Predicting Change in Autism Symptomatology in Young Children at Risk for Autism Spectrum Disorder: Fragile X Syndrome, Down Syndrome and Non-Syndromic ASD

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PREDICTING CHANGE IN AUTISM SYMPTOMATOLOGY IN YOUNG CHILDREN AT RISK FOR AUTISM SPECTRUM DISORDER: FRAGILE X SYNDROME, DOWN SYNDROME AND NON-SYNDROMIC ASD

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ABSTRACT

This dissertation is comprised of two manuscripts which examine the longitudinal development of autism symptomatology in young children at risk for developing autism spectrum disorder (ASD); individuals with Fragile x syndrome (FXS) and Down syndrome (DS). The first study is a within group analysis of the longitudinal development of ASD symptomatology in young children with FXS, and how diagnostic stability, language and non-verbal cognitive functioning may predict these trajectories. This paper provides insight into ASD diagnostic stability patterns within FXS, and how symptoms change over time across these groups. The second paper will extend this work by presenting a pilot cross-syndrome comparison, which includes young children with FXS, DS and non-syndromic ASD to examine hypothesis of unique ASD symptom trajectories between the disorders. This pilot examination will set the stage for future larger scale cross syndrome comparisons to target these important questions. Together, the findings presented here inform the theoretical understanding of ASD within FXS, as well as support clinical recommendations for screening, monitoring and diagnosing ASD within FXS.
TABLE OF CONTENTS

Abstract ..................................................................................................................................................... iii

List of Tables ............................................................................................................................................... v

List of Figures ........................................................................................................................................... vi

List of Abbreviations ............................................................................................................................... vii

Chapter 1: Predicting Change in Autism Symptomatology in Young Children with
Fragile X Syndrome .................................................................................................................................... 1

Chapter 2: Cross-Syndrome Contrasts of Autism Symptoms in Young Children: Fragile
X, Down Syndrome and Non-Syndromic Autism ................................................................................... 32

References ............................................................................................................................................... 54
LIST OF TABLES

Table 1.1 Participant Demographics for FXS Males ......................................................... 29

Table 1.2 Results of a taxonomy of multilevel models for change in BOSCC scores over time ........................................................................................................................................ 30

Table 2.1 Mean BOSCC scores by group across time ............................................................... 52
LIST OF FIGURES

Figure 1.1. Uncontrolled effects of diagnostic stability group on BOSCC scores over time .......................................................... 31

Figure 2.1. Individual spaghetti plots of BOSCC total score trajectories between groups ................................................................. 53
LIST OF ABBREVIATIONS

ADI ................................................................. Autism Diagnostic Interview
ADOS ............................................................. Autism Diagnostic Observation Schedule
ASD ................................................................. Autism Spectrum Disorder
ASIB ................................................................. Autism Infant Sibling
BOSCC ......................................................... Brief Observation of Social Communication Change
CBE ................................................................. Clinical Best Estimate
DS ................................................................. Down Syndrome
ELC ............................................................... Mullen Early Learning Composite
FXS ................................................................. Fragile X Syndrome
ID ................................................................. Intellectual Disability
NSASD ........................................................... Non-syndromic Autism Spectrum Disorder
SCQ ............................................................... Social Communication Questionnaire
CHAPTER 1
PREDICTING CHANGE IN AUTISM SYMPTOMATOLOGY IN YOUNG CHILDREN WITH FRAGILE X SYNDROME

Autism Spectrum Disorder (ASD) is a behaviorally defined disorder characterized by core impairments in social communication and the presence of atypical restricted interests and repetitive behaviors (American Psychiatric Association, 2013). ASD is a heterogeneous disorder, with cognitive, language and adaptive impairments ranging significantly between individuals. This significant heterogeneity has led the field to shift focus to the identification of possible distinct phenotypes within the autism spectrum, labeled the “autisms” by some (Geschwind & Levitt, 2007). Early work aimed at identifying mechanisms targeted genetic factors given the established heritability present in twin studies (Geschwind, 2011). However, genetic biomarkers underlying ASD are complicated with the identification of over 1000 potential risk genes (Vorstman et al., 2013), and at least 4 single gene disorders with significant relationships to ASD (Geschwind, 2011).

Fragile X syndrome (FXS) has the highest penetrance of any single gene disorder implicated in ASD, with between 60-75% of individuals meeting criteria for ASD (Harris et al., 2008; Klusek, Martin, & Losh, 2014) and upwards of 90% exhibiting at least one symptom (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000). Males with FXS and ASD also exhibit a more homogenous behavioral phenotype (e.g. intellectual disability, language delay) than individuals with non-syndromic ASD (nsASD), making it an ideal
model for the study of the development of ASD. By examining a more homogenous
group of individuals with ASD, targeted treatments, similar to the precision medicine
approach in the physical health field, may be achieved.

FXS is caused by an expansion on the \textit{FMR1} gene of the CGG repeat sequence on
the X chromosome. In the typical population, average CCG repeat length is less than 55,
with expansions of 55-200 repeats considered the fragile X premutation. When the
expansion exceeds 200 repeats, a reduction in Fragile X Mental Retardation Protein
(FMRP) ensues. FMRP is essential for cognitive development (Garber, Visootsak, &
Warren, 2008). Males and females with FXS are differentially impacted by the disorder
given the protective factor afforded to females by the presence of a second, typically
unaffected, X chromosome. Therefore, females with FXS are often less severely impaired
across a range of domains than males (Hagerman & Hagerman, 2002).

**Autism Features in FXS**

Core features of ASD including social impairments and restricted and repetitive
behaviors are generally accepted as common behavioral features in FXS, however
questions remain as to the whether or not these features may occur on a spectrum within
individuals with FXS or if they represent a true comorbidity of ASD. These questions
have been part of an ongoing discussion in the literature for the last 30 years in an
attempt to explain the elevated proportion of males with FXS and ASD. Initial theories to
explain the elevated features of ASD in FXS (Cohen et al., 1991) proposed possible
pathways driven by biological markers of FXS (e.g. FMRP) or secondary consequences
of almost universal intellectual disability. Recent work has sought to provide clarity on
this issue by directly testing these relationships. Hall and colleagues examined the
relationships between parent reported autism symptoms and biological markers (i.e. FMRP) and cognitive functioning in a sample of 120 individuals with FXS 5-25 years of age. Cognitive functioning, but not FMRP, was related to autism symptomatology with lower cognitive functioning associated with greater parent reported autism symptomatology (Hall, Lightbody, Hirt, Rezvani, & Reiss, 2010). The null relationship between FMRP and autism symptomatology is consistent with previous work reporting similar findings (Donald B Bailey Jr, Hatton, Skinner, & Mesibov, 2001; Rogers, Wehner, & Hagerman, 2001).

Others have approached the question of unique and shared ASD features in FXS and non-syndromic ASD through a comparative behavioral lens. This approach explores whether behavioral features across individuals with nsASD and FXS with comorbid autism (FXS+ASD) are parallel, which would provide evidence that ASD in both groups could arise from a similar etiology. Findings from this body of work suggest a developmental effect in which autism symptomatology is remarkably similar between 21-48 month olds with FXS+ASD and nsASD (Rogers et al., 2001), with a unique behavioral phenotype of strengths in the quality and frequency of social overtures in those with FXS+ASD, that is not seen in nsASD, becoming evident over time (McDuffie, Thurman, Hagerman, & Abbeduto, 2014; Wolff et al., 2012). These findings have led some researchers to hypothesize that the features of ASD seen in FXS do not arise from the same mechanistic underpinnings (Abbeduto, McDuffie, & Thurman, 2014; Hall et al., 2010). This is supported by evidence of structural brain abnormalities in young children with nsASD that differ from those with FXS+ASD despite similar behavioral features of ASD across the two groups (Hazlett et al., 2009).
Despite possible mechanistic differences giving rise to shared features of ASD across groups, consistency exists in findings that individuals with FXS+ASD are more impaired across a range of domains that those with FXS without ASD (FXS-noASD) including cognition (Bailey, Hatton, Tassone, Skinner, & Taylor, 2001; Hogan et al., 2017; Kaufmann et al., 2004), adaptive behavior (Caravella & Roberts, 2017; Hahn, Brady, Warren, & Fleming, 2015), language (Klusek et al., 2014), motor skills (Bailey et al., 2000) and social approach behaviors (Roberts, Weisenfeld, Hatton, Heath, & Kaufmann, 2007). Additionally, children with FXS+ASD receive more hours of speech-language and behavioral therapy than those without an ASD diagnosis (Martin et al., 2013). Therefore, identifying ASD features in FXS is essential for providing access to the appropriate dose and type of intervention required to reduce functional impairment. Identifying predictors of ASD in young children with FXS is essential towards efforts to improve treatment with several features emerging as salient. Sex is one of the most well-established predictors of ASD in FXS, with males being significantly more likely to show impairing symptoms of ASD as well as meet diagnostic criteria (Hall et al., 2010; Klusek et al., 2014). Cognitive level and language ability have also been identified to predict autism symptomatology in FXS beginning in infancy (Hogan et al., 2017) and maintaining through childhood and early adolescence (Klusek et al., 2014). Greater pragmatic language impairments have also been found as predictors of increased autism symptom severity (Lee, Martin, Berry-Kravis, & Losh, 2016).

**Stability of Autism Symptomatology in nsASD and FXS**

Stability of a positive ASD diagnosis in non-syndromic populations has been reported to be between 53-100% (Woolfenden, Sarkozy, Ridley, & Williams, 2012). In
community samples, autism is generally stable (~90%) by 2 years of age when using a clinical best estimate (CBE) procedure informed by gold standard measures of autism symptomatology (e.g. Autism Diagnostic Observation Schedule), developmental measures and clinical judgment (Chawarska, Klin, Paul, & Volkmar, 2007). Instability in ASD diagnoses presents in individuals who display milder features or inconclusive presentations at 2 years old (Guthrie, Swineford, Nottke, & Wetherby, 2013). In a sample of 418 high risk infant siblings of children with ASD assessed at 18, 24 and 36 months, diagnostic stability at 24 months was 82% (Ozonoff et al., 2015). However, when the sample was followed to age 3, 41% of the sample who met diagnostic criteria for ASD at 3 years old were determined to have been false negatives at the 24 month visit (Ozonoff et al., 2015). Early false negatives were found to be behaviorally atypical at 24 months (e.g., developmental delays, presence of autism symptomatology), however, their presentation was not yet clear enough to meet diagnostic criteria for ASD. Taken together, this research suggests that diagnostic stability in toddlers with nsASD is dependent on clarity of the clinical presentation, which may not occur until 3 years of age or later, highlighting the need for the monitoring of autism symptomatology into the third year of life and beyond. Also, evidence suggests when instability occurs, it more often represents false negatives at a young age than false positives.

