-2 (i.e. 25.5 and 28).

Our third research question was to examine the relationship between ASD characteristics and other child characteristics. Therefore, we subdivided our participants according to the children who were and were not classified as having ASD on all three of the assessments (ADOS, ADI-R, and CARS-2) and compared their chronological age and performance on the nonverbal IQ and language measures. Finally, we used the ADOS algorithm score, the ADI-R total score, and the CARS-2 total score to examine ASD characteristics as a continuous variable. We first examined correlations between the ADOS, ADI-R, and CARS-2 with chronological age, nonverbal IQ, and our measures of language production. After identifying the variables that were most strongly related to ASD symptom severity, we tested three linear regression models to examine predictors of ADOS, ADI-R, and CARS-2 scores.

Results

Research question 1: ASD rates

Thirty-two of the 33 boys (96.97%) with FXS met criteria for ASD on the ADOS. Thirty of the 33 boys (90.91%) met criteria on the ADI-R using the algorithm proposed by Risi and colleagues (2006). In terms of combined agreement, 29 of the 33 boys with FXS (87.88%) met criteria for ASD on both the ADOS and ADI-R. In contrast, 17 of the 33 boys (51.52%) met criteria for ASD on the CARS-2. Sixteen of the boys with FXS (48.48%) met criteria for ASD on all three measures (see Figure 1). Table 3 provides participant-level classifications.
Research question 2: ASD rates according to different CARS-2 cut-off scores

Next, we explored how the alternative CARS cut-off scores that have been suggested in the literature would influence the co-occurring ASD classifications in our FXS group. Using the 28-cut-off value, 20 of the 33 males with FXS (60.61%) were classified as having ASD. Based on this revised score cut-off, 19 of the participants (57.58%) met criteria for ASD across the CARS, ADOS, and ADI-R, compared to 48.48% with a CARS cut-off score of 30. In contrast, the 25.5 cut-off value resulted in 25 of the 33 males with FXS (75.76%) being classified as having ASD. Across all three assessments, 23 of the 33 participants (69.70%) met score cut-offs for an ASD classification with the 25.5 cut-off-value for the CARS.

Research question 3: Associations between ASD severity and child characteristics

To address our second research question, we first compared child characteristics between the 16 males with FXS who were classified as having co-morbid ASD (FXS+ASD) across the three ASD assessments and the 17 males with FXS who did not meet criteria for ASD on all three measures (FXS-Only, using the 30 cut-off for the CARS). We evaluated the significance of the six t-tests with a p threshold of .008 (Bonferroni corrected alpha = .05). The boys did not significantly differ in chronological age (p = .546) or TEGI scores (p = .069). However, the boys with FXS+ASD had significantly lower nonverbal IQ, MLU, number of different words (NDW), and EVT-2 standard scores relative to boys with FXS-Only (ps < .003; see Table 4).

Following our categorical ASD comparison approach, we examined our variables using a continuous approach. Bivariate correlation analyses examined the relationship among chronological age, nonverbal IQ and EVT-2 standard scores, TEGI scores, MLU, NDW, ADOS algorithm scores, ADI-R total scores, and CARS-2 total scores. Table 5 provides the full correlation matrix. We found differences in associations between child characteristics and ASD severity scores across the three ASD assessments. Notably, none of the variables were significantly associated with the ADI-R scores. However, nonverbal IQ, MLU, NDW, and EVT-2 values were significantly and negatively associated with ADOS algorithm scores. In addition, MLU, NDW, and EVT-2 scores were significantly and negatively correlated with CARS scores. See Table 5 for the full correlation matrix.

Three linear regression analyses were used to examine the amount of variance in scores on the ADOS, ADI-R, and CARS-2 that was explained by significant

