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Examining Risk, Resilience, and Protection in the Study of Autism Spectrum Disorder in Infancy

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An abstract of
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Abstract

Examining Risk, Resilience, and Protection in the Study of Autism Spectrum Disorder in Infancy

By Rebecca Burger-Caplan

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and the presence of restricted interests and repetitive behaviors. These symptoms present with enormous heterogeneity among individuals with ASD, though the developmental pathways toward such variable presentation are not well studied. ASD is one of the most highly heritable of all psychiatric disorders, suggesting a genetic etiology for the disorder, however current means of identifying infants at risk for ASD remain limited to variables that mark the probability for ASD outcome, rather than a risk factor marking experienced insults to the typical developmental trajectory. The absence of a truly measurable risk factor is limiting both clinically and methodologically. Without a clear quantifier of risk, it is impossible to identify processes that may ameliorate or protect against such risk and contribute to the heterogeneity of clinical presentation. The current dissertation introduces a framework for clearly quantifying and characterizing processes of risk, resilience, and protection into the study of ASD, aiming to reveal the mechanisms by which some infants at risk do not develop full ASD. Infant participants were drawn from a large longitudinal study and included a sample of infant siblings of children with ASD who had a higher likelihood of developing the disorder. The first study applies a risk, resilience, and protection framework to test several variables for their usability and potency as markers of measurable, experienced risk for ASD in infancy. Results reveal four factors as usable and potent predictors of ASD, though highlight the paucity of truly measurable markers of individual experienced risk. The second study introduces social visual engagement in the first 2-6 months of life as a marker of measurable, experienced risk. The risk, resilience, and protection framework is applied to assess this measure's usability and potency as a risk factor and to test elements of early social communicative development as mechanisms of resilience, promoting adaptive development in the presence of risk. Results indicate receptive language and communication skill developed in the first year of life as a resilience factor. Results from both studies suggest future directions toward clarifying the role of protective factors, and the impact of risk and resilience results presented here for the hypothesized protective role of female biological sex.

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Table of Contents

Examining Risk, Resilience, and Protection in the Study of Autism Spectrum Disorder in Infancy

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder marked by deficits in social communication and interaction as well as by the presence of restricted and repetitive interests and behaviors that impair typical social and adaptive functioning (American Psychiatric Assocaition, 2013). ASD is among the most heritable of all psychiatric disorders (Constantino et al., 2013), indicating a strongly genetic etiology (Abrahams & Geschwind, 2008). Perhaps the biggest challenge in understanding the mechanisms by which ASD and its associated social impairment emerge is the broad heterogeneity of outcomes. The range in the degree to which social-communicative deficits are present or impairing across individuals is a central problem, limiting the feasibility of a unitary theory of the direct genetic or developmental path to autism. This heterogeneity, however, also creates a central opportunity within this field to clearly characterize these varying developmental profiles, and to prospectively examine their impact on clinical outcome and symptom severity. This dissertation will outline the known and unknown factors contributing to such heterogeneity, and will introduce a well-defined, existing theoretical and methodological framework to the study of risk, resilience, and protection in autism. Specifically, this dissertation will apply what has been learned about risk, resilience, and protection in other fields of study to frame and address important questions and research gaps in ASD.

Known Genetics of ASD

A substantial literature strongly supports a genetic cause for autism and the existence of congenitally present genetic atypicalities in children who are diagnosed with ASD in childhood.

High familial recurrence rates in ASD speak to a significant genetic etiology of the disorder. Studies of monozygotic twins indicate nearly 90% concordance (Rosenberg et al., 2009). The recurrence rate in families is substantial, with nearly 50% of siblings of children with ASD developing atypically (Ozonoff et al., 2011, 2014) and nearly 1 in 5 of these later-born siblings developing ASD (Ozonoff et al., 2011). This recurrence rate is higher than that of any other neurodevelopmental disorder.