Within the FXS literature, less is known about diagnostic stability across early childhood with no published studies using a CBE process to inform diagnosis of ASD. Research into the stability of ASD diagnoses and symptomatology in FXS to date has largely relied on diagnostic categorizations determined by measure cutoffs, instead of clinician judgment. Hernandes and colleagues (2009) used the ADI-R, a parent interview
about lifetime autism symptomatology, to inform diagnoses of ASD in a sample of 56 males with FXS, who had a mean age of 56 months at baseline. They reported general diagnostic stability (68%) when participants were tested over the course of 3 years, with far less stability in individuals with milder symptomatology (21%). At the symptom level, the two diagnostic groups became less differentiated over the course of the study; participants in the FXS+ASD group evidenced a reduction in autism symptomatology while those with FXS-noASD, showed an increase in autism symptoms.

In a slightly older sample of 65 children with FXS (31 male, 34 female), ages 8-11 years, Lee and colleagues (Lee et al., 2016) used the ADOS to report diagnostic stability and symptom change over time across 2 visits that were on average 2.5 years apart. Across the 2.5 years, the percentage of male participants meeting criteria for ASD increased from 54% to 80%, with most of the increasing impairment found in the social communication domain. Rates of ASD were remarkably stable in the female participants, which were reported to be 41.5% at time 1, and 41.2% at time 2. The study did not report if any participants meeting criteria for ASD at time 1 failed to meet criteria at time 2, so exact stability estimates are unknown.

In the largest longitudinal study to date (n=116), Hatton and colleagues (2006) examined Childhood Autism Rating Scales (CARS-2, Schopler, Reichler, & Renner, 2002) total scores in a sample of 1.5 to 15 year olds. The CARS-2 is a clinician rated measure that is completed after an observation and interactions with a child to rate symptoms of ASD. It provides clinical categorizations into three groups based on raw score totals; non-autistic, mild-to-moderate autistic behaviors and severe autistic behaviors. The CARS-2 is considered to be a screener for ASD symptoms and is not a
diagnostic tool unless used in conjunction with other measures. When examining raw scores over time, the authors reported an average increase of 2 points over 10 years. Using the clinical categorizations provided by the CARS-2, the authors reported that about 54% of the participants maintained their diagnostic category throughout the course of the study, with 13% evidencing decreasing symptomatology and 33% evidencing increases. Taken together, these studies suggest ASD symptom stability rates of 50-70% within FXS. However, inferences about the diagnostic stability of clinical diagnoses of ASD within FXS are limited due to the reliance on the use of measure cutoffs to create diagnostic groups, rather than clinical decision making, in most current published work.

A more recent series of studies has emerged examining the stability of autism features in infants with FXS. One of these studies used a case study approach to compare 8 participants between 9-24 months of age with FXS+ASD and FXS-noASD outcomes at 24 months of age (Hogan et al., 2017). Although the sample size was small, comparisons suggested that autism symptomatology as measured by the Autism Observation Scale for Infants (AOSI, Bryson, Zwaigenbaum, McDermott, Rombough, & Brian, 2008) decreased in all participants between 9 and 12 months of age, with lower mean scores for the participants in the FXS-noASD group. In a complementary study, similar patterns were found in consistency of autism symptomatology between the 12 and 24 month time points, in that infants with ADOS scores above diagnostic cutoffs at 24 months evidenced higher AOSI scores at 12 months of age, than those who did not meet ADOS diagnostic cutoffs at 24 months (Roberts, Tonnsen, McCary, Caravella, & Shinkareva, 2016). These papers are important additions to the literature, in that they are the first to begin to
explore autism symptomatology and stability in infants with FXS syndrome, when autism symptoms often first emerge.

Taken together, these studies suggest variability in autism symptomatology across development in young males with FXS, leading to inconsistent reports of diagnostic stability. This variability may reflect the dynamic nature of development suggesting that symptoms of ASD vary across time in individuals with FXS as other development processes are occurring, such as increases in language/communication abilities or increasing social demands. However, the significant variability in measurement tools and diagnostic practices (i.e. measure cutoffs vs. clinician judgment) may also be contributing to the inconsistent findings. Lastly, change in autism symptomatology in individuals with FXS may be subtle throughout development and therefore poorly captured by measures designed to produce categorical outcomes.

Challenges measuring change in autism symptomatology over time is not a unique problem in FXS research (Anagnostou et al., 2015). Studies examining change in core autism symptomatology in nsASD are also limited given that the gold standard measures used in many studies such as the ADOS or ADI-R are diagnostic measures, with limited score ranges that are intended to measure presence or absence of ASD symptoms. A new measure, the Brief Observation of Social Communication Change (BOSCC, Grzadzinski et al., 2016) has been designed specifically to address these limitations in the ability to detect subtle change in autism symptomatology over time in current commonly used measures. The BOSCC measures many of the core features identified on the ADOS and ADI-R, however the coding ranges allow for greater variability, allowing for detections of subtler changes. Currently, two studies using the
BOSCC have been published; a paper examining the psychometric properties of the BOSCC (Grzadzinski et al., 2016) and a paper examining ASD symptom change following an early intervention program in 21 children with nsASD (Kitzerow, Teufel, Wilker, & Freitag, 2016). In both studies, changes in BOSCC scores were detected with small to medium effect sizes in as little as 6 months to 1 year, suggesting that the measure is robust to detecting findings over short periods of time, and in small samples (n = 21; Kitzerow et al., 2016).

**Current Limitations**

Across this body of work significant inconsistencies are present in the measures used to quantify change in autism symptomatology, which make comparisons across studies challenging. This is especially difficult given that some are parent report measures while others are based on trained clinician observations. Additionally, all measures reported (e.g. CARS-2, ADOS, ADI-R, AOSI), were not developed to measure change, making clinically significant change harder to identify. For example, Hatton and colleagues (Hatton et al., 2006) finding that CARS-2 scores increased 2 points over 10 years may have limited functional significance. Given that ASD is theoretically a lifelong condition, this small change in total score over a 10-year period may instead reflect reliability.

Other commonly used measures, such as the ADOS and ADI, are diagnostic tools that are not sensitive enough to capture change over short periods of time (Anagnostou et al., 2015; Grzadzinski et al., 2016). Given the diagnostic intention of these tools, some items are considered to be “low threshold”, in that if the symptom is present, it is coded. This approach, similar to a “present” or “absence” rating, it is not designed to
differentiate nuances in autism symptomatology that may be expected to change gradually over time. This is in contrast to the design of the BOSCC, which includes a greater scoring range (0-5 vs. 0-3) and the ability to capture subtleties in the frequency and quality of social communication behaviors and restricted interests/repetitive behaviors. Additionally, the commonly used practice of using the ADOS or ADI to both create the groups of interest (i.e., FXS+ASD vs FXS-noASD) and measure differences (i.e., how do these groups differ on ASD symptoms as measured by the ADOS?), is inherently flawed in the circular nature of these questions (i.e., if the ADOS was used to differentiate the groups of interest, we would expect the groups to be different on the ADOS). Therefore, a tool such as the BOSCC, which measures related but not identical constructs to gold standard diagnostic tools of ASD, reduces the consequences of the previously described practice.

**The Present Study**

The present study aims to address these gaps by utilizing a new measure designed to capture change in autism symptomatology in a sample of 2 to 6-year-old males with FXS. The BOSCC has been designed specifically to address limitations of current measures’ ability to accurately measure change in core features of autism. Additionally, predictors of these trajectories will be examined to determine both risk and protective factors of the development of impairing autism symptomatology within FXS. By looking at a range of predictors that have been previously identified as being related to co-morbid autism symptomatology in FXS, we aim to identify risk factors that may be important targets for intervention to improve functioning in young children with FXS. Lastly, this
study aims to add to the literature by examining trajectories based on diagnostic stability patterns, rather than using a single diagnostic timepoint to determine grouping status.

The present study aims to address the following research questions:

1) Do patterns of change in autism symptomatology differ in young males with FXS dependent on their CBE diagnosis of ASD, and the stability of that diagnosis?
   a. We hypothesize that children with FXS who have a CBE diagnosis of ASD will show increasing ASD symptom severity over time, while participants with FXS who do not have ASD will show minimal change in the severity of ASD symptomology.

2) Do cognitive and language functioning in males with FXS predict change in autism symptomatology over time?
   a. Consistent with previous literature, we hypothesize that lower cognitive and language functioning will be predictive of increasing autism symptom severity.

Methods

Participants

This study included 28 males with FXS assessed between the ages of 2-8 years. Females were excluded from the analysis because of small sample available for analysis (n=11), with only 1 female meeting diagnostic criteria for ASD. Each participant had at least 2 diagnostic assessments using the ADOS-2. The participants are drawn from a larger ongoing longitudinal study, herein referred to as the “parent study”, measuring the developmental trajectories of infants and preschool age children with FXS. Inclusion criteria for the study included gestational age >36 weeks, English as the primary language
in the home and no other known medical conditions. Participants were recruited primarily from research and medical sites, as well as social media sites, specializing in FXS.

