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Parsing Heterogeneity in the Emerging Autism Phenotype:
Effects of Familial Risk for Autism Spectrum Disorder

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of the requirements for the degree Master of Arts
in Education

by

Torrey Lynn Cohenour

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ABSTRACT OF THE THESIS

Parsing Heterogeneity in the Emerging Autism Phenotype:
Effects of Familial Risk for Autism Spectrum Disorder

by

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Master of Arts in Education
University of California, Los Angeles, 2020
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Autism spectrum disorder (ASD) is a heterogeneous, highly heritable neurodevelopmental disability. Evidence suggests individuals with ASD who are at high familial risk for autism (i.e., those from multiplex families containing two or more ASD-affected children), exhibit distinct clinical characteristics from individuals with ASD from simplex families. The aim of the present study was to examine the effects of familial risk for autism on the emerging autism phenotype among infants and toddlers showing signs of ASD. Participants (N = 137) were 12- to 36-month-olds referred to two larger autism intervention studies due to autism concerns. Overall, simplex children showing signs of ASD demonstrated more severe cognitive delays, were more likely to present with expressive language delays, and had more severe autism symptoms than multiplex children with emerging ASD symptoms. This study is among the first to examine the effects of familial risk for autism among infants and toddlers showing early symptoms of ASD, and
demonstrates that simplex-multiplex differences are present in the first years of life and before autism symptoms fully emerge.
The thesis of Torrey Lynn Cohenour is approved.

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2020
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Autism spectrum disorder (ASD) is a neurodevelopmental disability characterized by persistent deficits in social-communication and the presence of restrictive interests or repetitive behaviors (American Psychiatric Association, 2013). Autism is highly prevalent, affecting an estimated 1 in 54 children in the United States (Maenner et al., 2020). Risk for autism is largely attributable to genetic factors, though the mechanisms underpinning the emergence of autism traits are complex. Indeed, the genetic mechanisms which give rise to ASD appear to be as heterogeneous as the behavioral manifestation of the disorder itself (D’abate et al., 2019; Gaugler et al., 2014; Grove et al., 2019; Klei et al., 2012; Robinson et al., 2015). Autism is highly heritable, with heritability estimates ranging from approximately 60% to 90% (Bai et al., 2019; Colvert et al., 2015; Sandin et al., 2017; Tick et al., 2016). Furthermore, autism recurrence within families and evidence for the presence of subthreshold autism traits among unaffected family members of individuals with ASD is suggestive intergenerational transmission of autism risk in at least a portion of cases (Constantino & Todd, 2003; Ozonoff et al., 2011; Virkud et al., 2009).

Over the past decade, researchers have begun to leverage the heritability of autism by designing prospective studies examining the development of infants who, by virtue of having an older sibling with an autism diagnosis, are at heightened familial risk for ASD (Szatmari et al., 2016). Such studies specifically target the later-born infant siblings of children with autism, who have an estimated 20% chance of later receiving an autism diagnosis themselves (Ozonoff et al., 2011). Such studies have been fruitful in advancing clinical and public health endeavors targeting early identification and intervention, and basic science endeavors advancing our collective understanding of the pathogenesis of ASD. However, prospective infant sibling studies include only children with autism who have an affected sibling at high familial risk (and thus, only children from multiple-incidence, or “multiplex” families), while children with autism without an affected sibling who are at low familial risk for autism (from single-incidence or “simplex” families) are typically excluded. Individuals with autism from multiplex families account for a relatively small share of the larger autism population – approximately 11% of individuals with ASD are from
multiplex families (Leppa et al., 2016). Thus, while estimates suggest the overwhelming majority of individuals with autism are from simplex families, children with ASD from simplex families are underrepresented in research, particularly in studies of development across the first years of life. This disparity is pronounced in infant studies largely for pragmatic reasons – in a sample of 100 infants at high familial risk for ASD, recurrence risk estimates indicate approximately 20 of those infants (1 in 5) will be later diagnosed with ASD. In contrast, risk for autism among infants at low familial risk is assumed to be approximately equal to population-level risk (1 in 54). However, the availability of screening and diagnostic tools designed for use in infants as young as 12 months of age (e.g., Autism Diagnostic Observation Schedule, Second Edition; Lord, Rutter, et al., 2012) has provided an opportunity for researchers to identify children at low familial risk with emerging ASD symptoms at a younger age than was previously possible.

**Theoretical Framework**

Increasing the representation of infants and toddlers with autism from simplex families in research is imperative, as there is increasing evidence to suggest that distinct genetic pathways underlie autism among individuals from multiplex families and those from simplex families. Critically, it has become increasingly clear that distinct genetic pathways to ASD map onto distinct behavioral phenotypes. This hypothesized link between genetic mechanisms and behavioral features aligns well with theoretical accounts of neurodevelopmental disabilities more broadly, as well as heterogeneity within diagnostic groups and individual differences among affected individuals. Neuroconstructivism posits that development unfolds in the context of dynamic interactions between “constraints” on development – including genetic, neural, behavioral and environmental mechanisms. The neuroconstructivist model provides a useful framework for examining atypical developmental trajectories – theorized to be the result of differential functioning of these constraints – which culminate in the ostensive symptoms of ASD early in life (Karmiloff-Smith et al., 2012; Westermann et al., 2007). For example, neuroconstructivism may posit that constraints which function at the genetic, neural, or behavioral level shape the manner
in which a child with autism interacts their social environment. Thus, a neuroconstructivist approach supports the notion that that familial autism risk status itself may function as a potent constraint on development of among individuals with ASD – possibly resulting in a distinct constellation of genetic, neural, and behavioral constraints between simplex and multiplex children with ASD, which ultimately result in observable phenotypic differences.

Literature Review

Genetic Architecture of Simplex and Multiplex Autism

Both inherited autism risk variants passed from parents to offspring and spontaneous, \textit{de novo} genetic events that are present in the affected individual but not family members have been implicated in autism (Ruzzo et al., 2019). Evidence for diverging genetic architecture of ASD in multiplex and simplex cases primarily emerges from behavioral genetic studies of ASD-affected families examining the relative influence of inherited and non-inherited genetic variance on autism risk. A relatively well-established distinction between the genetic architecture of simplex and multiplex ASD is the rate of rare \textit{de novo} autism risk variants among affected individuals. Rare \textit{de novo} genetic events occur less frequently among multiplex individuals with ASD relative to simplex individuals, with a recent study reporting nearly half the rate of such risk variants in multiplex ASD relative to simplex ASD (Ruzzo et al., 2019; Sebat et al., 2007). In contrast, multiplex individuals with ASD are thought to harbor majority of liability for autism in \textit{inherited} autism risk variants – including polygenic risk attributable to common variants that operate additively, and rare inherited risk variants that are detectable in both affected individuals as well as their unaffected family members (Klei et al., 2012; Ruzzo et al., 2019). Further underscoring the familial nature of multiplex autism, family studies find that unaffected family members of children with ASD tend to show evidence of subthreshold autistic traits or broader developmental delays – a phenomenon observable within multiplex families, but not simplex families (Constantino & Todd, 2005; Gerdts et al., 2013; Sebat et al., 2007). This distinction is noteworthy, given that rare \textit{de novo} mutations contributing to ASD risk, which are observed at a higher
frequency among simplex individuals, are of larger effect size and higher penetrance than inherited genetic risk for ASD seen among individuals from multiplex families (Kosmicki et al., 2017; Ruzzo et al., 2019). These findings suggest that individuals with autism from multiplex families are less likely to be impacted by the pathogenic de novo mutations of large effect which are seen at a higher frequency in simplex cases, and even in the presence of inherited rare risk variants, their penetrance appears lower, and effect, smaller, given they are detectable in both affected and unaffected members of multiplex families (Ruzzo et al., 2019). Taken together, this evidence is suggestive of a distinct genetic architecture of autism in simplex and multiplex individuals.