Also well-known in the field is the wide genetic heterogeneity in ASD. While several specific gene mutations have been identified as conferring risk for ASD (Geschwind, 2011), each only accounts for a very small fraction of children with ASD, and no single gene can account for more than 1% of ASD cases (Krumm, O'Roak, Shendure, & Eichler, 2014; State & Šestan, 2012). Much of the genetic liability for ASD is accounted for by common variance (Gaugler et al., 2014; Klei et al., 2012), and a substantial proportion of the mutations and variants identified are *de novo*, occurring at vulnerable loci on candidate genes (Gaugler et al., 2014; Krumm et al., 2014; Sanders et al., 2013; Sebat et al., 2007). Further, ASD is thought to be multi-genic, resulting from a collection of gene mutations, further expanding the likely heterogeneity of mutations implicated. The effects of individual common variants in accounting for ASD are minimal, suggesting that many need to be present in concert to be manifest as ASD (Anney et al., 2012). The suggested target size (the number of functionally disruptive mutations occurring in concert in an individual) of mutations with potentially causal consequences is estimated to be as high as 500 genes (Ronemus, Iossifov, Levy, & Wigler, 2014).

Known Phenotypic Presentation of ASD

The considerable heterogeneity in phenotypic presentation of ASD is well-documented. From its earliest definition (Kanner, 1943), autism was considered a syndrome, and thus a

collection of co-occurring symptoms. At present, it remains a largely behaviorally and symptomatically defined disorder. ASD is only diagnosable once discrete and clinically recognizable symptoms begin to emerge in the second year of life, and is rarely recognized so early (Mandell, Novak, & Zubritsky, 2005). As symptoms emerge, the phenotypic presentation of children with ASD becomes vastly heterogeneous. The range of intellectual, adaptive, and communicative skill and ability profiles among individuals with ASD is enormous, on top of which behavioral and disruptive symptoms expand such phenotypic diversity even further (Christensen, Baio, Braun, et al., 2016; Klin et al., 2007; Klin, Volkmar, & Sparrow, 1992; Ventola, Saulnier, Steinberg, Chawarska, & Klin, 2011). Several attempts at parsing the heterogeneity evident in individuals with ASD have yielded suggested subtypes, though each of these groups of clustered cognitive and symptom severity profiles retains a significant degree of heterogeneity within-cluster (Cantio, Jepsen, Madsen, Bilenberg, & White, 2016; S. H. Kim, Macari, Koller, & Chawarska, 2015; Rice, Moriuchi, Jones, & Klin, 2012).

What remains consistent among phenotypic presentations of children and adults with ASD are the core diagnostic features of social-communication deficits and present atypical interests and behaviors that impede social functioning. The unitary syndrome is defined by challenges in social and communicative skills that require clinical and other support. Across the wide range of verbal and cognitive ability profiles in ASD, social and communicative adaptive skill holds up as a consistently impacted area of functioning that is similarly impairing across the autism spectrum (Burger-Caplan, Saulnier, Jones, & Klin, 2016; Klin et al., 2007).

Heterogeneity Presents Challenges and Unknowns

It is highly likely that infants who are later identified as having ASD are born with relevant genetic mutations, and it would follow that genetic factors would be an ideal marker of

likely atypical development. Though a genetic point of origin for ASD is strongly supported, its enormously heterogeneous phenotypic presentation suggests similar heterogeneity of genetic vulnerabilities, thus muddying any possibility of identifying a single genetic marker of risk that could predict a majority of ASD outcomes. This conclusion is hardly surprising given most past attempts to relate genotypic characterization to complex neurodevelopmental or neuropsychiatric disorders: studies of pleiotropy, variable penetrance, variable additive burden accruing from multiple small effect risk alleles and interactions thereof, all demonstrate that genotype-phenotype studies have so far yielded only modest replicated results (Meyer-Lindenberg & Weinberger, 2006). The identification of close to 50 'high-likelihood' genes for autism (Geschwind, 2011) indicates nothing about the exact genes at play in a common direct pathway to ASD. Heterogeneity of the developmental trajectories that emerge out of presumed common risk, measured in deviations from typical development, is evident from early in a child's life.

Direct paths from genotype to phenotype are unknown. Taken together, the number of potentially contributing genetic mutations identified, the large hypothesized target size, and the fact that none can explain more than 1% of ASD cases suggest limited utility of specific identified genetic mutations as direct indicators of likely ASD development. Further, no coherent pathway has been identified to explain how genetic liabilities are translated into phenotypic outcomes. Genetic mutations present at birth may contribute to development differently at different time-points in infancy. Epigenetic study of early mammalian development suggests that translation and transcription of particular genes can be time-locked to particular points in development (Millan, 2013; Oyama, 2000; Sanders, 2015; Smith & Thelen, 2003). Biological processes such as synaptogenesis, which directly links to emerging behavioral and developmental capacity, happen reliably at specific developmental time-points (Friedman et al., 2015; Tau & Peterson, 2010). As

such, the direct path from genetic mutation to later-emerging atypicalities is dependent on—and convoluted by—the timing and robust presence of other facets of development.