The initial parent study measured the development of autism symptomatology in infants with FXS (R01MH90194, PI: Roberts). Participants in this study were seen at 9, 12 and 24 months with a diagnostic assessment for ASD completed at the 24-month time point. The subsequent renewal of this study allowed for the continued longitudinal examination of the development of ASD in these participants through the preschool years with diagnostic assessments for ASD targeted annually at 3, 4 and 5 years-of-age. Therefore, the majority of participants had their first ASD diagnostic assessment at 24 months-old and follow up assessments at 3, 4 or 5 years-of-age. There was variability in the number of assessments and in the age at which assessments occurred, however, given the age of participants at onset of the study renewal and inclusion of a handful of participants who were recruited for enrollment starting at 4 or 5 years-of-age. For inclusion in the present study, participants were required to have at least 2 video recorded diagnostic assessments which included the Autism Diagnostic Observation Schedule-2.

**Measures**

**Brief Observation of Social Communication Change.** The Brief Observation of Social Communication Change (BOSCC, Lord et al., 2016) is a coding scheme designed to measure change in autism symptomatology over time, when compared across 2 or more time points. The BOSCC is coded based on 10-12 minutes of semi-structured play between an adult and the target child. The BOSCC can be applied to a range of play interactions that meet basic criteria by providing engaging and developmentally appropriate materials and allowing the child to move freely around the room and explore
toys. Additionally, whatever setting and structure is selected for the initial observation should be kept consistent for all future play sessions to which the BOSCC will be coded and compared. The coding scheme consists of item level decisions trees that allow the coder to answer a series of yes or no questions based on the behaviors in the observation. Observations are split into two 6-minute segments, which are coded independently. Fifteen items are coded, and items 1-12 are included in the total score. Total scores across the two segments are then averaged to determine a participant’s total score on the BOSCC. For the present study, the BOSCC coding scheme will be applied to video clips selected from the participant’s ADOS-2 administrations. Video clips include the following tasks from the ADOS-2 administrations: 3 minutes of Free Play, 3 minutes of Bubble Play, 3 Minutes of Birthday Party or Bath Time and 3 Minutes of Balloon Play. Alternative tasks are available to supplement (e.g. Snack, Response to Joint Attention) if these tasks are less than 3 minutes in length. This collection of tasks and time lengths is recommended by the authors and has been previously published by Kitzerow and colleagues (Kitzerow et al., 2016). The first author of the present study has attended research training on the coding of the BOSCC and has been given authorization to use it for research purposes by the authors. According to measure use procedures, a team of undergraduate research assistants were trained up to reliability standards with the first author. To achieve reliability with the first author, coders must be within 1 point for more than 80% of items on each coding segment and within 4 points on each segment total score. To minimize coding drift, 20% of videos were coded by the first author and another coder. Intraclass correlations were used to examine reliability of absolute agreement, which was high (ICC = .911). BOSCC scores collected from the same child
over time are compared to measure change. BOSCC scores will be the primary outcome variable in the analyses.

**Autism Diagnostic Observation Schedule-2.** The Autism Diagnostic Observation Schedule-2 (ADOS-2) is a standardized semi-structured play-based measure used to assess behaviors consistent with a diagnosis of ASD. To administer the ADOS-2 within a research setting, examiners are required to attend research training and achieve reliability in the administration and coding of the measure with group of certified trainers at 80% reliability or become reliable with a research reliable examiner at the research site. The ADOS-2 has 5 modules, which are administered based on an individual’s chronological age and expressive language level. Throughout the ADOS-2, the examiner provides opportunities for social interaction through a series of standardized behavioral presses and activities. Some of these activities include free play, bubble play, balloon play and imaginative play with a baby doll. Behaviors observed during these presses are then coded on items measuring core features of ASD. Items are generally scored on a scale of 0-3, with scores of 0 indicating a normative response or the absence of atypicality, and a 3 indicating severe atypicality indicative of ASD. Items identified as being the most specific to an autism diagnosis from each module are used to create an algorithm score. A calibrated severity score can be derived from the algorithm score to allow comparison across the ADOS modules. Participant scores on the ADOS-2 are used to inform the CBE diagnostic procedures to determine the presence or absence of autism at each visit. The ADOS-2 reports strong inter-rater reliability of 84% (Lord et al., 2012). Within lab inter-rater reliability is calculated on a randomly selected sample of 20% of administrations as part of the completed and ongoing studies. Current within lab inter-
rate reliability is 83.3%. As previously mentioned, segments of the videotaped ADOS-2 administrations will be used for the BOSCC coding scheme.

**Mullen Scales of Early Learning.** The Mullen Scales of Early Learning (Mullen, 1995) is a standardized measure of development normed for young children ages 0-60 month, that measures development in five areas; Gross Motor, Fine Motor, Receptive Language, Expressive Language, and Visual Reception. Each domain produces a raw score, which can be transformed into a T-Score and an Age Equivalent Score. T-Scores have a mean of 50 and a standard deviation of 10. A summary standard score, the early learning composite (ELC), represents a composite score across all domains, with the exclusion of Gross Motor. The ELC has a mean of 100, and standard deviation of 15. Limitations exist in the use of T-scores for young children with developmental disabilities due to floor effects. In this sample, in the domains of Visual Reception, Expressive Language and Receptive Language, 46%, 54% and 64% were at the floor for each domain respectively. Therefore, Mullen raw scores will be used in the analysis as predictors of change scores on the BOSCC. The sum of the scores on the Expressive and Receptive language domains will be used as a measure of language functioning. The Visual Reception domain will be used as a measure cognitive functioning. Standard scores will be used for describing the sample. Internal consistency for each of the skills ranges from 0.75 to .08, and coefficients of test-retest reliability range from .70-.80 (Mullen, 1995).

**Procedure**

The USC institutional review board has approved all data collection procedures. Assessments were conducted primarily in the participant’s homes, with occasional visits.
at the Neurodevelopmental Disorders Lab at USC based on family preference. Conducting visits in the participants’ homes allowed for decreased travel burden on families with children with disabilities and supports increased ecological validity as it removes the potential confound associated with a child’s adjustment to a novel laboratory setting. Procedures followed in the home and lab settings were identical. Assessments were conducted over the course of one to two days, in 3-4-hour sessions each day, depending on the child’s age and attention span. Breaks were given throughout the assessment as needed. Participants were compensated for participation. All in-person measures were administered by research staff that included bachelors level research assistants, graduate students and post-doctoral researchers. ADOSs were only administered by staff who had achieved independent research reliability or within lab reliability with a research reliable counterpart. For all assessments included in the present study, a CBE diagnosis of ASD was determined at each time point by a team of at least 3 members of the research staff who were all research reliable on the ADOS, one of whom was a licensed psychologist. The CBE procedure is standard in the field of ASD research (Ozonoff et al., 2015). Data from the Mullen, Vineland Adaptive Behavior Scales, and ADOS was integrated to determine whether a child met criteria for ASD. Additionally, all research reliable staff on the ADOS attended monthly consensus coding meetings for ongoing calibration of scoring.

Clinical best estimates of ASD were determined for participants at each time point and the ADOS-2 videotapes were scored using the BOSCC. In addition to the evaluation of ASD, global developmental delay was evaluated for each participant. All participants in the study met criteria for global developmental delay at each assessment which is
consistent with the phenotype of young males with FXS. Therefore, all participants in this study who meet criteria for ASD also have co-morbid developmental delay at all time points.

**Data Analytic Plan**

At the time of analysis, 28 males with FXS had 2 or more ADOS administrations available for BOSCC coding and were included in the analysis (total of 73 assessments, range of 2-4 assessments per participant). Multilevel models are robust to variability in the number of assessments. As an initial step, data were plotted longitudinally using spaghetti plots. A linear pattern was evident, therefore linear models are used. Multilevel models were used to examine change over time in BOSCC scores, specifying time as the level 1 clustering variable, with individual variables entered at level 2. Across all models, age was centered at 24 months, given that the majority of participants had their first time point at 24 months. Therefore, intercepts are interpreted as the average BOSCC score at 24 months of age. Slopes will be interpreted as the average change in BOSCC scores per month.

As an initial step, participants were grouped in terms of the stability of ASD diagnoses. A stable diagnosis was defined as meeting or not meeting ASD diagnostic criteria at all assessments while an unstable diagnosis was defined as meeting ASD diagnostic criteria for one or more assessments but not across all assessments. In the total sample, 54% percent had stable ASD positive diagnoses, 21% percent had stable ASD negative diagnoses, 11% percent changed from an ASD positive diagnosis to an ASD negative diagnosis, and 14% percent changed from an ASD negative diagnosis to an ASD positive diagnosis. Thus, 75% demonstrated a stable positive or negative ASD diagnosis.
and 25% demonstrated an unstable ASD diagnostic trajectory representing both initial false positives and false negatives. Based on these diagnostic stability patterns, participants were split into 4 groups: 1) stable ASD positive (Stable ASD; n= 15), 2) stable ASD negative (Stable noASD; n= 6), 3) participants with an initial diagnosis of noASD which changed to ASD (Unstable ASD; n=4), and 4) participants with an initial diagnosis of ASD which changed to noASD (Unstable noASD; n =3). Fit statistics (e.g., AIC and BIC) for models using the diagnostic stability groups described previously were compared to models that dichotomized diagnosis to ASD or noASD based on the participants “outcome” diagnosis (i.e., their diagnosis at the last time point for the participant in the study). The model using the diagnostic stability grouping structure evidenced the best statistical fit and aligned with the theoretical interest of the study, and previous work in nsASD where diagnostic stability has been examined (Ozonoff et al., 2015). Therefore, to answer research question one, the stability diagnostic group was entered into the model as a level 2-time invariant predictor, to determine how diagnostic group impacts initial BOSCC scores at 24 months and change over time.

To answer research question two, raw scores on the Mullen were entered in the model as level 2-time invariant predictors. Raw scores were selected from the participants assessment closest to the age of 24 months ($m = 32.5$, range = 23-78 months). Raw scores for expressive and receptive language were highly correlated ($r = 0.79$), therefore they were combined (i.e., summed) to create one language variable. A sum, rather than an average, was selected to create the composite language variable given that scores across the two language domains do not share an equivalent scale (i.e., 6 raw score points on the Expressive Language domain is not necessarily developmentally
equivalent to 6 raw score points on the Receptive Language domain). Visual Reception raw scores were used as a measure of early non-verbal cognitive functioning.