Clinical Significance of Differing Genetic Pathways to Autism

Variation in the genetic architecture of autism is clinically relevant, given the likely role of genetic mechanisms in shaping clinical manifestation of the disorder. Autism risk attributable to de novo influences is associated with increased incidence of intellectual disability and broader neurological and developmental anomalies, including seizures and neuromotor delays (Robinson et al., 2014; Weiner et al., 2017). Conversely, autism risk largely attributable to inherited genetic liability for autism is associated with less severe ASD symptoms and higher cognitive functioning, relative to cases with significant de novo influences (Robinson et al., 2014). In fact, some inherited autism risk variants, as seen in multiplex families, have been found to be positively associated with cognitive ability and greater educational attainment in the general population (Clarke et al., 2016; Weiner et al., 2017). Both findings are compelling, particularly when considering the potential impact on resultant phenotypes: may enhanced inherited polygenic risk for ASD account for the wide range in cognitive ability among individuals with ASD, and are multiplex individuals overrepresented on the more cognitively-able end of the autism spectrum? Might the genetic overlap of enhanced ASD risk and greater educational attainment reflect a continuous distribution, where intense interests fuel educational attainment towards the center of the distribution, and manifest as circumscribed interests at the extreme? It stands to reason that mechanism of genetic
transmission – whether largely attributable to *de novo*, or non-familial influences as in simplex cases, or inherited, familial influences as in multiplex cases – may contribute to the phenotypic heterogeneity in the broader population of individuals with autism.

**Behavioral Phenotypes in Simplex and Multiplex ASD**

Fueled by clinical observation and behavioral genetics findings, few studies have sought to further probe simplex-multiplex differences through behavioral phenotyping by examining cognitive and behavioral features of individuals from simplex and multiplex families.

**Cognitive Functioning**

Among existing studies, there is consistent evidence for a relative “cognitive advantage” among children and adults with autism from multiplex families relative to simplex families. Using data collected as part of the Autism Genetic Resource Exchange (AGRE), Davis and colleagues (2013) found that simplex individuals with autism (those who had no ASD-affected siblings) had significantly lower nonverbal and verbal IQ scores relative to multiplex individuals with autism (those who had one or more ASD-affected siblings). They further found that among all participants who had nonverbal IQ scores below 70, nearly 90% were simplex, while 90% of participants with nonverbal IQ scores above 110 were multiplex (Davis et al., 2013). A study of children and adolescents with ASD aged 2 to 17 years revealed similar findings – Berends and colleagues (2019) report that multiplex participants had significantly higher verbal, non-verbal, and full-scale IQ scores relative to simplex younger siblings (those who had a typically-developing older sibling and no family history of autism) as well as simplex first-born children (who had no siblings and no family history of autism). However, the authors found no differences between the simplex younger siblings and simplex first-born children in cognitive ability. These findings were partially replicated in a study conducted of toddlers recently diagnosed with ASD by Dissanayake and colleagues (2019) – the only known study to date to specifically examine simplex and multiplex differences among toddlers with ASD. The authors found that multiplex toddlers with ASD had stronger verbal and nonverbal cognitive skills relative to simplex younger siblings. However, no differences were
detected between multiplex toddlers and simplex first-born toddlers, nor between simplex younger siblings and simplex first-born toddlers (Dissanayake et al., 2019). Notably, a study conducted by Oerlemans et al. (2016) reports no differences in cognitive ability between simplex and multiplex individuals with ASD; however, this sample diverged substantially from those previously reported in that it included only youth with a full-scale IQ greater than 70 who were of European Caucasian descent (Oerlemans et al., 2016).

**Autism Symptom Severity**

Studies examining multiplex-simplex differences in autism symptom severity are slightly more numerous. In an early study, researchers compared autism symptom severity among individuals with ASD from simplex families and multiplex families (Cuccaro et al., 2003). Whether the simplex group included participants without a typically-developing older sibling, or participants who had an affected second or third-degree relative, was not reported. As with previously reported studies, with the exception of Dissanayake et al. (2019), this sample included a wide age range, with participants ranging in age between 2.5 years to nearly 21 years. Using the Autism Diagnostic Interview-Revised (ADI-R; Rutter et al., 2003), a semi-structured diagnostic interview administered to parent/caregivers, the authors found no differences between simplex and multiplex groups in autism symptoms. However, these results must be interpreted in the context of the sole assessment used: a *parent-report* measure of autism symptoms. In the absence of a standardized, clinician-administered diagnostic assessment using direct observation, it is difficult to disentangle the possible effects of simplex or multiplex risk status at the level of the affected individual (i.e., phenotypic differences) from the possible effects of multiplex or simplex status on parent and caregiver impressions and report of their child’s symptoms. Thus, signal related to true phenotypic differences between simplex and multiplex individuals with ASD may be distorted by noise introduced at the level of measurement. In line with this theory, a later 2015 study reported discrepancies between parent and clinician reports of social-communication impairments among children with ASD (Taylor et al., 2015). Researchers found that, per parent report, multiplex
children had more severe social-communication impairments than simplex children. In contrast, scores from the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, et al., 2012), a clinician-administered autism diagnostic assessment, indicated that children from simplex families had more severe autism symptoms, including social-communication deficits (Taylor et al., 2015). Despite the results reported in Taylor and colleagues (2015), which found less severe symptoms among multiplex children relative to simplex children as measured on the ADOS, both Berends et al. (2019) and Dissanayake et al. (2019) failed to detect differences in autism symptom severity using the same standardized assessment, despite finding substantial differences between simplex and multiplex groups on cognitive functioning.