| Table 3. Diagnostic classification for each measure and age and nonverbal IQ for each child. |
| Case no. | Chronological age in years | Nonverbal IQ | CARS-2 | ADOS | ADI-R |
|----------|---------------------------|-------------|--------|------|-------|
| 01       | 15.25                     | 56          | ASD    | ASD  | No ASD |
| 02       | 9.33                      | 54          | No ASD | ASD  | No ASD |
| 03       | 12.00                     | 60          | No ASD | ASD  | ASD   |
| 04       | 14.00                     | 38          | No ASD | ASD  | ASD   |
| 05       | 12.42                     | 42          | No ASD | ASD  | ASD   |
| 06       | 13.58                     | 52          | No ASD | ASD  | ASD   |
| 07       | 14.58                     | 44          | ASD    | ASD  | ASD   |
| 08       | 12.25                     | 50          | ASD    | ASD  | ASD   |
| 09       | 9.25                      | 56          | ASD    | ASD  | ASD   |
| 10       | 12.33                     | 48          | No ASD | ASD  | ASD   |
| 11       | 12.42                     | 52          | No ASD | ASD  | ASD   |
| 12       | 9.17                      | 65          | ASD    | ASD  | ASD   |
| 13       | 13.58                     | 38          | No ASD | ASD  | ASD   |
| 14       | 14.33                     | 40          | No ASD | ASD  | ASD   |
| 15       | 10.58                     | 52          | ASD    | ASD  | ASD   |
| 16       | 9.00                      | 58          | ASD    | ASD  | ASD   |
| 17       | 11.08                     | 44          | ASD    | ASD  | ASD   |
| 18       | 12.33                     | 40          | No ASD | ASD  | ASD   |
| 19       | 13.75                     | 38          | No ASD | ASD  | No ASD |
| 20       | 10.08                     | 48          | ASD    | ASD  | ASD   |
| 21       | 16.42                     | 48          | ASD    | ASD  | ASD   |
| 22       | 15.83                     | 36          | No ASD | ASD  | ASD   |
| 23       | 10.25                     | 36          | ASD    | ASD  | ASD   |
| 24       | 13.42                     | 38          | ASD    | ASD  | ASD   |
| 25       | 11.67                     | 36          | ASD    | ASD  | ASD   |
| 26       | 9.67                      | 58          | ASD    | ASD  | ASD   |
| 27       | 14.33                     | 40          | No ASD | ASD  | ASD   |
| 28       | 11.50                     | 36          | ASD    | ASD  | ASD   |
| 29       | 11.33                     | 40          | No ASD | ASD  | ASD   |
| 30       | 13.00                     | 36          | ASD    | ASD  | ASD   |
| 31       | 12.33                     | 42          | No ASD | ASD  | ASD   |
| 32       | 11.92                     | 46          | ASD    | ASD  | ASD   |
| 33       | 12.00                     | 36          | No ASD | No ASD | ASD |

CARS-2: Childhood Autism Rating Scale, second edition; ADOS: Autism Diagnostic Observation Schedule; ADI-R: Autism Diagnostic Interview, Revised; ASD: autism spectrum disorder.

[Figure 1. Percentage of ASD classifications by assessment. ASD: autism spectrum disorder; ADOS: Autism Diagnostic Observation Schedule; ADI-R: Autism Diagnostic Interview, Revised; CARS: Childhood Autism Rating Scale.]

[Table 3. Diagnostic classification for each measure and age and nonverbal IQ for each child.]

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Following our categorical ASD comparison approach, we examined our variables using a continuous approach. Bivariate correlation analyses examined the relationship among chronological age, nonverbal IQ and EVT-2 standard scores, TEGI scores, MLU, NDW, ADOS algorithm scores, ADI-R total scores, and CARS-2 total scores. Table 5 provides the full correlation matrix. We found differences in associations between child characteristics and ASD severity scores across the three ASD assessments. Notably, none of the variables were significantly associated with the ADI-R scores. However, nonverbal IQ, MLU, NDW, and EVT-2 values were significantly and negatively associated with ADOS algorithm scores. In addition, MLU, NDW, and EVT-2 scores were significantly and negatively correlated with CARS scores. See Table 5 for the full correlation matrix.

Three linear regression analyses were used to examine the amount of variance in scores on the ADOS, ADI-R, and CARS-2 that was explained by significant
child variables. For each regression model, the dependent variable was the ADOS severity score, ADI-R total score, or CARS-2 total score. Independent variables were mean-centered nonverbal IQ standard scores, EVT-2 standard scores, or NDW. We limited the number of independent variables within each model to no more than three given our sample size.

When examining associations between child characteristics and ADOS severity scores, we observed that NDW, MLU, nonverbal IQ, and EVT-2 scores were significantly correlated with ADOS severity score. We limited our independent variables to NDW and nonverbal IQ, and left out MLU and EVT-2 because MLU was highly correlated with NDW \( (r = .86) \) and EVT-2 scores were highly correlated with nonverbal IQ \( (r = .71) \). Together, NDW and nonverbal IQ accounted for a significant amount of variance in ADOS algorithm scores, \( F(2, 29) = 7.16, p = .003, R^2 = .331 \). When looking at the contribution of each independent variable to the model, NDW accounted for a significant amount of unique variance in ADOS scores, but nonverbal IQ only approached significance.