Results

First, ANOVA tests were run to determine if the groups differed on demographic variables or predictor variables of interest. All means and standard deviations are presented in Table 1. More participants in the Stable ASD and Unstable noASD groups were older than 24 months at their first ADOS/BOSCC time point (2/3 in the Unstable noASD group, and 4/15 in the Stable ASD group), however differences in mean ages between groups were not found to be statistically significant. Additionally, no statistically significant differences were found between groups on age at Mullen, Receptive Language T-scores or Fine Motor T-scores. For the remaining domains, overall F-tests were significant. Therefore, Tukey HSD post-hoc tests were run to determine where differences lie between groups. Only significant post-hoc tests are reported. On the Expressive Language domain, the Unstable ASD group scored 11 points higher than the Stable ASD group (p = .015). On the Visual Reception domain, the Stable noASD group scored 8 points higher than the Stable ASD group (p = .053). The Early Learning Composite was lowest in the Stable ASD group, which was 9.31 points lower than the Stable NoASD group (p = .000), 8.79 points lower than the Unstable ASD group (p = .00), and 12.11 points lower than the Unstable noASD group (p = .000). Participants in the Stable ASD group had an average ADOS calibrated severity score (CSS) of 8.6, which was 6 points higher than the Stable NoASD group (p = .000), and 4 points higher than the Unstable ASD group (p = .000). Additionally, the Unstable NoASD group had
an average CSS score of 7.67, which was 5 points higher than the Stable NoASD group \( (p = .000) \), and 3 points higher than the Unstable ASD group \( (p = .015) \).

A taxonomy approach to the multilevel modeling was taken to address the proposed research questions (Singer, Willett, & Willett, 2003). Results of the series of models are presented in Table 2. First, the unconditional means model and unconditional growth model were run. The unconditional means model (Model A) produced an intraclass correlation (ICC) of 67.33, suggesting that 67% of the variance in BOSCC scores are attributable to differences among participants. Next, the unconditional growth model, including age centered at 24 months, was run. Within-person variance declined between Model A and Model B, producing a pseudo \( R^2 \) of 0.38, suggesting that 38% of the within-person variability is associated with the linear effect of time.

Next, diagnostic group was added to the model as a predictor of initial status (i.e., average BOSCC score at 24 months) and change over time (Model C). The Stable ASD group was selected as the reference group. On average, participants in the Stable ASD group had initial BOSCC scores of 31.64 \( (t = 14.40, p = .00) \). Participants in the Stable NoASD group \( (b = -16.49, t = -4.20, p = 0.00) \), and Unstable ASD group \( (b = -9.86, t = -2.24, p = .034) \) both had lower initial BOSCC scores than the Stable ASD group. Initial BOSCC scores did not differ between the Unstable NoASD group and the Stable ASD group \( (b = 0.68, t = 0.10, p = .924) \). Slopes did not differ between the Stable ASD group and the Stable NoASD or Unstable ASD groups. However, participants in the Unstable NoASD group evidenced a negative slope \( (b = 0.42, t = -1.90, p = .065) \), suggesting that their BOSCC scores decreased by 0.42 points per month, compared to the Stable ASD group. These trajectories are displayed in Figure 1. Between person variance declined
from Model B to Model C producing a pseudo $R^2$ of 0.60, which suggests that 60% of the variability in initial BOSCC scores is associated with diagnostic group.

To answer research question 2, additional models were run to examine the effects of language and non-verbal cognitive ability on initial BOSCC scores and change over time. Language was determined to be negatively related to initial BOSCC scores ($b = -0.49, t = -3.93, p = .001$), but not change over time. Non-verbal cognitive ability was not related to initial BOSCC scores or change over time. When non-verbal cognitive ability was added to the model, language remained as a significant predictor, however model fit declined (i.e., AIC and BIC estimates increased), so non-verbal ability was removed for the final model. Variance estimates for Model D suggest that language accounts for an additional 8% of the variability in initial BOSCC scores.

**Discussion**

This study examined the development of autism symptomatology over time in young males with FXS, using a novel measure of social communication change. Multilevel models were used to examine linear change based on diagnostic group, including early language and non-verbal cognitive ability as predictors of initial BOSCC scores and change over time. The data included in this study are drawn from a parent study that is the first study to track the stability of the clinical best estimate diagnosis of ASD in young children with FXS, beginning in toddlerhood (NIH R01MH90194, PI: Roberts). Therefore, this study is the first to explore the longitudinal development of autism symptomatology change in toddler and preschool aged children with FXS accounting for instability in the diagnoses of ASD at the individual level. While limits exist with this approach (i.e., namely, small sample sizes), the information gathered from
this study is essential for furthering the understanding of the heterogeneity of autism

Participants comprised 4 groups, Stable ASD (54%), Stable NoASD (21%), Unstable ASD (14%) and Unstable NoASD (11%). For those who received their first diagnosis at 24 months (n=22), 77% had stable diagnostic trajectories and maintained the diagnosis of either ASD or non-ASD across time. Of the 5 participants who displayed ASD diagnostic instability, 1 was a false positive, and 4 were false negative. This suggests that it is more likely for young males with FXS to be false negative than false positive for ASD at 2 years old. This is consistent with patterns in nsASD work of a 5:1 false negative to false positive ratio (Ozonoff et al., 2015). At 24 months, 83% of participants who received a diagnosis of ASD maintained that diagnosis across time which is consistent with the nsASD literature, which reports a diagnostic stability of ASD at 24 months of 82% (Ozonoff et al., 2015).

Our results suggest that there is significant variability in the presence and severity of ASD symptomatology within the FXS phenotype at 24 months-of-age. This variability can be partially explained by diagnostic categorization and stability of ASD. As a group, young children with FXS who have early and stable diagnoses of ASD throughout childhood display the most severe symptomatology, with symptom levels remaining high and stable across time. In a seemingly parallel pattern, participants with an early and stable diagnosis of FXS noASD, evidence much lower levels of autism symptomatology at 24 months with symptoms remaining low and stable over time. This finding is contradictory to the results published by Hernandez and colleagues (2009), who reported that autism symptomatology across their two diagnostic groups (FXS+ASD and FXS-noASD) became less differentiated across time. These contrasting findings may be
attributable to differing methodologies across the studies in the measurement of autism symptoms (i.e., BOSCC in the present study vs. ADI-R in the Hernandes study), diagnostic categorization procedures (i.e., CBE in the present study vs. ADI-R cutoffs at Time 1 in the Hernandes study), or their slightly older sample at Time 1 (i.e., 33 months in the present study vs. 56 months in the Hernandes study). Additionally, Hernandes and colleagues reported that 32% of participants in their sample changed diagnostic categories over the course of the study, however their diagnostic grouping was based on their Time 1 data. Therefore, their dichotomous approach subsumed participants with instability in their diagnostic trajectories into the diagnostic group determined at their initial visit. The findings presented in the current study may represent the trajectories for more “pure” diagnostic groups of FXS+ASD and FXS-noASD, given that the Stable ASD and Stable NoASD groups exclude participants who display diagnostic instability.

Compared to the Stable ASD group, participants in the Unstable ASD group had initial BOSCC scores that were 10 points lower, however there were no measurable differences in slopes. In theory, the UnstableASD group represents a group of males with FXS whose early symptom presentation did not warrant a diagnosis of ASD, with symptoms appearing more consistent with ASD over time (i.e., false negative cases). Contrary to what was hypothesized, these participants’ BOSCC scores didn’t significantly “worsen” with time, rather they follow a flat trajectory. Clinically, this group may represent a group of males with FXS who exhibit a milder presentation of ASD and whose symptoms of ASD are harder to distinguish from global developmental delay in toddlerhood. The flat BOSCC trajectories would suggest that their diagnostic instability may be influenced by clinician factors (e.g., a more conservative approach to
diagnosing ASD in FXS in toddlerhood when the symptom presentation is less severe and global developmental delay is present), rather than a result of a significant “worsening” in symptom presentation with time.

This finding is distinct from what has been described in the nsASD literature, where the “false negative” participants evidenced a worsening of autism symptomatology over time. This worsening is hypothesized to represent the later emergence of ASD, rather than clinician level variables or true misdiagnoses at earlier timepoints (Ozonoff et al., 2015). While autism symptom trajectories appear to differ, the false negative groups across the current study and the nsASD literature share a common feature of being slightly higher functioning in language and cognitive abilities than those with early and stable diagnoses of ASD.

The only group to display a declining slope (i.e., fewer ASD symptoms over time) was the Unstable NoASD group. These participants are similar to participants described as “false positives” in other work, who receive a diagnosis of ASD early on in development, however they no longer meet criteria for ASD as they age. This group was the minority of the sample, with only 3 participants falling into this group. Clinically, these participants are important as they represent a group of children who appear to “get better” with time, who may hold answers to effective interventions or protective factors within the FXS phenotype. In contrast to false positive cases in the nsASD literature, these children do not show a milder or “intermediate” presentation of ASD. Initial ADOS severity scores for the Unstable NoASD group were 7.67, which is on the cusp between moderate to high levels of ASD related symptoms and did not differ from the Stable ASD group. However, these two groups did differ on global development, with the Unstable
NoASD group scoring almost 1 standard deviation higher on a measure of broad
development at their initial time point, measured by the Mullen Early Learning
Composite.

In addition to understanding developmental trajectories of autism
symptomatology, this study aimed to discover predictors of early symptom presentation
and trajectory, with non-verbal cognitive functioning and language being the primary
domains of interest. Language and non-verbal cognitive impairment have long been
established as prominent features of the FXS phenotype, often developing at similar
levels (Abbeduto, Brady, & Kover, 2007). These delays complicate the diagnosis of
ASD in FXS given the need to differentiate global delays in development from a specific
social communication impairment consistent with ASD. For example, a clinician may
have trouble determining whether a lack of gesture use or poor integration of
communication forms (e.g., eye-contact, gestures, vocalizations) is the result of an
immature social communication system or an impaired one resulting from ASD. Given
this concern, clinicians may attribute autism symptomatology seen within FXS to
developmental delay, more broadly.