**Developmental Trajectories**

Though not explicitly focused on simplex-multiplex comparisons, a longitudinal study tracking autism symptom severity across toddlerhood suggests that the course and trajectory of symptom development may differ between children with ASD from multiplex and simplex families (Lord, Luyster, et al., 2012). In a clinic-referred sample of toddlers, some of whom were the younger siblings of a child with ASD, researchers identified four distinct trajectories, or classes, of describing symptom development: a class containing children with severe autism symptoms that remained severe over development (Severe-Persistent), a class containing those whose ASD symptoms became more severe over time (Worsening), a class containing those whose symptoms became less severe over time (Improving), and a class containing children who were ultimately found to not meet autism criteria (Non-Spectrum). Of the 34 toddlers with an affected older sibling (multiplex toddlers), 14 (41%) were categorized into the Non-Spectrum class, while nine (29%) of the 31 toddlers without siblings with ASD (simplex toddlers) were categorized as Non-Spectrum. Put differently, of the 23 simplex and multiplex children in the Non-Spectrum class, the majority (61%) were multiplex. These findings suggest that toddlers with autism from multiplex families may be more likely than simplex children to follow a developmental trajectory that is characterized by persistent low-levels of autism features (despite initial ASD concerns) and
a developmental outcome that is less severe and may more closely resemble typical development than autism.

In summary, research to date examining effects of familial risk for autism on behavioral phenotypes has been rather limited, particularly among very young children showing early signs of autism. Within the existing literature, a few key themes can be identified. First, as predicted by genetic findings, multiplex individuals with ASD tend to demonstrate stronger cognitive skills compared to simplex individuals with ASD. Interestingly, this effect was present in samples restricted to toddler- and preschool-age children with ASD, as well as samples containing school-age children and adolescents with ASD. Second, findings pertaining to group differences in symptom severity seem to be influenced by informant. There was a trend toward children with ASD from multiplex families demonstrating more severe autism symptoms compared to simplex children as reported by parents, however, when a gold-standard, clinician-administered diagnostic assessment was used, group differences were either no longer detectable, or simplex individuals were found to have more severe symptoms. This pattern speaks both to potential effects of family affectedness on parents (characteristics of parents themselves, or parent perceptions of autism symptoms), and by extension, the limitations of parent-report measures to index symptom severity. However, even when comparing studies using the same clinician-administered direct assessment of autism symptom severity, findings are still quite mixed, perhaps in part due to the wide age range of participants included in many of the reviewed studies.

The Present Study

Despite availability of gold-standard diagnostic instruments designed to identify children as young as 12 months of age showing behavioral signs of autism, very little is known about infants and toddlers who are later diagnosed with ASD from simplex families. This is suggestive of a substantial blind spot in our broader understanding of autism that has yet to be addressed. It remains unclear whether early development among infants presumed to be at low familial risk for autism, as in children from simplex families, resembles that reported in infants and toddlers at high
familial risk for autism from multiplex families, nor whether early symptom development in simplex ASD resembles that observed in multiplex ASD. Understanding differences in between multiplex and simplex autism very early in development has important implications for our understanding of the relationship between distinct genetic etiologies (e.g., mechanisms of genetic transmission of ASD) and behavioral phenotypes early in symptom development. With increasing calls for the development of “pre-emptive” interventions for infants at developmental risk for autism, addressing this gap also has important implications for the development of effective interventions. Proposed autism endophenotypes (or perhaps more appropriately, markers of risk for ASD) which manifest as “developmental liabilities” – attention problems, motor coordination difficulties, and atypical social visual attention – are often a proposed target for such pre-emptive interventions (Constantino, 2018; Jones & Klin, 2013; Pohl et al., 2019). However, little is known about whether, and how, these liabilities manifest in infants and toddlers with autism from simplex families, given these findings are derived from infant sibling studies of multiplex children. Thus, examining clinical characteristics that distinguish simplex and multiplex autism may be in service of both basic science endeavors to understand biological mechanisms underpinning autism, as well as those clinical endeavors seeking to optimize care for affected individuals and their families. This study seeks to address this gap by characterizing the emerging autism phenotype among infants and toddlers early in symptom development, and comparing the clinical characteristics among simplex infants and toddlers at low familial risk for autism and multiplex infants and toddlers at high familial risk for autism. Examining behavioral phenotypes across groups shortly after the onset of behavioral symptoms provides a unique opportunity to describe and parse heterogeneity in the earliest behavioral manifestations of autism in the first years of life.
Research Aims

Aim 1: Examine whether infants and toddlers with emerging autism symptoms from multiplex families differ from infants and toddlers with emerging autism symptoms from simplex families in nonverbal and verbal cognitive ability.

Aim 2: Test whether there is an association between familial autism risk status and language delay among infants and toddlers with emerging autism symptoms from multiplex and simplex families.

Aim 3: Evaluate whether infants and toddlers with emerging autism symptoms from multiplex families differ from infants and toddlers with emerging autism symptoms from simplex families in autism symptom severity.

Method

Procedure

The current study uses secondary data derived from two larger autism intervention studies for infants and toddlers showing early behavioral features of ASD conducted at University of California, Los Angeles. The first study was conducted between October 2012 and June 2016 and the second ongoing study began in June 2018 (NICHD P50HD055784-5843, NICHD P50HD055784-8487, PI: Connie Kasari). The University of California, Los Angeles Institutional Review Board approved the studies, and parents of participating infants and toddlers provided written consent to participate. Upon referral to the intervention studies, children were screened for the presence of elevated autism symptoms, a requirement for inclusion in the intervention studies, using the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2; Lord, Rutter et al., 2012). At screening, parents or caregivers were also asked to complete a demographic questionnaire, which included family sociodemographic information as well as information about family history of autism or other neurodevelopmental disabilities.

Criteria for Determining Familial Risk Status

For the present study, parent-reported family history of autism was assessed in order to
construct familial risk groups. Multiplex status (high familial risk status) was determined by the presence of at least one sibling with an autism diagnosis. While those who do not have a sibling with ASD are, by definition, from single-incidence families, for the purpose of the present study, simplex status was more stringently defined in order to reflect those from simplex families who were likely also at low familial risk for autism. Simplex status was defined by the absence of “suspected” autism in first degree relatives, and no family history of autism in second- or third-degree relatives. These additional criteria were used in order to minimize the likelihood of misclassifying children who may have harbored some inherited genetic risk for autism that has simply not manifested in the family unit.

Participants

Participants were deemed eligible for participation in the original intervention studies if they were between 12 and 36 months of age, demonstrated elevated autism features as measured on the ADOS-2, and had no co-occurring neurological, sensory, or known genetic conditions. Participants in the initial intervention studies were recruited from community-based healthcare providers (e.g., pediatricians) and autism diagnostic or intervention service providers. Complete autism family history information and ADOS-2 screening outcome was available for 152 participants.

Of the 152 participants with available ASD family history data, a total of $n = 36$ participants had at least one sibling with an ASD diagnosis, and were categorized as multiplex (MPX). Of the remaining 116 participants who did not have an affected sibling, children with a parent or sibling with “suspected” ASD were excluded ($n = 4$), as were those with a family history of ASD in second- or third-degree relatives ($n = 11$). The remaining 101 simplex participants were then split into two groups: simplex younger siblings who had at least one typically-developing older sibling (SPX-Sib, $n = 41$), and simplex first-born children who had no older siblings (SPX-FB, $n = 60$). Thus, a total of 137 participants were categorized according to familial risk status. Of this group, a total of 121 infants and toddlers (88.3%) met ADOS-2 criteria at screening. See Table 1 for participant
characteristics.