As reported above, none of the variables were significantly correlated with the ADI-R total scores; however, the variables that had the highest Pearson’s \( r \) values included nonverbal IQ and EVT-2 standard scores \( (r = -.24 \text{ and } r = -.24) \), respectively. Given that nonverbal IQ and expressive language have been found to explain variance in ADI-R scores (Kaufmann et al., 2004), we included nonverbal IQ and EVT-2 scores in our regression model. In this analysis, we shifted our aim from testing for unique predictors of ADI-R scores, to testing whether nonverbal IQ and EVT-2 scores together explained variance in ADI-R scores.

### Table 4. Differences in age, language and nonverbal IQ in boys with FXS who did (FXS + ASD) versus did not (FXS-Only) meet ASD diagnostic criteria on all three ASD measures.

|                  | Boys with FXS + ASD | Boys with FXS-Only | t (df) | \( p^a \) | Cohen’s \( d \) |
|------------------|---------------------|--------------------|-------|-------|-------------|
| Chronological    |                     |                    |       |       |             |
| age in years     |                     |                    |       |       |             |
| Nonverbal IQ     | 40.88 (6.24)        | 49.94 (7.97)       | 3.62  | .001  | 1.27        |
| MLU              | 2.71 (0.94)         | 3.92 (1.14)        | 3.25  | .003  | 1.16        |
| NDW              | 121.47 (44.28)      | 172.00 (40.58)     | 3.37  | .002  | 1.19        |
| EVT-2            | 44.50 (15.03)       | 69.65 (7.83)       | 6.08  | .000  | 2.10        |
| TEGI Screener    | 53.26 (34.28)       | 72.65 (21.00)      | 1.90  | .069  | 0.68        |

\( ^a \)Significant \( p \) values were set at a \( p \) threshold of .008 (Bonferroni corrected alpha = .05).

Nonverbal IQ = Leiter-R (Roid & Miller, 1997); EVT-2: Expressive Vocabulary Test, second edition (Williams, 2007); MLU: mean length of utterances in morphemes; NDW: number of different words; TEGI Screener: Test of Early Grammatical Impairment Screener (Rice & Wexler, 2001).

Source: reproduced with permission from Roid & Miller, 1997; Williams, 2007; Rice & Wexler, 2001.

### Table 5. Bivariate Correlations.

|       | ADOS | ADI-R | CARS-2 | Age | NV IQ | MLU | NDW | EVT-2 | TEGI |
|-------|------|-------|--------|-----|-------|-----|-----|-------|------|
| ADOS  | -    | .25   | .75*   | .13 | -.41b | -.43b| -.51a| -.39b | -.12 |
| ADI-R | -    | .10   | .06    | -.28| .11   | .09 | -.28| .07   |      |
| CARS-2| -    | -.04  | -.34   | -.56a| -.60a | -.63a| -.28|       |      |
| Age   | -    | -.43b | .13    | .28 | .26   | .71a| .19 |       |      |
| NV IQ | -    | .28   | .86a   | .52a| .47b  | .49a|     |       |      |
| MLU   | -    | .86a  | .50a   | .58a|       |     |     |       |      |
| NDW   | -    | -.52a | .52a   | .47b|       |     |     |       |      |
| EVT-2 | -    | -.49a | -.49a  | .47b|       |     |     |       |      |
| TEGI  | -    |       |        |     |       |     |     |       |      |

\( ^* p < .010. \)

\( ^b p < .050, \) two-tailed.

ADOS: Autism Diagnostic Observation Schedule algorithm score; ADI-R: Autism Diagnostic Interview, Revised total score; CARS-2: Childhood Autism Rating Scale, second edition total score; NV IQ: Nonverbal IQ = Leiter-R (Roid & Miller, 1997); EVT-2: Expressive Vocabulary Test, second edition (Williams, 2007); MLU: mean length of utterances in morphemes; NDW: number of different words; TEGI: Test of Early Grammatical Impairment Screener (Rice & Wexler, 2001).