In this study, higher language scores at 24 months, predicted lower initial BOSCC
scores (i.e. less autism symptomatology), but not slopes over time. In contrast, non-verbal
cognitive ability was not predictive of initial BOSCC scores or slopes over time.
Therefore, early language abilities, but not non-verbal functioning, may serve as a
protective factor for children with FXS resulting in more robust and advanced social
communication abilities. Language abilities have also been found to predict ASD
symptom severity in adolescents with FXS, suggesting that this is a persistent
relationship within the FXS+ASD phenotype (Abbeduto et al., 2019). This finding has important implications for early intervention targets in young children with FXS, with recommendations to focus on language development before 24 months as a possible protective factor to the development of autism symptomatology.

Given that scoring on two BOSCC items includes vocalizations (i.e. vocalizations directed to others, integration of vocal and non-vocal modes of communication), it is prudent to consider whether the relationship between higher language abilities and BOSCC scores may be influenced by these items. The current sample is not large enough to conduct this type of analysis, however future research may consider examining if overlap in the measurement of language on the Mullen and BOSCC is able to partially explain this relationship. However, vocalizations need not be complex to receive credit on the BOSCC, therefore it is unlikely that the relationship between language abilities and initial BOSCC scores can be completely accounted for by these items.

While the variables of interest in this study accounted for a significant amount of variance in this data, unexplained variance remains to be understood. Specifically, we were unable to explain some of the variance associated with change over time in autism symptomatology. Future work may choose to focus on time-varying predictors which may be able to provide additional insight into predictors of individual growth trajectories.

This study presents important and novel findings to enhance the understanding of autism symptom trajectories within the early developmental period in males with FXS. Individuals with FXS and co-morbid ASD are at greater risk for more profound impairment in language, cognition and adaptive skills, and require greater levels of service to achieve optimal outcomes. The findings presented here inform not only
possible surveillance and diagnostic practice of ASD in FXS, but also inform early intervention recommendations. The vast majority (83%) of participants who received a diagnosis of ASD at age 2, maintained this diagnosis over time. This finding supports the clinical practice of screening for, evaluating, and diagnosing ASD within FXS as early as age 2, should symptoms be clear and compelling. Additionally, clinicians should remain vigilant and follow children whose presentation at 24 months is atypical, albeit milder than FXS peers with more impaired cognitive and language abilities. This study highlights that these individuals may not necessarily “get worse” over time or grow into a diagnosis of ASD. Rather, a lack of improvement in social communication or a lack of any clear reduction in autism symptomatology may be evidence of co-morbid ASD. It is important to note that the clinicians in this study were highly experienced with both ASD and FXS, and this level of expertise is unlikely to be present in all community settings where families of young children with FXS may be seeking an evaluation for ASD. This may increase the risk for diagnostic overshadowing in these settings, where clinicians may be less likely to diagnose a co-morbid condition instead attributing behavioral symptoms of ASD to the FXS phenotype.

Early language abilities, distinct from non-verbal abilities, were predictive of BOSCC scores at 24 months with higher language scores being associated with lower BOSCC scores. Language abilities at 24 months, however, did not make a child more or less likely to have a declining or inclining slope. Given the longstanding knowledge of language impairment in FXS, and the relationship identified between language and early autism symptomatology in this study, language intervention should be prioritized as an early intervention for young children with FXS.
This study is limited by a few factors. Primarily, the small sample size. Fragile X syndrome is a rare syndrome, making recruitment of large samples especially at young ages, challenging. This sample capitalizes on longitudinal data from 28 males which results in 73 data points providing more power to the analysis. Admittedly, the diagnostic grouping structure (i.e., stability groups) results in very small samples for comparison. However, this structure aligned with the theoretical questions of this study and resulted in a better statistical fit than a dichotomous approach of FXS+ASD and FXS-noASD. Future work should assess generalizability of the findings in a larger sample. Females were intentionally excluded from this study given the significant heterogeneity within the female FXS phenotype. Therefore, we must acknowledge that the results presented here can only be generalized to young males with FXS.
Table 1.1 Participant Demographics for FXS Males (n = 28)

<table>
<thead>
<tr>
<th></th>
<th>Stable ASD</th>
<th>Stable noASD</th>
<th>Unstable ASD</th>
<th>Unstable noASD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 15</td>
<td>n = 6</td>
<td>n = 4</td>
<td>n = 3</td>
</tr>
<tr>
<td>ADOS-2/BOSCC(^1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in Months</td>
<td>35.15 (18.08)</td>
<td>25.63 (0.75)</td>
<td>24.77 (1.35)</td>
<td>43.32 (16.06)</td>
</tr>
<tr>
<td>ADOS CSS</td>
<td>8.6 (1.4)</td>
<td>2.33 (1.21)</td>
<td>3.75 (2.99)</td>
<td>7.67 (0.58)</td>
</tr>
<tr>
<td>BOSCC Total</td>
<td>30.63 (9.41)</td>
<td>15.08 (6.76)</td>
<td>20.25 (1.04)</td>
<td>25.50 (11.79)</td>
</tr>
<tr>
<td>Mullen(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in Months</td>
<td>29.15 (11.35)</td>
<td>25.65 (0.70)</td>
<td>24.57 (1.35)</td>
<td>39.21 (12.90)</td>
</tr>
<tr>
<td>ELC</td>
<td>52.33 (6.62)</td>
<td>60.83 (7.96)</td>
<td>59.75 (3.20)</td>
<td>63.33 (13.58)</td>
</tr>
<tr>
<td>Visual Reception T-Score</td>
<td>22.87 (5.11)</td>
<td>31.00 (7.21)</td>
<td>31.00 (2.58)</td>
<td>33.33 (11.55)</td>
</tr>
<tr>
<td>Exp. Language T-Score</td>
<td>22.53 (5.37)</td>
<td>28.00 (7.18)</td>
<td>34.00 (3.37)</td>
<td>30.33 (10.50)</td>
</tr>
<tr>
<td>Rec. Language T-Score</td>
<td>22.53 (6.52)</td>
<td>29.50 (12.71)</td>
<td>22.50 (3.00)</td>
<td>29.00 (8.54)</td>
</tr>
<tr>
<td>Fine Motor T-Score</td>
<td>21.47 (3.23)</td>
<td>24.33 (4.59)</td>
<td>22.75 (1.89)</td>
<td>25.00 (5.57)</td>
</tr>
</tbody>
</table>

\(^1\) ADOS-2 Autism Diagnostic Observation Schedule, 2\(^{nd}\) Edition, BOSCC Brief Observation of Social Communication Change, CSS Calibrated Severity Score, Mullen Mullen Scales of Early Learning, ELC Early Learning Composite

\(^2\) Data corresponds to the first ADOS available for coding

Data corresponds to the Mullen administered closest to the 24-month timepoint; standard scores are presented.
Table 1.2 Results of a taxonomy of multilevel models for change in BOSCC scores over time

<table>
<thead>
<tr>
<th></th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
<th>Model D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>25.80** (1.94)</td>
<td>25.06** (2.16)</td>
<td>31.64** (2.20)</td>
<td>41.32** (3.07)</td>
</tr>
<tr>
<td>Stable noASD</td>
<td>--</td>
<td>--</td>
<td>-16.49** (3.92)</td>
<td>-10.22** (4.07)</td>
</tr>
<tr>
<td>Unstable ASD</td>
<td>--</td>
<td>--</td>
<td>-9.86** (4.40)</td>
<td>-4.80 (4.33)</td>
</tr>
<tr>
<td>Unstable noASD</td>
<td>--</td>
<td>--</td>
<td>-0.68 (7.12)</td>
<td>11.45 (7.47)</td>
</tr>
<tr>
<td><strong>Rate of Change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>--</td>
<td>0.04 (0.06)</td>
<td>0.07 (0.08)</td>
<td>0.12 (0.08)</td>
</tr>
<tr>
<td>Stable noASD</td>
<td>--</td>
<td>--</td>
<td>-0.21 (0.23)</td>
<td>-0.33 (0.22)</td>
</tr>
<tr>
<td>Unstable ASD</td>
<td>--</td>
<td>--</td>
<td>-0.00 (0.16)</td>
<td>-0.04 (0.16)</td>
</tr>
<tr>
<td>Unstable noASD</td>
<td>--</td>
<td>--</td>
<td>-0.42* (0.23)</td>
<td>-0.41* (0.22)</td>
</tr>
<tr>
<td><strong>Variance Components</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within person</td>
<td>42.62 (6.52)</td>
<td>26.48 (5.14)</td>
<td>26.63 (5.16)</td>
<td>26.05 (5.10)</td>
</tr>
<tr>
<td>Level 2 (b/t person)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In initial status</td>
<td>87.86 (9.37)</td>
<td>85.74 (9.25)</td>
<td>34.08 (5.84)</td>
<td>27.73 (5.27)</td>
</tr>
<tr>
<td>In rate of change</td>
<td>--</td>
<td>0.04 (0.21)</td>
<td>0.05 (0.22)</td>
<td>0.05 (0.22)</td>
</tr>
<tr>
<td><strong>Pseudo R^2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within person</td>
<td>--</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>In initial status</td>
<td>--</td>
<td>--</td>
<td>0.60</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Model Fit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIC</td>
<td>534.07</td>
<td>536.62</td>
<td>512.71</td>
<td>504.96</td>
</tr>
<tr>
<td>BIC</td>
<td>540.90</td>
<td>550.36</td>
<td>538.80</td>
<td>533.03</td>
</tr>
</tbody>
</table>

**p < .05, *p < .1
Figure 1.1 Uncontrolled effects of diagnostic stability group on BOSCC scores over time
CHAPTER 2
CROSS-SYNDROME CONTRASTS OF AUTISM SYMPTOMS IN YOUNG CHILDREN: FRAGILE X, DOWN SYNDROME AND NON-SYNDROMIC AUTISM

Autism spectrum disorder (ASD) is a developmental disorder that is characterized by deficits in social communication and the presence of atypical restricted and repetitive behaviors and interests (American Psychiatric Association, 2013). ASD is one of the most common and impairing childhood conditions, with prevalence rates of about 1.5% in the general population (Centers for Disease Control, 2014). Heritability of ASD is estimated to be around 70-80% with more than 1000 identified risk genes as well as a handful of single gene disorders that carry a high risk for developing ASD (Geschwind, 2011).