Measures

**Autism Diagnostic Observation Schedule, Second Edition (ADOS-2).** The ADOS-2 is a gold-standard diagnostic instrument developed to inform the diagnosis of autism spectrum disorder and characterize the severity of autism symptoms. The Toddler Module of the ADOS-2 was developed specifically to assess autism symptoms in children aged 12 to 30 months, and provides separate scoring algorithms depending on a child’s age and language level. The Toddler Module demonstrates satisfactory sensitivity and specificity in identifying very young children with early-emerging autism symptoms (Luyster et al., 2009). The ADOS-2 Modules 1 and 2, designed for older children and those with limited language, demonstrate satisfactory sensitivity and specificity in identifying young children with ASD (Lord, Rutter, et al., 2012). The ADOS-2 produces two domain scores: a Social Affect domain score, which captures social-communication deficits, and a Restricted, Repetitive Behavior (RRB) domain score. Additionally, calibrated severity scores (scaled from 1 to 10) serve as an index of the overall severity of autism-specific behaviors (Esler et al., 2015).

**Mullen Scales of Early Learning (MSEL).** The MSEL is a standardized, developmental assessment examining cognitive and motor development in children between 3 and 68 months of age (Mullen, 1995). The assessment probes five domains of development: gross motor skills, fine motor skills, visual reception, expressive language, and receptive language. Age equivalent scores for each domain as well as T-scores are provided. Using age equivalent scores, nonverbal developmental quotient (NVDQ) and verbal developmental quotient (VDQ) are calculated. Developmental quotients (DQ) are calculated by dividing the average age equivalent score across domains by chronological age and multiplying the resulting value by 100. Visual reception and fine motor age equivalent scores are used to calculate NVDQ, while receptive language and expressive language age equivalent scores are used to calculated VDQ. The purpose of utilizing DQ, as is commonly used to examine developmental level in studies of children with ASD or other
developmental disabilities, is to avoid possible floor or ceiling effects while also providing an age-appropriate metric of cognitive functioning akin to IQ (Munson et al., 2008). Additionally, T-scores are used as an indicator of developmental delay relative to age, particularly in the context of language delay. As is common practice in previous studies of infants and toddlers with ASD, language delay is defined as a T-score less than or equal to 35 on receptive language or expressive language domains of the MSEL (Marrus et al., 2018).

**Demographic Form.** Parents or caregivers were asked to complete a brief questionnaire about child and family demographic characteristics, and information about the participating child’s siblings including their age, grade, sex, and whether they had an autism diagnosis. Parents or caregivers were asked to indicate whether any other family members have a history of learning disabilities or developmental disabilities, including autism.

**Analytic Strategy**

Analysis of variance and chi-square tests were used to assess for group differences on pertinent sociodemographic variables. Multivariate analyses of covariance (MANCOVA) were used to test for group differences in clinical characteristics after controlling for background variables. Given the unequal sample sizes across familial risk groups, equality of covariance matrices was assessed using Box’s $M$, which was evaluated at $p < .001$. Pillai’s criterion, which is more robust than other multivariate statistics in the presence of unequal sample sizes and heterogeneity of covariance matrices, is reported (Olson, 1974). Significant multivariate tests were further decomposed into separate univariate analyses with pairwise contrasts, corrected for error inflation with Bonferroni adjustment, to further parse group differences. Binary logistic regression was performed to test for group differences in receptive and expressive language delay after controlling for background variables.
Results

Descriptive Analyses

ADOS-2 Screening Outcome

Of the 137 children screened with the ADOS-2, mean age at screening was 20.04 months ($SD = 5.70$). Screening age was lowest for the MPX group ($M = 18.94$ months, $SD = 4.16$), followed by SPX-Sib ($M = 21.44$ months, $SD = 6.23$) and SPX-FB groups ($M = 22.02$ months, $SD = 5.88$). One-way analysis of variance revealed significant group differences in age at referral (Welch’s $F(2,84.02) = 4.93, p = .009$). Games-Howell post-hoc tests indicate MPX participants were significantly younger than SPX-FB participants at referral ($p = .010$), though no other groups differed significantly. Of the 137 children screened, the proportion of children who did not meet ADOS-2 screening criteria was greatest among the MPX group ($n = 7, 19.4$%), followed by SPX-FB ($n = 6, 10.0$%), and SPX-Sib ($n = 3, 7.3$%) groups. Binary logistic regression was conducted to test the association between familial risk status and screening outcome, controlling for age at screening. The model demonstrated appropriate goodness-of-fit (Hosmer and Lemeshow’s test, $\chi^2(8) = 6.04, p = .643$). The omnibus test of the overall model fit was not significant ($\chi^2(3, N = 137) = 4.03, p = .258$). The model explained only 5.6% of the variance in screening outcome ($Nagelkerke R^2 = .056$), with familial risk status accounting for about 5% of that figure.

Final Sample Description

The final sample includes 121 participants who met ADOS-2 criteria for elevated autism symptoms and thus, enrollment in the original intervention study. Among infants and toddlers who had met ADOS-2 criteria for enrollment, mean age at enrollment was 20.92 months ($SD = 5.55$). There were no differences between familial risk groups in age at enrollment, gender, primary language heard at home, or the proportion of infants and toddlers who had already received a community diagnosis of ASD prior to screening for the intervention studies. The male-to-female ratio was 3.48 (22.3% female, 77.7% male), which resembles that of recent epidemiological reports (Shaw et al., 2020). The majority of participants were from racial/ethnic minority families.
(57.9%). Significant group differences were detected in the proportion of children who reported White versus non-White ethnicity, \( \chi^2(2, N = 121) = 7.67, \ p = .022 \). Follow-up analyses revealed that the MPX group contained a significantly lower proportion participants who were White relative to the SPX-FB group \( (p = .018) \). Income was reported for 113 participants, with the majority of families (62.8%) reporting a household income over $100,000. There were no group differences in the proportion of families who reported an income over $100,000, \( (p = .397) \).