Source: reproduced with permission from Roid & Miller, 1997; Williams, 2007; Rice & Wexler, 2001.
This alternative approach was more fitting given that nonverbal IQ and EVT-2 scores were highly intercorrelated ($r = .71$) and were unlikely to reveal unique predictors when included in a model together. Nonverbal IQ and EVT-2 scores did not predict significant variance in ADI-R total scores, $F(2, 30) = 1.11, p = .344, R^2 = .069$. Also, as expected, neither variables were significant unique predictors.

Finally, NDW, MLU, and EVT-2 were significantly correlated with CARS-2 total scores; however, NDW and MLU were highly correlated ($r = .86$) and NDW and EVT-2 were highly correlated and conceptually similar ($r = .52$). Given the multicollinearity between NDW and MLU and moderate association between NDW and EVT-2 scores, we examined whether incorporating NDW into the regression model significantly increased the explained variance in CARS-2 total scores. Our model comparison, comparing a model that included only EVT-2 and MLU as independent variables and a model that included EVT-2, MLU, and NDW as independent variables, indicated that the inclusion of NDW did not significantly increase the explained variance in CARS-2 total scores ($p = .209$). Together, EVT-2 and MLU explained 45% of variance in CARS-2 total scores, $F(2, 29) = 11.81, p < .001, R^2 = .449$. Furthermore, both variables accounted for a significant amount of unique variance in CARS-2 total scores. See Table 6 for full regression findings. Although EVT-2 and MLU scores were derived from assessments that used considerably different procedures (standardized norm-referenced assessment vs. naturalistic conversation language sample, respectively), the two measures were moderately associated. This association did not exceed a Pearson’s $r$ of .50. As such, to address potential concerns of multicollinearity, we conducted follow-up analyses (Fox, 2008). First, we regressed CARS-2 total scores on MLU scores. Then we calculated the residuals (i.e., unexplained variance in CARS-2 total scores) and then we regressed these residuals on EVT-2 scores, which revealed that EVT-2 scores still explained unique variance in CARS-2 total scores ($\beta = -0.39, 95\% CI (-0.19$ to $-0.01))$.

Discussion

Although the high rates of co-occurring ASD in FXS are well-documented in the literature, the specific rates vary substantially. These varying rates may be influenced by the different ASD assessments that have been used across the studies. The current study sought to develop a better understanding of how these rates compare across three commonly used ASD assessments. Therefore, the current study compared ASD scores and classifications from the ADOS, ADI-R, and CARS-2 in a sample of 33 boys with FXS. It was important to compare the classification rates in the same sample of participants, given that it is well-documented that child characteristics may influence scores on the ASD assessments.

Rates of ASD and FXS

In the current study, overall rates of ASD found in boys with FXS were highest when using the ADOS and ADI-R; these assessments classified the majority of boys as having co-occurring ASD. In contrast, the CARS-2 classified just over half of the participants as having co-occurring ASD. This difference is particularly striking given that the ADOS, ADI-R, and CARS-2 have high sensitivity and specificity in idiopathic ASD. Despite the contrast between the CARS-2 and the ADI-R and ADOS, the categorization rates for each assessment were mostly within the range of the rates reported across different studies. It is important to note though, that the rates of ASD categorization in the current study were fairly high relative to some of the other rates that have been reported, especially for the ADOS and ADI-R (outlined in Table 1).

Furthermore, previous studies have found high agreement between the ADOS and CARS-2 in children with idiopathic ASD (i.e. above 88%; Ventola et al., 2006). In our study, there was only 58% agreement between the ADOS and CARS-2 when using a

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**Table 6. Summary of multiple regression analyses of expressive language and vocabulary predicting ADOS, ADI-R, and CARS-2 scores.**

| Variables | $B$   | $SE_{B}$ | $\beta$  | 95% CI        |
|-----------|-------|----------|----------|---------------|
| ADOS      |       |          |          |               |
| Intercept | 15.38 | 0.80     | 13.74 to 17.01 |
| Nonverbal IQ | -0.17 | 0.10     | -0.37 to 0.03 |
| NDW       | -0.05 | 0.02     | -0.08 to -0.01 |
| ADI-R     |       |          |          |               |
| Intercept | 41.12 | 1.77     | 37.51 to 44.74 |
| Nonverbal IQ | -0.21 | 0.30     | -0.39 to 0.21 |
| EVT-2     | -0.09 | 0.15     | -0.34 to 0.44 |
| CARS-2    |       |          |          |               |
| Intercept | 29.30 | 0.69     | 27.89 to 30.70 |
| MLU       | -1.43 | 0.67     | -2.80 to -0.06 |
| EVT-2     | -0.13 | 0.05     | -0.34 to -0.02 |

*p < .050.