ASD is a heterogeneous disorder, with core impairments of social communication and restricted and repetitive behaviors accompanied by a myriad of cognitive, language and adaptive behavior presentations. One of the most impairing co-morbid features of ASD is intellectual disability (ID), which occurs in approximately 37-55% of individuals with ASD (Charman et al., 2017; Rivard, Terroux, Mercier, & Parent-Boursier, 2015). The presence of ID in young children at risk for ASD can make diagnosing ASD more complex, given that it requires diagnosticians to differentiate delays associated with ID from those better explained by ASD (Matson & Shoemaker, 2009). Diagnostic differentiations are also complicated by the overlap in symptomatology between ASD
and ID including symptoms often thought to be classic features of ASD such as repetitive behaviors and echolalia occurring commonly in individuals with ID more broadly (Wallander, Dekker, & Koot, 2003). However, accurate and early diagnosis of the co-morbidity of ASD and ID is essential, given that their co-occurrence causes individuals to experience greater impairments and less optimal outcomes than individuals with either condition in isolation.

**Single-Gene Models of ASD**

Single gene disorders account for about 1-2% of all ASD cases (Abrahams & Geschwind, 2008). While mechanisms for impairment across the single-gene disorders are varied (e.g. excess gene expression, protein deficit) consistency exists in the almost universal ID present in many conditions. Therefore, single gene disorders are an excellent model for studying the behavioral phenotype of ASD in individuals with ID. Fragile X syndrome (FXS) and Down syndrome (DS) are two excellent models to examine these relationships given that ID is nearly universal in both disorders, and they are relatively common in the population at 1 in 5000 (Coffee et al., 2009) and 1 in 1500 (Kazemi, Salehi, & Kheirollahi, 2016) respectively.

**Autism Symptomatology in FXS**

FXS is the most common form of inherited ID (Hagerman & Hagerman, 2002). FXS results from a repeat expansion of the CGG sequence on the FMR1 gene on the X chromosome. Repeats on the FMR1 gene that exceed 200 result in methylation of the gene, which significantly reduces or eliminates production of Fragile X Mental Retardation Protein (FMRP), which is essential for cognitive development. Females with FXS are often less impaired than males with FXS due to the presence of a second X
A significant body of work has been developed examining the relationship between ASD and FXS. Consistent across these studies are findings that the rates of ASD in FXS are high, with estimates ranging from 60-75% (Harris et al., 2008; Klusek, Martin, & Losh, 2014). The presence of ASD in FXS results in greater impairment across a range of domains including cognition (Bailey Jr, Hatton, Tassone, Skinner, & Taylor, 2001; Hogan et al., 2017; Kaufmann et al., 2004), adaptive behavior (Caravella & Roberts, 2017; Hahn, Brady, Warren, & Fleming, 2015), language (Klusek et al., 2014), motor skills (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000) and social approach behaviors (Roberts et al., 2019). There is consensus that features consistent with ASD are commonly observed in individuals with FXS (Hagerman & Hagerman, 2002), however there continues to be debate about the etiology of the symptoms, and whether or not they constitute the presence of a true comorbidity of ASD or represent phenotypic features associated with FXS. Evidence across biological and behavioral studies suggests that symptoms of ASD in FXS may be behaviorally similar to non-syndromic ASD (nsASD), however different patterns of strengths and weaknesses may emerge with time (Wolff et al., 2012) that could be driven by unique brain mechanisms (Hazlett, Poe, Lightbody, Gerig, Macfall, et al., 2009). These strengths in FXS and comorbid ASD (FXS+ASD) are typically identified in the social domain, marked by increased frequency and quality of social overtures even in those diagnosed with ASD (McDuffie, Thurman, Hagerman, & Abbeduto, 2015; Wolff et al., 2012). However, evidence suggests that strengths in social communication in FXS+ASD may not emerge until around the age of 4 or 5 (Wolff et al.,
2012), with little differentiation between nsASD and FXS+ASD observed in the toddler years (Hazlett, Poe, Lightbody, Gerig, Macfall, et al., 2009; Rogers, Wehner, & Hagerman, 2001).

Longitudinal examinations of ASD symptomatology in individuals with FXS+ASD suggest variability in the presentation of ASD symptomatology over time. In infancy, ASD symptoms appear to remain elevated, albeit decreasing over time (Hogan et al., 2017), followed developmentally by reports of both increasing (Hatton et al., 2006; Lee, Martin, Berry-Kravis, & Losh, 2016) and decreasing (Hernandes et al., 2009) ASD symptomatology in early childhood. This variability is intriguing and may be attributable to the wide range of measures used to assess change in ASD symptomatology across these studies, variability present in the methods used to determine ASD diagnostic status (i.e. parent report vs. measure cutoffs vs. clinician judgment) or developmental effects and age related changes in symptomatology.

**ASD Symptomatology in DS**

Down syndrome is the most common form of ID, caused by the presence of three copies of the 21st chromosome, in either all or a portion of the body’s cells. DS occurs at a rate of between 1 in 400 to 1 in 1500 and affects males and females similarly (Kazemi et al., 2016). Three types of DS are possible: trisomy 21 (95%), translocation (4%) and mosaicism (2%). Children with the mosaicism form of DS are often less severely impaired than the 2 other types of DS because the third copy of chromosome 21 is not present in all cells in the body (Papavassiliou, Charalsawadi, Rafferty, & Jackson-cook, 2014).
Research into symptomatology and features of ASD in DS is significantly more limited than in FXS. This may be partly attributable to the historically held belief that individuals with DS “could not have” ASD because of the documented sociability in many with the syndrome, with some hypothesizing that this high sociability may serve as a protective factor against ASD for these individuals (Rasmussen, Borjesson, Elisabet, & Gillber, 2001; Reilly, 2009). However, research has suggested that rates of ASD in DS are elevated compared to the general population, ranging from 7-39% (Diguiseppi et al., 2010; Hepburn, Philofsky, Fidler, & Rogers, 2008; Kent et al., 1999; Nærland, Bakke, Storvik, Warner, & Howlin, 2017), and that the presence of comorbid DS and ASD (DS+ASD) results in greater functional impairment (Diguiseppi et al., 2010; Dressler, Perelli, Bozza, & Bargagna, 2011; Moore Channell et al., 2019).

Similar to young children with nsASD (Landa, 2008), research examining individuals with DS+ASD suggests that parents report observing symptoms consistent with ASD in infancy and toddlerhood and that their behavioral phenotype is distinct from those with DS without ASD (DS-noASD) (Rasmussen et al., 2001). Despite symptoms being present in infancy, most children with DS are not diagnosed with ASD until they are between 6-14 years-of-age (Rasmussen et al., 2001) highlighting a significant missed opportunity for targeted early intervention. Similar to findings in FXS, ASD symptomatology may occur on a spectrum within DS, with some individuals meeting full criteria while others show evidence of sub-threshold symptomatology. Social strengths, including social reciprocity, using a range of facial expressions, imitation and eye-contact have been identified in individuals with DS+ASD when contrasted to nsASD (Hepburn et al., 2008; Starr, Berument, Tomlins, Papanikolaou, & Rutter, 2005; Warner, Howlin, 2017).
Salomone, Moss, & Charman, 2017). Similarly, individuals with FXS have also been noted to display social strengths when compared to individuals with nsASD, however their strengths are identified in the areas of social smiling, showing and directing attention, and sharing (McDuffie et al., 2015).

Studies comparing children with DS+ASD directly to nsASD have produced inconsistent findings. In one of the largest studies examining school age children 6-15 years old, Warner and colleagues used the clinical cutoffs on the Social Communication Questionnaire (SCQ) to define their DS+ASD group (n=183), and identified overall lower SCQ scores in the DS+ASD group compared the nsASD group (n=189) (Warner et al., 2017), with the greatest strengths in the social domain including imitation skills and gesture use. Due to methodological limitations in this study (i.e., survey data), they were unable to control for developmental or cognitive functioning between the nsASD and DS+ASD groups.

In contrast, Moss and colleagues who also utilized the SCQ to measure ASD symptomatology and to create their diagnostic groups, reported no significant differences in the symptom profiles between the DS+ASD and nsASD groups (Moss, Richards, Nelson, & Oliver, 2013). The Moss et al., study examined a much smaller sample (i.e., 17 DS+ASD, 17 nsASD), a much broader age range (4-62 years) and matched the groups on overall ASD symptom severity (+/- 2 points on total SCQ). Therefore, it may be the case that when overall symptom severity between nsASD and DS+ASD are similar, differences in social communication strengths are not measurably different.

The literature reviewed thus far relies heavily on parent report screening tools to determine diagnostic status of ASD within their DS samples. These findings are therefore
limited due to possible biases in parent report of symptoms. More critically, however, these tools are limited by their inability to take into consideration a participant’s developmental level and how that may explain the presence or absence of certain social communicative skills. Rather, they are blunt measures which when used with children with ID, may not necessarily perform as designed. This is especially problematic in very young children, or those with severe or profound ID (Starr et al., 2005).

There are only 2 published studies in DS research that utilize gold standard diagnostic tools of ASD (e.g., Autism Diagnostic Observation Scheduled (ADOS) and Autism Diagnostic Interview (ADI)) (Godfrey et al., 2019; Hepburn et al., 2008), with only 1 using a clinical best estimate (CBE) procedure to examine the co-morbidity of ASD within DS. Hepburn and colleagues (2008) assessed 20 children with DS longitudinally, at 2 and 4 years old using a CBE procedure informed by the ADOS and ADI, and reported a prevalence of 10%. Within the sample, 2 participants met CBE criteria for ASD at age 2 and maintained that diagnoses at their follow up visit 2 years later, with an observed worsening of symptoms. All participants without a co-morbid diagnosis of ASD at their first visit, remained without ASD at their follow up appointment. Thus, this study showed very high consistency in the diagnostic stability across the preschool years both in those with and without diagnoses of ASD. Importantly, the authors noted that if they had relied on ADOS scores alone, they would have overidentified participants with DS+ASD as a number of the participants had scores on the ADOS that fell in the ASD range but clinician view was that those features were better accounted for by ID or other factors.
In a more recent study, Godfrey and colleagues (2019) matched 66 participants with nsASD to 22 participants with DS-noASD and 11 with DS+ASD on sex, age and verbal mental age. Participants ranged in age from 30 months to 99 months. Diagnoses of ASD were determined by clinical cutoffs on the ADOS, not CBE, and the ADI-R was used as the outcome variable of interest. The study produced similar findings to the Warner et al. study (2017) in that children with ASD+DS displayed less severe symptomatology than the nsASD group in both the social communication and restricted and repetitive behavior domains, however more severe symptomatology than the DS-noASD group.