**Primary Aims**

**Cognitive Ability**

Cognitive scores derived from the MSEL were available for 117 participants. To test for significant group differences in cognitive ability, a MANCOVA was conducted with NVDQ and VDQ as dependent variables. Chronological age, sex, ethnicity, and study cohort were treated as covariates. Box’s test indicated the assumption of equality of covariance matrices was met \( (p = .160) \). There was a significant multivariate effect of familial risk status on cognitive ability (Pillai’s Trace = .116, \( F(4,220) = 3.37, \ p = .011, \eta_p^2 = .058 \)). Chronological age also significantly contributed to the multivariate model \( (p = .005) \). Univariate follow-up analyses were conducted following the significant omnibus multivariate test, and revealed significant group differences in both NVDQ, \( (F(2,110) = 5.39, \ p = .006, \eta_p^2 = .089) \), and VDQ \( (F(2,110) = 5.72, \ p = .004, \eta_p^2 = .094) \). Chronological age was also significant for NVDQ, \( (p = .011) \). To further specify the nature of group differences, pairwise contrasts were evaluated for both NVDQ and VDQ. Comparisons reveal that MPX children had significantly higher NVDQ scores than SPX-FB children \( (p = .015, \eta_p^2 = .070) \) and SPX-Sib children \( (p = .009, \eta_p^2 = .077) \). As expected, there were no significant differences between the two simplex groups \( (p > .99) \). A similar pattern was revealed for VDQ scores: MPX children had significantly higher scores than both SPX-FB children \( (p = .046, \eta_p^2 = .052) \) and SPX-Sib children \( (p = .003, \eta_p^2 = .093) \), and simplex groups did not differ from one another \( (p = .710) \).
**Language Delay**

The majority of toddlers presented with a language delay: 81.2% of toddlers presented with a receptive language delay, and 72.8% of toddlers presented with an expressive language delay. A series of binary logistic regressions were conducted predicting presence of language delay while controlling for chronological age, sex, ethnicity, study cohort, and NVDQ. The model predicting receptive language delay was significant ($\chi^2(7, N = 117) = 50.48, p < .001$). The model demonstrated appropriate goodness of fit (Hosmer and Lemeshow Test, $\chi^2(8) = 7.57, p = .476$), and accounted for 56.6% of the variance in receptive language delay (Nagelkerke $R^2 = .566$). The effect of age was significant ($p = .003$, Odds Ratio [OR] = .750) as was the effect of NVDQ ($p < .001$, OR = .882), suggesting that older age and higher NVDQ were associated with lower odds of receptive language delay. The overall effect of familial risk status was not significant ($p = .201$). The second binary logistic regression model was conducted testing for the association between familial risk status and expressive language delay. The overall model was significant ($\chi^2(7, N=117) = 41.04, p < .001$). The model demonstrated appropriate goodness of fit (Hosmer and Lemeshow Test, $\chi^2(8) = 4.46, p = .813$), and accounted for approximately 42.5% of the variance in expressive language delay (Nagelkerke $R^2 = .425$). The overall effect of familial risk status was significant (Wald $\chi^2(2) = 6.966, p = .031$). Specifically, the model indicates that after controlling for the effects of age, sex, study cohort, ethnicity, and NVDQ, SPX-Sib children were 8.20 times more likely than MPX children to present with an expressive language delay at enrollment (Wald $\chi^2(1) = 6.84, p = .009$). As with the model predicting receptive language delay, the effect of NVDQ was significant ($p < .001$, OR = .926), such that higher NVDQ predicted lower odds of expressive language delay.

**Autism Symptoms**

A MANCOVA was conducted to test for differences across familial risk groups in autism symptoms. ADOS-2 Social Affect (SA) and Restricted and Repetitive Behavior (RRB) domain raw
scores were entered as dependent variables. Chronological age, sex, ethnicity, and study cohort were treated as covariates. Box’s test indicated the assumption of equality of covariance matrices was met \( (p = .982) \). Analyses reveal significant group differences in autism symptoms across familial risk groups (Pillai’s Trace = .106, \( F(4,228) = 3.20, p = .014, \eta_p^2 = .053 \)). The significant omnibus multivariate test was followed with univariate analyses to test for group differences in SA and RRB domain scores separately. Univariate omnibus tests revealed significant group differences in SA domain scores \( (F(2,114) = 4.077, p = .019, \eta_p^2 = .067) \) and RRB domain scores \( (F(2,114) = 3.73, p = .027, \eta_p^2 = .061) \). The effect of chronological age was also significant for both SA \( (p = .011) \) and RRB \( (p = .009) \) scores. Pairwise comparisons with Bonferroni correction were used to further specify the nature of group differences. Compared to MPX children, SPX-Sib children had significantly higher SA scores \( (p = .020, \eta_p^2 = .062) \) and RRB scores \( (p = .024, \eta_p^2 = .060) \). There were no differences between MPX and SPX-FB children on SA \( (p = .066) \) or RRB scores \( (p = .135) \) after Bonferroni adjustment. SPX-FB and SPX-Sib groups also did not differ significantly on SA \( (p > .99) \) nor RRB scores \( (p > .99) \).

An ANCOVA was conducted to test for differences across familial risk groups in overall autism symptom severity, as indexed by ADOS-2 calibrated severity scores, after controlling for age, sex, ethnicity, and study cohort. The model reveals significant group differences in severity scores \( (F(2,114) = 5.413, p = .006, \eta_p^2 = .087) \). Pairwise comparisons indicate MPX children had significantly lower severity scores than both SPX-FB \( (p = .016, \eta_p^2 = .066) \) and SPX-Sib children \( (p = .008, \eta_p^2 = .077) \). Simplex groups did not differ from one another \( (p > .99) \).

**Discussion**

The present study revealed clinically-meaningful phenotypic differences in simplex and multiplex autism, and further specified the effect of familial risk status on clinical characteristics of infants and toddlers with emerging ASD symptoms from simplex and multiplex families.
Age at Referral and Screening Outcome

There were significant differences in age at referral between familial risk groups among all families referred to the study (i.e., those who did and did not meet ADOS-2 criteria), with children from multiplex families completing screening at a younger age. This may reflect a tendency among multiplex parents to more quickly to identify delayed or atypical development, and express concerns to providers more promptly. Indeed, parents who have at least one older child with ASD tend to express concerns about their younger child’s development significantly earlier than parents with either typically-developing older children, or no older children (Herlihy et al., 2015). In a sample of infants who screened positive and were subsequently diagnosed with ASD, Herlihy and colleagues (2015) found that, despite the fact that infants from multiplex families showed the fewest and least pronounced developmental delays relative to infants from simplex families, multiplex parents still reported first concerns about their child’s development four to six months earlier than simplex parents. This combination of earlier concern and increased ease of access to services relative to simplex families (as a result of having an older affected child) may be driving the younger age at referral and screening among infants and toddlers from multiplex families.

However, the lack of significant group differences in age at screening after removal of the 16 participants who did not meet ADOS-2 criteria was unexpected. For descriptive purposes, age at screening was reinspected as a function of both familial risk group and screening outcome (thus, yielding six groups). In doing so, an intriguing pattern emerged: the average age of SPX-FB and SPX-Sib participants who did not meet ADOS-2 criteria was greater than the average age of their counterparts who did meet ADOS-2 criteria. In contrast, the average age of MPX participants who did not meet ADOS-2 criteria was younger than that of their MPX counterparts who did meet ADOS-2 criteria. Though this pattern is difficult to interpret in the absence of a larger sample, it may signal different factors are service-seeking behavior of parents, and potentially, provision of referrals among care providers. For one, so-called “diagnostic suspicion bias”, may
lead to multiplex children presenting with more mild developmental delays being referred immediately for autism-specific services due to their known elevated risk for ASD, while parents and care providers of simplex children presenting with similar developmental delays may be more likely to take, or be advised to take, a “wait-and-see” approach. Furthermore, while not statistically significant, it could be argued that the discrepancy between multiplex and simplex groups in the proportion of children who did not meet ADOS-2 criteria (19.4% of multiplex children versus 8.9% of all simplex children) is still substantively meaningful. Important contextual factors, such as referral source, severity of parent concerns, age at first concerns, service utilization history, and, among multiplex families, the severity of the affected sibling’s ASD symptoms, may underlie parents’ decision to seek out and enroll in an autism intervention study.