B: Unstandardized regression coefficient; $\beta$: standardized coefficient; CI: confidence interval; $SE_{B}$: standard error of the coefficient; EVT-2: Expressive Vocabulary Test, second edition (Williams, 2007); Nonverbal IQ: Leiter-R (Roid & Miller, 1997); MLU: mean length of utterances in morphemes; NDW: number of different words; ADOS: Autism Diagnostic Observation Schedule; ADI-R: Autism Diagnostic Interview, Revised; CARS-2: Childhood Autism Rating Scale, second edition.

Source: reproduced with permission from Williams, 2007 and Roid & Miller, 1997.
Participant 33 was in the younger half of our sample, other boys with FXS had lower ADI-R total scores. The children who were not classified as ASD on the CARS-2 had lower ADOS severity scores (mean = 6.19) than the children with FXS who were classified as ASD on the CARS-2 (ADOS severity score mean = 7.82; t(31) = 2.98, p = .006). Additionally, the last item on the CARS-2 (item 15) asks the rater to indicate the general impression of the child's ASD characteristics, with a scores ranging from 1 indicating “no autism spectrum disorder” to 4 indicating “severe autism spectrum disorder”. Although item 15 on the CARS-2 is not supposed to represent an average of the scores for the previous items that assess specific characteristics, the scores for CARS-2 items 1–14 were on average higher for the children who were classified as having ASD on the CARS-2 (t(31) = 8.75, p < .001) and the scores for item 15 were highly correlated with the sum of the scores for CARS-2 items 1–14 (r = .84) and the ADOS severity score (r = .58). These results suggest that, although the scores for item 15 are subjective and influence the overall score on the CARS-2, the ratings were in line with the scores on the other items on the CARS-2 and aligned with the ADOS severity scores.

The ADI-R did not significantly correlate with the ADOS or the CARS-2. The absence of a correlation between the ADI-R and the ADOS and CARS-2 score is likely associated with the fact that questions on the ADI-R focuses on behaviors that were primarily exhibited when the child was 4–5 years of age; in contrast, the ADOS and CARS-2 scores reflect current behavior. However, as expected, the ADI-R diagnostic classification had excellent agreement with the ADOS diagnostic classification. Additionally, there was overlap in the child variables that were correlated with the ADOS and CARS-2. Although the ADOS and CARS-2 scores reflect some overlap, the relatively low categorical agreement suggests that these assessments may not pick up on the same ASD-related characteristics or may weigh these features differently.

One striking finding was that the ADOS identified every participant except participant 33 as having co-occurring ASD. To attempt to gain insight into potential reasons why this participant was the sole individual not classified as having ASD on the ADOS, we further examined his other characteristics. In comparison to the other children, participant 33 had the second lowest CARS-2 total score (21.5). Interestingly, six other boys with FXS had lower ADI-R total scores. Participant 33 was in the younger half of our sample, though he was two years older than the youngest participant (participant 33: 11.08 years, youngest participant: 9.0 years). His nonverbal IQ (participant 33: 44, group mean: 45.55) was slightly lower than the group’s mean IQ, as was his TEGI Screener score (participant 33: 62.78, group mean: 63.60). He also scored in the lower half of the sample on MLU (participant 33: 2.89, group mean: 3.55; however, here again, he was well above the lowest MLU score (1.12). Finally, his vocabulary scores (participant 33: EVT-2 = 67, group mean: 57.45, and participant 33: NDW = 170, group mean: 148.31) were slightly higher than the sample means, placing him in approximately the top 70% of the sample. Although the participant’s age, nonverbal IQ, and language characteristics do not clearly explain why he was the only participant who was not classified as ASD on the ADOS, it provides additional information that could be compared to participant samples with FXS-Only.