**Comparative Research Between FXS and DS**

Currently, there are no studies comparing the development of autism symptomatology in young children with FXS and DS. Comparative research between FXS and DS has generally focused on language, cognitive development and adaptive behavior suggesting unique patterns of strengths and weaknesses between the disorders (Rice, Warren, & Betz, 2005; Valencia-naranjo & Robles-bello, 2017; Will, Caravella, Hahn, Fidler, & Roberts, 2018). Although not directly comparing core features of ASD, Abbeduto and colleagues compared theory of mind, a social cognitive construct often found in deficit in individuals with ASD, in a sample of 11 to 23 year-olds with DS (n=25) and FXS (n=18) groupwise matched on chronological age, mental age and nonverbal IQ (Abbeduto et al., 2001). Participants with DS were found to have greater impairment in theory of mind functioning than individuals with FXS who performed similarly to the typically developing control group. This finding is surprising given that rates of ASD are
higher in FXS than DS, and theory of mind deficits are often seen in individuals with ASD.

Taken together, these bodies of research suggest that individuals with some forms of syndromic ASD (i.e. FXS and DS) may evidence preservation of skills in the social communication domain that are not typically seen in individuals with nsASD, with possible distinct profiles between the two syndromes. It is unknown, however, when these differences emerge or if they are consistent over time. Only one study has examined the longitudinal development of autism symptomatology in DS, and no work has compared the development of autism symptomatology beginning in toddlerhood, in individuals with FXS and DS.

Comparing developmental trajectories of autism symptomatology across these etiologically distinct, but high-risk groups, is important to determine symptom overlap and divergence to inform the clinical phenotype of ASD in single gene disorders. Across both bodies of work, concern exists in the potential overidentification of ASD, given almost universal ID and possible overlap in symptoms. Indeed, concerns also exist about the bias to underdiagnose ASD in syndromic groups, known as diagnostic overshadowing, (Jopp & Keys, 2001), which can result in missed access to specialized services when they are needed.

While behavioral phenotypes of DS and FXS may overlap with nsASD, evidence suggests that identifying specific ASD phenotypes within single gene disorders is critical to providing treatment that targets possible underlying impairments (e.g., attention, anxiety, cognitive function, social amotivation) that give rise to the behavioral phenotype of ASD, which may be distinct from nsASD (Glennon, Karmiloff-Smith, & Thomas,
Understanding how these symptoms develop over time will provide important insight into the symptom presentation of ASD in high-risk syndrome groups that can inform screening and diagnostic practices and targets for intervention.

The Present Study

To our knowledge, there is no published literature comparing early autism symptom trajectories in children with FXS, DS and nsASD. Given this gap in the literature, this study aims to present preliminary findings from a pilot sample of 2-4-year-old children with FXS, DS and nASD, measured longitudinally. While expansion of this sample is ongoing and will be used for a complete analysis and interpretation, this initial exploration provides insight into possible differences between groups in autism symptomatology at 24 months. Additionally, patterns of symptom trajectories will be examined graphically, and initial hypothesis will be drawn to inform the larger study that will be built upon findings from this initial pilot study. Specifically, this pilot study will report rates of clinical best estimate diagnosis of ASD in a pilot sample of males with DS and FXS, and compare FXS, DS and nsASD groups with respect to, 1) BOSCC scores at 24 months and 2) trajectories of autism symptoms (i.e., measured by the BOSCC) from 2-4 years.

Methods

Participants

The sample included 32 male participants aged 2-4, 17 FXS, 10 DS, and 5 nsASD. Participants each had between 2 and 3 assessments, with a total of 74 assessments in the sample (38 FXS, 25. DS, 11 nsASD). Participants were drawn from a larger longitudinal study (R01MH90194) examining the early developmental trajectories
of children with FXS compared to children at high and low risk of developing ASD (e.g., low risk controls, and infant siblings of children with ASD). A supplemental study allowed for the addition of a pilot sample of infants with DS, to serve as a mental age match to the FXS sample. Participants with FXS all had full mutation FXS, confirmed by genetic report. The nsASD group includes participants from the low risk and high risk ASIB groups diagnosed with ASD at their 24-month visit, where a genetic syndrome was ruled out. To reduce variability due to differences in cognitive functioning, all nsASD participants were required to show developmental delays, measured by an Early Learning Composite less than 85 on the Mullen Scales of Early Learning. The DS group was confirmed by genetic report; 9 had Trisomy 21 and 1 had mosaicism. To be included in the current study, participants needed to have at least 2 comprehensive diagnostic assessments that included an ADOS, with the first occurring no later than 36 months of age. Data was only included for assessments that occurred at ages 2, 3 and 4 years old, given that this was the available range for the DS participants.

Exclusionary criteria for all participants included gestational age less than 37 weeks, a language other than English as the primary language in the home, and female sex. Females were excluded because of the significant heterogeneity within the female FXS phenotype, and the inability to account for that variability in such small pilot samples.

**Procedure**

The USC Institutional Review Board approved all studies from which data will be drawn for the present. As part of the parent study, participants were seen at 24 months for a comprehensive diagnostic assessment for ASD. Through a renewal of the parent study,
participants were seen annually through the age of 5 with recurrent annual comprehensive evaluations. Variability in the number of participants assessed at each time point occurred based on the time between a participants’ completion of the initial study (i.e., at 24 months) and the funding of the renewal. For some, this resulted in a missed comprehensive evaluation at 36 months (n = 6).

Visits for the parent study were primarily conducted in the participant’s homes, to minimize travel burdens for families with children with disabilities and to reduce effects due to novelty of the research lab. When convenient for the family, assessments were conducted at the Neurodevelopmental Disorders Lab at USC. At each assessment a standard battery was completed. A CBE diagnosis of ASD (Ozonoff et al., 2015) was determined at each evaluation by a team of at least 3 members of the research staff who were all research reliable on the ADOS, one of whom was a licensed psychologist. All available clinical information including the Mullen Scales of Early Learning, Vineland Adaptive Behavior Scales and the Autism Diagnostic Observation Schedule, were used to determine whether the child met criteria for a diagnosis of ASD.

Measures

Autism Diagnostic Observation Schedule-2. The Autism Diagnostic Observation Schedule-2 (ADOS-2) is a standardized semi-structured play-based measure used to observe behaviors consistent with a diagnosis of autism spectrum disorder. The ADOS-2 has 5 modules (i.e. Toddler, Modules 1-4), which are administered based on an individual’s chronological age and expressive language level. Items are generally scored on a scale of 0-3, with scores of 0 indicating a normative response or the absence of atypicality, and a 3 indicating severe atypicality. Items identified as being the most
specific to an autism diagnosis from each module are used to create an algorithm score. To allow for comparison across modules, algorithm scores can be converted to a calibrated severity score, which fall on the same scale, 0-10, across all modules. The ADOS-2 reports strong inter-rater reliability of 84% (Lord et al., 2012). Within lab inter-rater reliability is maintained above 80%. In the present study, the ADOS was used for two purposes. First, the ADOS was used to inform the CBE diagnosis of ASD in all groups. Second, video clips of tasks within the ADOS were coded using the Brief Observation of Social Communication Change (described below).

Brief Observation of Social Communication Change. The Brief Observation of Social Communication Change (BOSCC, Grzadzinski et al., 2016) is a coding scheme designed to measure change in autism symptomatology over time in individuals with minimal language, when compared across 2 or more time points. The BOSCC is coded based on 10-12 minutes of semi-structured play between an adult and the child being assessed. The BOSCC can be applied to a range of play interactions, however they must include developmentally appropriate play and toy selection. The child must also be allowed to freely move around the room and explore toys. Additionally, whatever setting and structure is selected for the initial observation should be kept consistent for all future play sessions to which the BOSCC will be coded and compared. Fifteen decision trees are used to derive codes based on behaviors observed during the session. Observations are split into two 6-minute segments, which are coded independently then averaged to determine a child’s total score on the BOSCC. For the present study, the BOSCC coding scheme will be applied to video clips selected from the participant’s ADOS-2. Video clips will include the following tasks from the ADOS-2 administrations; 3 minutes of
Free Play, 3 minutes of Bubble Play, 3 Minutes of Birthday Party or Bath Time and 3 Minutes of Balloon Play. This collection of tasks and time lengths is recommended by the authors and has been previously published on by Kitzerow and colleagues (Kitzerow, Teufel, Wilker, & Freitag, 2016). The first author of the current study has attended research training on the coding of the BOSCC and has been given authorization to use it for research purposes by the authors. Consistent with measure use guidelines, a team of undergraduate research assistants were trained to reliability standards with the first author. To achieve reliability with the first author, coders must score within 1 point for more than 80% of items on each coding segment and within 4 points on segment total scores. This requirement was met for 3 consecutive videos before coders were able to code independently. Additionally, approximately 20% of videos were coded for reliability to minimize coding drift. Intraclass correlations were used to examine reliability of absolute agreement, which was high (ICC = .911).