**Cognitive Ability and Language Delay**

Among children who met ADOS-2 criteria, results revealed clinically meaningful differences in cognition and broader developmental status of infants and toddlers from multiplex and simplex families. In line with findings reported by Dissanayake and colleagues (2019), children from multiplex families demonstrated a distinct advantage relative to simplex children in both nonverbal and verbal cognitive ability, and to some extent, in language ability. Given group differences in expressive language delay were detected between multiplex and simplex younger siblings, the lack of significant differences in receptive language delay was somewhat unexpected, though this may speak to the atypical language profile and trajectory of receptive and expressive language development observed in young children with ASD. Some evidence suggests toddlers and preschoolers with ASD are more likely than typically-developing children to show an “atypical” language profile, characterized by stronger expressive language skills relative to receptive language skills (Hudry et al., 2010). While the evidence for atypical expressive-receptive language profiles among children with ASD is somewhat mixed (see Kwok et al., 2015), it is possible that there are differences across familial risk groups in the proportion of children presenting with an atypical language profile, or alternatively, differences in the
trajectories of receptive relative to expressive language development across groups. Future work using more targeted assessments of language development is needed to better characterize and compare the language profiles of toddlers with ASD from multiplex and simplex families.

**Autism Symptom Severity**

These results also suggest multiplex-simplex differences extend beyond cognitive and language skills to also encompass autism-specific behaviors and symptom severity, in contrast with previous reports. Analyses revealed significant group differences in autism-specific social-communication deficits and restricted and repetitive behaviors between multiplex children and simplex younger siblings, though effect sizes were relatively small across both domains. When examining overall symptom severity, multiplex participants demonstrated less severe symptoms than both simplex groups, with particularly marked differences between multiplex children and simplex younger siblings. In contrast, previous work in older children found no differences in social-communication deficits and restricted or repetitive behaviors, or overall autism severity as measured using the same direct assessment. One possible explanation for this finding is that trajectories of symptom development differ between multiplex and simplex infants and toddlers. It is possible that behavioral features of autism among infants and toddlers from simplex families are more severe at the outset, whereas autism symptoms among multiplex toddlers are initially milder but become more pronounced later in childhood. This could explain why the present study detected such differences, but not previous research studies that included significantly older participants. Longitudinally tracking the emergence of autism symptoms among infants from simplex and multiplex families across toddlerhood would be valuable in testing this possibility.

**Limitations and Future Directions**

These findings should be interpreted in light of a few limitations. For one, as with previous studies comparing multiplex and simplex autism, there is the possibility that some participants at elevated familial risk for autism were inappropriately categorized as simplex, given familial risk status was determined using parent-reported family history of autism. This is particularly true for
simplex children without an older sibling to serve as a reference when determining risk status, as
was the case for infants and toddlers in the SPX-FB group. Of similar consequence, the
curtailment of reproduction, known as “reproductive stoppage”, or the extension of inter-
pregnancy interval among participating families may have distorted true multiplex familial risk
among families who were initially believed to be simplex. This limitation is difficult to reconcile
directly, however by excluding simplex participants with a family history of autism in second-, or
third-degree family members as well as those with a parent or sibling with “suspected” autism,
every effort was made to minimize the likelihood of including children in the simplex group who
may have harbored undetected familial risk for autism.

The possibility that ascertainment bias may have influenced group differences must be
noted. It is possible that the simplex group was sampled from the more severely affected end of
the spectrum while the multiplex group was sampled from the relative less severely affected end
of the spectrum. For example, multiplex children be more likely to be enrolled in community-based
interventions than same-age simplex children with similar severity of autism symptoms, and thus,
were less motivated to enroll in an experimental intervention study since they were already
receiving services. However, previous studies using different ascertainment methods, including a
sample of clinic-referred children who had screened positive for autism on a pediatrician-
administered universal screening measure (Dissanayake et al., 2019) and a sample of
participants derived from a large autism genetics database (Berends et al., 2019), found similar
results to those reported in the present study, suggesting ascertainment bias likely did not account
for these observed group differences.

The current study was not able to address the potential effects of simplex or multiplex
familial risk status on the caregiving environment, and the subsequent role caregiving
environments may have on clinical presentation. While multiplex-simplex differences have largely
been attributed to biological and genetic differences, the potential for environment effects, and
interactions between biological (genetic) and environmental forces, has largely been neglected.
For one, there is currently a dearth of research on autism service-seeking and utilization among simplex and multiplex families with at-risk infants and toddlers. While evidence suggests multiplex parents tend to develop concerns about their child’s development earlier than simplex parents (Herlihy et al., 2015), less is known about parent behavior (seeking of services and service utilization) following these first concerns. Relatedly, very little is known about parenting practices and home environments of simplex and multiplex families, and the potential effects that these factors may have on child development. It is possible that experience with raising an older child with autism, as is the case among multiplex parents in the present study, influences the manner in which parents care for and interact with their younger child showing signs of ASD. For one, multiplex parents may have knowledge about developmentally-supportive caregiving practices or behavioral intervention strategies that they have acquired through caring for their older child with ASD, which they, in turn, utilize when caring for their younger, at-risk infant. In the absence of an older affected child with ASD, simplex parents have not yet had exposure to or practice with using these supportive strategies and techniques. Taken together, the influence of familial risk status on the caregiving environment, and subsequent effects on child development, is an area ripe for further study.