Across the literature, weaknesses in each ASD assessment have been discussed. Notably, the items on the CARS-2 are based on the narrower definition of autism (Creak, 1961; Kanner, 1943). Additionally, the assessment items of the CARS-2 have not been updated since the first edition, which was published in 1988, despite the fact that the definition of ASD has evolved in the DSM (DSM-III, Revised 1987, DSM-IV 1994, DSM-V 2013). Additionally, unlike the ADOS, the CARS-2 does not contain specific questions assessing pragmatic language. Also, the CARS-2 does not have stringent training protocols, in contrast to the ADOS and ADI-R. However, previous studies that have reported good psychometric properties of the CARS also note that it is easy to train clinicians to reliably use the CARS fairly quickly (Filipek et al., 1999; National Research Council, 2001). Although the ADOS and ADI-R are considered to be the gold-standard diagnostic tools for assessing idiopathic ASD, previous research has questioned the appropriateness of these tools in examining ASD in individuals with FXS. For instance, Hall et al. (2010) suggested that the ADOS may produce false positives when used with males with FXS. In fact, FXS researchers have suggested that adaptations may need to be made when using these assessments in order to more accurately characterize ASD characteristics in individuals with FXS, in order to avoid scores being overly influenced by low nonverbal IQ or behaviors associated with anxiety which are characteristic of FXS (Abbeduto et al., 2019; Harris et al., 2008; Hogan et al., 2017). Alternatively, it may be more appropriate to use these assessments as a guide for documenting ASD characteristics, and more heavily emphasize the importance of best clinical estimate of classification. Best estimates have been used in previous ASD
research (e.g. Bal, Kim, Fok, & Lord, 2018; Ray-Subramanian, Huai, & Ellis Weismer, 2011) and have begun to be used in the FXS literature (e.g. Caravella & Roberts, 2017; Hogan et al., 2017).

**Associations between ASD assessments and child characteristics**

Contrary to our hypothesis, although nonverbal IQ was significantly correlated with ADOS algorithm scores, nonverbal IQ did not explain a significant amount of variance in ADOS, ADI-R, or CARS-2 scores. However, as in other studies, our participants who were classified as having ASD using all three assessments, had significantly poorer nonverbal IQ scores (Bailey et al., 2001; Kau et al., 2004). Thus, our findings partially align with the association between nonverbal IQ and ASD scores using the ADOS, ADI-R, and CARS-2 (Bailey et al., 2001; Demark et al., 2003; Kaufmann et al., 2004; Lee et al., 2016; Thurman et al., 2015). In considering the lack of strong relationships between nonverbal IQ and ASD scores, it is important to note that all participants had an intellectual disability, limiting the variance in scores.

In terms of language, our bivariate correlations revealed significant associations between ADOS and CARS-2 scores and language scores (MLU, NDW, and EVT-2). Given the high correlation values for the EVT-2 and NDW, the association between language and ADOS and CARS-2 scores seemed most notable for expressive vocabulary abilities. Interestingly, significant correlations appeared for language scores that came from a standardized test (EVT-2) and a language sample (MLU, NDW). Therefore, although the two assessment protocols differ, it does not appear to be the case that these specific measures derived from language samples overly highlight social deficits, rather than structural language skills. Because we were careful to avoid problems with multi-collinearity, we did not include all significant variables from the bivariate correlations into our regression models. Therefore, although there were slight differences in the specific unique predictors of ADOS and CARS-2 scores, language abilities (vocabulary abilities in particular) predicted scores on both measures. The ADOS algorithm (Gotham et al., 2009) was intended to minimize the influence of language abilities on ASD scores; however, previous studies in FXS have reported associations between language and ADOS scores (Abbeduto et al., 2019; Rogers et al., 2001; Thurman et al., 2017, 2015). In contrast, the CARS-2 is an observational measure that does not differ based on the child’s language skills. Furthermore, it only has one item that specifically prompts examiners to evaluate the child’s language abilities. Scores on this language item can be influenced by multiple aspects of language, including repetitive language, pronoun reversal, neologisms, tonal quality, as well as vocabulary and sentence structure (Schopler et al., 2010). The ADOS incorporates many items that more specifically assess different aspects about expressive communication (e.g. conversation, quality of social overtures and responses, reciprocal social communication). Despite these differences, both the CARS-2 and the ADOS scores are associated with language scores. This relationship may indicate that individuals with FXS who have low language abilities are more likely to display ASD characteristics and obtain higher scores on the ADOS and CARS-2.

Additionally, counter to our predictions, none of the child variables correlated with the ADI-R scores. Fewer studies have examined the association between child characteristics and ADI-R scores, but of the studies that have, nonverbal IQ has been found to be negatively associated with ADI-R scores (Kau et al., 2004; Kaufmann et al., 2004). Very few studies have examined the association between language and ADI-R scores. Klusek et al. (2014b) found non-significant associations between ADI-R scores and receptive and expressive vocabulary scores. These findings may stem from the fact that the ADI-R is heavily influenced by the child’s ASD characteristics when the child was younger, whereas the predictor variables represent concurrent abilities in language or cognitive skills.