Mullen Scales of Early Learning. The Mullen Scales of Early Learning (Mullen, Mullen, 1995) is a standardized measure of development normed for young children ages 0-60 months. The Mullen measures development across five domains; Gross Motor, Fine Motor, Receptive Language, Expressive Language, and Visual Reception. A summary standard score, the early learning composite (ELC) represents a composite score across all domains, with the exclusion of gross motor. The ELC has a mean of 100, and standard deviation of 15. The Mullen was administered by trained research staff. Internal consistency for each of the skills ranges from 0.75 to .08, and coefficients of test-retest reliability range from .70- .80 (Mullen, 1995). The Mullen ELC was used as inclusionary for the nsASD group (i.e., nsASD participants needed to have an ELC < 85.)
Data Analytic Plan

Given small samples, descriptive analysis and simple comparisons were utilized in lieu of complex inferential models. BOSCC scores from the first time point were compared across groups using an ANOVA and Tukey HSD post hoc tests. Tukey HSD tests analyze all possible pairwise comparisons and provides p-values that are corrected for multiple comparisons. Comparisons at 36 and 48 months were not conducted; due to the smaller samples at 36 and 48 months, a repeated measures ANOVA was not appropriate. Third, BOSCC change scores were calculated as: Last BOSCC Total – First BOSCC Total / Time in Months Between Assessments. One sample T-tests were conducted to determine if change scores differed from 0.

Results

Clinical Diagnoses of Autism within FXS and DS participants

Within the FXS group, 65% of participants (n=11) met CBE criteria for ASD. One participant was a false negative for ASD at his 24-month visit and was diagnosed with ASD at both subsequent visits (36 and 48 months). Therefore, he is included in the FXS+ASD group for all comparisons. All other participants displayed stable diagnoses of either FXS+ASD or FXS-noASD throughout the duration of their participation in the study.

Within the DS group, 20% of participants (n = 2) met CBE criteria for ASD. These two participants were false negatives at their 24-month visit and diagnosed with ASD at subsequent visits. The remaining participants did not meet criteria for ASD at any time during their participation in the study.
Examination of BOSCC Means and Trajectories

All but 1 participant had their first available BOSCC at 24 months. Means, standard deviations and sample sizes at each age point are presented in Table 2.1. A Shapiro-Wilk’s tests of normality was conducted on BOSCC scores at each time point and determined that BOSCC score distributions did not differ from normality at any time point. An ANOVA comparing BOSCC means at 24 months identified differences across groups, $F(4,26) = 4.94$, $p = .004$. Post hoc comparisons using a Tukey HSD test, which produce p-values corrected for multiple comparisons, indicated that the FXS+ASD ($M = 29.68$) group scored higher than the DS-noASD group ($M = 17.86$, $p = .021$) and higher than the FXS-noASD group ($M = 15.08$, $p = .005$) on BOSCC scores at 24 months. The average BOSCC score in the nsASD group at 24 months was 25.6, which is not statistically different than any of the comparison groups. The DS+ASD group had a mean BOSCC score of 22, which was the lowest of all groups with a co-morbid diagnosis of ASD, although this was not determined to be statistically different from any comparison group.

Longitudinal trajectories of BOSCC data by group are presented in Figure 2.1. Change scores for each group, which represented the average change in BOSCC score per month, are presented in Table 2.1. One sample T-tests were used to compare change scores to 0. Results of the T-tests suggest that change trajectories for each group are not different than 0 (i.e., all groups evidence flat BOSCC trajectories). However, these results are likely affected by the small sample sizes. Visual examination of individual growth trajectories highlights the heterogeneity within each group, which may be not best captured by group level means.
Discussion

This study reports findings from a pilot cross-syndrome longitudinal study of ASD symptom trajectories in toddlers and preschoolers with DS, FXS or nsASD. This study has two primary contributions. First, these pilot data provide important information to guide the design of a more systematic and large-scale study with additional participants. Second, given that no study has been published to contrast ASD trajectories across these syndromes, the findings are important to the field. Given the pilot nature of this study, caution is taken with regard to conclusions and inferences with limitations on generalizability recognized. As such, the patterns observed from the DS+ASD group will be described, however conclusive inferences will not be made given the sample size.

At the diagnostic level, rates of syndromic ASD in this pilot sample were consistent with rates reported in the literature across both the FXS and DS groups, at 65% and 20% respectively. Interestingly, both participants in the DS sample were false negatives for ASD at their 24 month visit and were not diagnosed with ASD until their 36 month assessment. In the only other study to conduct comprehensive assessments for ASD longitudinally in the toddler years in a DS sample, both participants with DS+ASD (n = 2) met criteria for ASD at 24 months and maintained this diagnosis over time (Hepburn et al., 2008). Future research should aim to identify what factors (e.g., individual level vs. clinician factors) may lead to these patterns of ASD diagnostic instability in DS, as they have significant implications for clinical practice in screening and surveillance.

This study suggests that by 24 months, participants in the FXS+ASD group are scoring highest on the BOSCC, with scores that are significantly higher than individuals
in both syndrome groups without ASD (i.e., FXS-noASD and DS-noASD). This finding aligns with the theoretical approach of a specific ASD phenotype within FXS, that is distinct from FXS without co-morbid ASD. In contrast, the FXS+ASD group could not be differentiated at 24 months from the DS+ASD and nsASD groups, suggesting that on average, these groups show similar levels of autism symptomatology at 24 months. This finding, if replicated in a larger sample, would suggest that toddlers with syndromic ASD and nsASD show similar composite levels of autism symptomatology during the toddler years. This lack of differentiation in autism symptomatology between FXS+ASD and nsASD groups in the toddler years is consistent with previous research (Hazlett, Poe, Lightbody, Gerig, MacFall, et al., 2009; Rogers et al., 2001).

Although participants with DS+ASD did not significantly differ from the nsASD group, they also did not differ significantly from the DS-noASD group. The DS+ASD group in this study display an intermediate autism symptom phenotype that falls between the nsASD and DS-noASD group (i.e., nsASD > DS+ASD > DS-noASD), although these differences did not rise to levels of statistical significance. However, this ASD symptom severity gradient within DS is consistent with what has been observed in larger studies (Godfrey et al., 2019; Warner et al., 2017). In contrast to the DS sample, participants in the FXS sample were distinguishable by almost 16 points on the BOSCC at their 24-month time point, based on their clinical diagnoses (i.e., FXS+ASD vs. FXS-noASD). This difference suggests there may be a much stronger distinction between ASD diagnostic groups within FXS, with more subtle differentiations in individuals with DS. This possible contrasting finding between the two syndrome groups will need to be replicated in a much larger sample.
At conception, this study was focused on comparing ASD symptom change trajectories across time between non-syndromic and syndromic forms of ASD. The BOSCC was utilized due to its ability to capture small and clinically relevant changes over a short period of time. In the present study, slopes across all groups could not be differentiated from 0 at the mean level. However, visual examination of BOSCC trajectories (Figure 2.1) highlights the variability in slopes that are most often non-zero. At a sample level, group does appear to explain variance in total BOSCC scores, evidenced by the descending pattern in mean trend lines plotted by group in Figure 2.1. However, the within group variability in slopes is masked at the mean level. Identifying predictors of within group variability will be essential to explaining and interpreting this heterogeneity.

**Limitations and Future Directions**

While this pilot study presents a novel comparison of ASD symptom trajectories in 2-4-year olds with nsASD, FXS and DS, it is limited by the small samples, and therefore generalizability is not yet possible. Since the inception of this pilot study, data collection across all three groups has continued through the parent study. Therefore, a follow up analysis is planned on a larger sample of participants, that is projected to include: 25 participants with nsASD with developmental delay, 16 participants with DS, and 21 FXS. The expanded sample will take advantage of multilevel models of change, which are robust to uneven time points, which are present in this dataset.

Prospective research interested in examining the development of autism symptomatology in young children with DS is inherently challenged by the relatively low prevalence rates of ASD within DS. With reported prevalence estimates ranging from 7-
39%, studies aiming for an outcome group of participants with DS+ASD need to enroll somewhere between 65 – 350 participants, to end up with a modest sample size of 25 participants. This sample size challenge is evident in the two studies that used gold standard in person diagnostic assessment of ASD, which included 2 (Hepburn et al., 2008) and 11 participants (Godfrey et al., 2019) with DS+ASD respectively.

This recruitment challenge is shared by researchers who prospectively study autism infant siblings at risk for developing ASD (ASIBs), where prevalence rates of ASD are similar, at approximately 20% (Ozonoff et al., 2011). To address this challenge in ASIB research, collaboration across sites and consortia (e.g., Baby Siblings Research Consortium, Infant Brain Imaging Study) are capitalized upon to produce large samples of children with outcome diagnoses of ASD. A recently launched NIH-Wide initiative “INCLUDE (Investigation of Co-occurring conditions across the Lifespan to Understand Down syndromE)”, lists ASD as a critical co-occurring condition that will be a target of this initiative. Hopefully, through targeted funding and collaborative research efforts, larger samples can be attained so that these important questions can be investigated to guide screening, diagnostic and treatment efforts to improve outcomes for individuals with DS and comorbid ASD.
Table 2.1 Mean BOSCC scores by group across time

<table>
<thead>
<tr>
<th></th>
<th>FXS</th>
<th>DS</th>
<th>Non-Syndromic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FXS+ASD</td>
<td>FXS-noASD</td>
<td>DS+ASD</td>
</tr>
<tr>
<td></td>
<td>n = 11</td>
<td>n = 6</td>
<td>n = 2</td>
</tr>
<tr>
<td>24 months</td>
<td>29.68 (9.42)*</td>
<td>15.08 (6.76)*</td>
<td>22.00 (4.24)</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 5)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td>36 months</td>
<td>32.71 (7.99)</td>
<td>12.6 (4.85)</td>
<td>21.25 (4.60)</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 5)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td>48 months</td>
<td>33.5 (7.26)</td>
<td>13.75 (5.30)</td>
<td>23.5 (-)</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 2)</td>
<td>(n = 1)</td>
</tr>
<tr>
<td>Average change</td>
<td>0.23 (0.50)</td>
<td>-0.05 (0.35)</td>
<td>-0.24 (0.62)</td>
</tr>
<tr>
<td>score per month</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*FXS+ASD > FXS-noASD
+ FXS+ASD > DS-noASD

FXS, Fragile X syndrome; DS, Down syndrome; ASD, autism spectrum disorder; nsASD, non-syndromic autism spectrum disorder; BOSCC, Brief Observation of Social Communication Change
Figure 2.1 Individual spaghetti plots of BOSCC total score trajectories between groups
REFERENCES


59