Conclusions

The present study provides novel insight into the nature and specificity of clinical and behavioral differences between infants and toddlers with emerging symptoms of ASD from simplex and multiplex families. Rather than manifesting later in development, these results suggest that diverging clinical profiles among infants and toddlers from simplex and multiplex families are present and detectable from very early in symptom development, and in some cases, before diagnosable autism symptoms have full emerged. These data also support the notion that the effects of familial risk status cross various domains of functioning, including cognition, language and autism-specific behavioral features. The present study underscores the need to prioritize the inclusion both simplex infants and multiplex infants in research, and particularly in
studies aimed at developing and optimizing behavioral interventions for infants showing signs of autism.
## Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>SPX-FB n = 54</th>
<th></th>
<th>SPX-Sib n = 38</th>
<th></th>
<th>MPX n = 29</th>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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<td>44</td>
<td>81.5</td>
<td>28</td>
<td>73.7</td>
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<td>75.9</td>
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<td>18.5</td>
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<td>26.3</td>
<td>7</td>
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<td>3.4</td>
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<td>3</td>
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<td>27.6</td>
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<td>7</td>
<td>24.1</td>
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<td>3</td>
<td>7.9</td>
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<td>6.9</td>
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<td>20.7</td>
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<td>5</td>
<td>17.2</td>
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<td>35</td>
<td>92.1</td>
<td>23</td>
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<td>Other</td>
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<td>14.8</td>
<td>3</td>
<td>7.9</td>
<td>6</td>
<td>20.7</td>
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<td><strong>Study Cohort</strong></td>
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<td>Study 1</td>
<td>33</td>
<td>61.1</td>
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<td>Study 2</td>
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<td>16</td>
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<td>7</td>
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<td>4</td>
<td>13.8</td>
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<td><strong>Maternal Education</strong></td>
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<tr>
<td>Some College or Less</td>
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<td>20.4</td>
<td>12</td>
<td>31.6</td>
<td>4</td>
<td>13.8</td>
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<td>College Degree</td>
<td>19</td>
<td>35.2</td>
<td>7</td>
<td>18.4</td>
<td>15</td>
<td>51.7</td>
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<tr>
<td>Graduate Degree</td>
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<td>44.4</td>
<td>19</td>
<td>50.0</td>
<td>10</td>
<td>34.5</td>
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<td><strong>Household Income</strong></td>
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<td>Less than $100,000</td>
<td>20</td>
<td>37.0</td>
<td>15</td>
<td>39.5</td>
<td>7</td>
<td>24.1</td>
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<td>$100,000+</td>
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<td>61.1</td>
<td>19</td>
<td>50.0</td>
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<td>65.5</td>
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<td>1.9</td>
<td>4</td>
<td>10.5</td>
<td>3</td>
<td>10.3</td>
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</tbody>
</table>

*Note. N = 121. Household income reported for n = 113 participants.*

SPX-FB = simplex first-born, SPX-Sib = simplex younger sibling, MPX = multiplex.
Table 2. MANCOVA of MSEL Developmental Quotients

<table>
<thead>
<tr>
<th>Effect</th>
<th>Pillai's Trace</th>
<th>F</th>
<th>df</th>
<th>p</th>
<th>Partial $\eta^2$</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.597</td>
<td>80.77</td>
<td>(2,109)</td>
<td>.000</td>
<td>.597</td>
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<tr>
<td><strong>Familial Risk Status</strong></td>
<td>0.116</td>
<td>3.37</td>
<td>(4,220)</td>
<td>.011</td>
<td>.058</td>
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<tr>
<td>Age (months)</td>
<td>0.094</td>
<td>5.64</td>
<td>(2,109)</td>
<td>.005</td>
<td>.094</td>
</tr>
<tr>
<td>Sex</td>
<td>0.002</td>
<td>0.11</td>
<td>(2,109)</td>
<td>.896</td>
<td>.002</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.017</td>
<td>0.94</td>
<td>(2,109)</td>
<td>.395</td>
<td>.017</td>
</tr>
<tr>
<td>Study Cohort</td>
<td>0.007</td>
<td>0.37</td>
<td>(2,109)</td>
<td>.690</td>
<td>.007</td>
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</table>

*Note.* $N = 117$. Multivariate effect of familial risk status on the linear combination of nonverbal and verbal developmental quotients, after controlling for effects of age (months), sex (male, female), ethnicity (non-White, White), and study cohort (Study 1, Study 2). MSEL = Mullen Scales of Early Learning.

$df =$ degrees of freedom (hypothesis, error).
Table 3. Univariate Follow-Up Tests of MSEL Developmental Quotients

<table>
<thead>
<tr>
<th>Variable</th>
<th>SPX-FB</th>
<th>SPX-Sib</th>
<th>MPX</th>
<th>F(2,110)</th>
<th>Partial η²</th>
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<tr>
<td></td>
<td>$M_{adj}$ (SE)</td>
<td>$M_{adj}$ (SE)</td>
<td>$M_{adj}$ (SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVDQ</td>
<td>78.96 (2.84)</td>
<td>77.56 (3.17)</td>
<td>91.56 (3.71)</td>
<td>5.39**</td>
<td>.089</td>
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<tr>
<td>VDQ</td>
<td>57.53 (3.78)</td>
<td>51.36 (4.21)</td>
<td>71.90 (4.93)</td>
<td>5.72**</td>
<td>.094</td>
</tr>
</tbody>
</table>

Note. $N = 117$. Estimated marginal means and tests for group differences on NVDQ and VDQ scores after controlling for age, sex, ethnicity, and study cohort. $F$ test statistics refer to the main effect of familial risk group on NVDQ and VDQ scores after controlling for effects of covariates (age, sex, ethnicity and study cohort).

MSEL = Mullen Scales of Early Learning.

NVDQ = Nonverbal developmental quotient, VDQ = Verbal developmental quotient.

$M_{adj} =$ Adjusted mean (estimated marginal mean), SE = standard error.

*p<.05 **p<.01
Table 4. Pairwise Group Differences in MSEL Developmental Quotients

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>t(110)</th>
<th>p</th>
<th>Partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NVDQ</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MPX vs. SPX-FB</td>
<td>12.6</td>
<td>4.4</td>
<td>2.87</td>
<td>.015</td>
<td>.070</td>
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<tr>
<td>MPX vs. SPX-Sib</td>
<td>14.0</td>
<td>4.6</td>
<td>3.04</td>
<td>.009</td>
<td>.077</td>
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<td>SPX-FB vs. SPX-Sib</td>
<td>1.4</td>
<td>3.9</td>
<td>-0.40</td>
<td>.99</td>
<td>.001</td>
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<td><strong>VDQ</strong></td>
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<tr>
<td>MPX vs. SPX-FB</td>
<td>14.4</td>
<td>5.8</td>
<td>2.46</td>
<td>.046</td>
<td>.052</td>
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<tr>
<td>MPX vs. SPX-Sib</td>
<td>20.5</td>
<td>6.1</td>
<td>3.35</td>
<td>.003</td>
<td>.093</td>
</tr>
<tr>
<td>SPX-FB vs. SPX-Sib</td>
<td>6.2</td>
<td>5.2</td>
<td>1.19</td>
<td>.710</td>
<td>.013</td>
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MSEL = Mullen Scales of Early Learning.

NVDQ = Nonverbal developmental quotient, VDQ = Verbal developmental quotient.

B = unstandardized estimated difference, SE = standard error.