Our results do not provide definitive evidence of the impact of nonverbal IQ on ASD severity. However, they raise interesting questions about the relationship between nonverbal IQ and ASD severity as measured by the ADOS and ADI-R versus the CARS-2 in this sample of school-age and adolescent males with FXS. Our findings also highlight the association between expressive language skills as ASD characteristics, particularly in studies using the CARS-2.

**Study limitations and future directions**

While the present study provides some important information in terms of agreement between ASD diagnostic measures, there are limitations. First, the sample size was limited and only included school-age males with FXS; as such, our study does not provide information regarding rates of ASD for younger children or adults with FXS, or females with FXS. Additionally, an inclusionary criterion required the boys to communicate verbally. Therefore, our study does not address the rate of ASD in individuals with FXS and associations between child characteristics and ASD features in individuals with FXS who are minimally verbal. Second, we did not collect inter-rater reliability data for the ADOS, ADI-R, or CARS-2 throughout the study. Each examiner who completed the ADOS and
ADI-R completed the clinical or research training and met research reliability (inter-rater reliability on the overall scores and algorithm scores of at least 80% across three consecutive administrations with a research-reliable examiner). Examiners either had research reliability or worked toward research reliability throughout the study and therefore, inter-rater comparisons and discussions occurred frequently. Examiners who scored the CARS-2 also had participated in the ADOS clinical training, thoroughly read the CARS-2 manual, and double-scored several CARS-2 protocols and discussed the scores with an examiner who was trained to use the CARS-2. Our reliability procedures were in line with previous published studies examining ASD in FXS (e.g. Demark et al., 2003; Hall et al., 2008; Harris et al., 2008; Klusek et al., 2014b; Thurman et al., 2015). Despite this, the lack of reliability scores is a limitation in the current study. Third, we did not assess other child characteristics, such as anxiety, which has previously been linked to ASD behavioral characteristics in males with FXS (Roberts et al., 2018), and would be an important next step. Fourth, we focused on ASD and non-ASD classifications according to the specific scores for each ASD assessment; we did not include a clinical judgment component, which would have more in line with best practice. Fourth, including a comparison group of participants with idiopathic ASD would provide important information as to how these language measures are related to the ASD diagnostic measures. Finally, we focused on expressive language measures. However, given that receptive language skills are also impaired in FXS, future studies should carefully consider receptive language.

Future studies should compare rates of ASD between measures in younger children with FXS to understand how these symptoms emerge at early ages. Following these same children over time will shed light on the question of the stability of ASD characteristics in individuals with FXS. Also, future studies should include a clinical best estimate for a diagnosis of ASD in addition to the assessment measures (see Klusek et al., 2014b) for a more in-depth examination of how these measures relate to and inform clinical diagnoses. Another important avenue for future studies is to expand the independent variables examined that may account for variance in scores on assessments of ASD. Language comprehension is often significantly impaired in idiopathic ASD, yet the relationship between receptive and expressive language in children with FXS + ASD has received little research attention (Haebig & Sterling, 2017). Future studies should include measures of vocabulary and grammatical comprehension and their role in accounting for ASD severity in individuals with FXS.

Summary and conclusions

The current study was the first to compare ASD classification rates in a sample of males with FXS using three commonly used ASD assessments—the ADOS, ADI-R, and CARS-2. We found that the ADOS and ADI-R classified most of our sample as having co-occurring ASD; conversely, when using the traditional cut-off score of 30, the CARS-2 classified just over half of the participants as having co-occurring ASD. Although the ADOS and CARS-2 categorical classifications only partially overlapped, their scores were strongly correlated, which may indicate that these assessments may weigh ASD-related features differently or may assess only partially overlapping characteristics. Interestingly, our study also found that expressive language abilities, especially expressive vocabulary, accounted for a significant amount of variance in ADOS and CARS-2 scores. The current findings allow for an enhanced understanding of the degree of overlap among the ADOS, ADI-R, and CARS-2 when used within the same sample of school-age and adolescent males with FXS. These findings will facilitate the interpretation of other studies that use one or more of these ASD assessments with males with FXS. Our knowledge of ASD symptomatology in males with FXS is growing, but additional research is needed to optimize our understanding of ASD characteristics within the context of FXS and the most appropriate way to assess these features.

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