*p < .05 **p < .01
### Table 5. Binary Logistic Regression Models Predicting Language Delay

<table>
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<tr>
<th>Model</th>
<th>B</th>
<th>SE</th>
<th>p</th>
<th>OR</th>
<th>95% CI for OR</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LL</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UL</td>
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<tr>
<td><strong>Receptive Language Delay</strong></td>
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<tr>
<td>Age (months)</td>
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<tr>
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<td>(Constant)</td>
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<td><strong>Expressive Language Delay</strong></td>
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<td>Sex</td>
<td>.477</td>
<td>.663</td>
<td>.471</td>
<td>1.61</td>
<td>.44</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>-.549</td>
<td>.542</td>
<td>.311</td>
<td>0.58</td>
<td>.20</td>
</tr>
<tr>
<td>Familial Risk Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.031</td>
</tr>
<tr>
<td>SPX-FB</td>
<td>.539</td>
<td>.621</td>
<td>.386</td>
<td>1.71</td>
<td>.51</td>
</tr>
<tr>
<td>SPX-Sib</td>
<td>2.104</td>
<td>.805</td>
<td>.009</td>
<td>8.20</td>
<td>1.69</td>
</tr>
<tr>
<td>(Constant)</td>
<td>7.301</td>
<td>2.382</td>
<td>.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. N = 117. Receptive and expressive language delay were defined as a receptive language or expressive language T-score ≤ 35 on the Mullen Scales of Early Learning. Familial risk status (reference group = MPX) was entered as a predictor. Age, NVDQ, study cohort (0 = Study 1, 1 = Study 2), sex (0 = male, 1 = female), ethnicity (0 = non-White, 1 = White) were treated as covariates. NVDQ = Nonverbal developmental quotient. SE = standard error, OR = odds ratio.*
### Table 6. MANCOVA of ADOS-2 Domain Scores

<table>
<thead>
<tr>
<th>Effect</th>
<th>Pillai’s Trace</th>
<th>F</th>
<th>df</th>
<th>p</th>
<th>Partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.452</td>
<td>46.57</td>
<td>(2,113)</td>
<td>.000</td>
<td>.452</td>
</tr>
<tr>
<td><strong>Familial Risk Status</strong></td>
<td>0.106</td>
<td>3.20</td>
<td>(4,228)</td>
<td>.014</td>
<td>.053</td>
</tr>
<tr>
<td>Age (months)</td>
<td>0.124</td>
<td>8.03</td>
<td>(2,113)</td>
<td>.001</td>
<td>.124</td>
</tr>
<tr>
<td>Sex</td>
<td>0.023</td>
<td>1.36</td>
<td>(2,113)</td>
<td>.261</td>
<td>.023</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.010</td>
<td>0.57</td>
<td>(2,113)</td>
<td>.568</td>
<td>.010</td>
</tr>
<tr>
<td>Study Cohort</td>
<td>0.007</td>
<td>0.42</td>
<td>(2,113)</td>
<td>.659</td>
<td>.007</td>
</tr>
</tbody>
</table>

*Note. N = 117. Multivariate effect of familial risk status on the linear combination of ADOS-2 Social Affect and RRB domain scores, after controlling for the effects of age (months), sex (male, female), ethnicity (non-White, White), and study cohort (Study 1, Study 2). ADOS-2 = Autism Diagnostic Observation Schedule, Second Edition. df = degrees of freedom (hypothesis, error).*
### Table 7. Univariate Follow-Up Tests of ADOS-2 Domain Scores

<table>
<thead>
<tr>
<th>Variable</th>
<th>SPX-FB $M_{adj}$ (SE)</th>
<th>SPX-Sib $M_{adj}$ (SE)</th>
<th>MPX $M_{adj}$ (SE)</th>
<th>$F(2,114)$</th>
<th>Partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA Domain</td>
<td>14.55 (0.63)</td>
<td>15.10 (0.69)</td>
<td>12.32 (0.82)</td>
<td>4.08*</td>
<td>.067</td>
</tr>
<tr>
<td>RRB Domain</td>
<td>3.44 (0.25)</td>
<td>3.76 (0.28)</td>
<td>2.66 (0.33)</td>
<td>3.73*</td>
<td>.061</td>
</tr>
</tbody>
</table>

*Note. N = 121. Estimated marginal means and tests for group differences on ADOS-2 Social Affect and RRB domain scores after controlling for age, sex, ethnicity, and study cohort. $F$ test statistics refer to the main effect of familial risk group on SA and RRB domain scores after controlling for effects of covariates (age, sex, ethnicity and study cohort). ADOS-2 = Autism Diagnostic Observation Schedule, Second Edition. SA = Social Affect, RRB = Restricted and Repetitive Behavior.

$M_{adj}$ = Adjusted mean (estimated marginal mean). SE = standard error.

*p<.05 **p<.01
Table 8. Pairwise Group Differences in ADOS-2 Domain Scores

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>t(114)</th>
<th>p</th>
<th>Partial $\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SA Domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPX vs. SPX-FB</td>
<td>-2.24</td>
<td>0.96</td>
<td>-2.23</td>
<td>.066</td>
<td>.045</td>
</tr>
<tr>
<td>MPX vs. SPX-Sib</td>
<td>-2.78</td>
<td>1.01</td>
<td>-2.76</td>
<td>.020</td>
<td>.062</td>
</tr>
<tr>
<td>SPX-FB vs. SPX-Sib</td>
<td>-0.55</td>
<td>0.85</td>
<td>0.64</td>
<td>.999</td>
<td>.004</td>
</tr>
<tr>
<td><strong>RRB Domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPX vs. SPX-FB</td>
<td>-0.79</td>
<td>0.39</td>
<td>2.03</td>
<td>.135</td>
<td>.035</td>
</tr>
<tr>
<td>MPX vs. SPX-Sib</td>
<td>-1.10</td>
<td>0.41</td>
<td>2.70</td>
<td>.024</td>
<td>.060</td>
</tr>
<tr>
<td>SPX-FB vs. SPX-Sib</td>
<td>-0.31</td>
<td>0.34</td>
<td>0.91</td>
<td>.365</td>
<td>.007</td>
</tr>
</tbody>
</table>

*Note.* $N = 121$. Pairwise differences in ADOS-2 domain scores based on estimates marginal means. Bonferroni adjustment applied to $p$ values to correct for multiple comparisons.


SA = Social Affect, RRB = Restricted and Repetitive Behavior.

B = unstandardized estimated difference, SE = standard error.
Figure 1. Group Differences in Nonverbal and Verbal Developmental Quotients

Note. $N = 117$. Error bars depict two standard errors. Pairwise group differences based on estimated marginal means. Bonferroni adjustment applied when interpreting $p$ values. There were no significant differences between SPX-Sib and SPX-FB groups.

NVDQ = Nonverbal developmental quotient, VDQ = Verbal developmental quotient.

*p<.05  **p<.01
Figure 2. Group Differences in ADOS-2 Domain Scores

Note. *p<.05 **p<.01

Figure 3. *Pairwise Group Differences in ADOS-2 Severity Score*

*Note. N = 121. Error bars depict two standard errors. Pairwise group differences based on estimated marginal means. Bonferroni correction applied when interpreting p values. There was no significant difference between SPX-Sib and SPX-FB groups. ADOS-2 = Autism Diagnostic Observation Schedule, Second Edition. CSS = calibrated severity score.*

* p<.05  ** p<.01
References


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