Impact of familial liability on individual risk is unknown. Heterogeneous outcome in the face of presumed risk for ASD is particularly well-documented for infant siblings of children already diagnosed with the disorder. The atypically high recurrence rate of ASD in families, along with strong evidence for genetic origins of the disorder, supports infant siblings of children with ASD as an enriched population for likely ASD outcome, given the higher probability that a sibling will receive a diagnosis (Zwaigenbaum et al., 2007). Even so, the majority of these siblings do not develop ASD.

Thus, while recurrence rates in families suggest that younger siblings of affected individuals carry a higher likelihood of developing ASD, this sibling status does not constitute the known presence of a genetic marker for ASD. Rather, this increased probability of ASD among siblings is made up of myriad potential genetic anomalies with a nearly 1 in 5 chance of occurring in a constellation indicative of ASD. It follows, therefore, that genetic events not based in inherited variance must account for a significant proportion of the ASD population. In fact, *de novo* coding variations, including copy number variants on vulnerable genetic loci account for approximately 30% of all simplex cases of ASD (in which there is only one individual with ASD within a set of siblings; Iossifov et al., 2014). In simplex families, only 13% and 43%, respectively, of identified missense and likely gene disrupting mutations—both *de novo* events—accounted for ASD-affected individuals. This suggests wide variability even in the genetic mutations that demonstrate incomplete penetrance and do not yield ASD-like symptoms (Iossifov et al., 2014; Ronemus et al., 2014).

Infant siblings of children with ASD carry a higher probability, by virtue of their sibling's diagnosis, of having a number of known and unknown genetic anomalies. These, in turn, constitute some level of inherent risk, at birth, of developing ASD. However, as reviewed here, it is currently impossible to prospectively conclude the extent of *experienced* risk for ASD imparted by genetic vulnerabilities for a given individual. There is enormous variability of outcome in infants at familial-high-risk of developing ASD. While the recurrence rate for diagnosis of ASD is nearly 1 in 5, those children who do not develop ASD are not all entirely neurotypical. Estimates suggest that nearly 3 in 10 infant siblings at familial-high-risk will develop atypically without developing ASD (Ozonoff et al., 2014). Atypical development for these children spans development of sub-threshold symptoms of ASD constituting the Broader Autism Phenotype (BAP), and non-ASD developmental delays (DD; including delayed cognitive development and delayed expressive or receptive language). Taken together, these rates of recurrence indicate that close to 50% of infant siblings at familial-high-risk will develop atypically, presumably reflecting heritable genetic anomalies indicative of ASD-risk. These variable outcomes across a population at increased probabilistic risk for ASD provide a rich array of developmental progressions for study, while the possibility of typical development (TD) underscores the challenges inherent in using sibling status as a proxy for congenital genetic atypicalities.

Sub-threshold symptomology in siblings is rare in single-incidence, simplex families where *de novo* mutations are more likely, and unaffected siblings may not carry any genetic risk for ASD. Presumed-unaffected siblings within multiplex families (in which more than one sibling holds an ASD diagnosis), however, are noted to present with a series of aggregated symptoms very early in development, particularly pronounced in male siblings, relative to female (Constantino, Zhang, Frazier, Abbacchi, & Law, 2010). Many prospectively identified siblings demonstrate early

language delays or 'autistic speech' which are resolved prior to later assessment yielding 'unaffected' status (Constantino et al., 2010)

ASD is one of the most heritable psychiatric conditions and has a well-substantiated genetic etiology. Yet, as reviewed above, the direct impact of such heritability and genetic origin on the development of the ASD phenotype, symptom profile, and severity of clinical outcome remains vastly ill-defined. It is clear that genetic vulnerability contributes to a higher probability of ASD among siblings, but any attempt to quantify such genetic risk is limited by the state of the field, whereby it remains impossible to prospectively ascertain an individual's experienced risk exposure.

Direct paths to heterogeneous phenotypic profiles are unknown. Among infant siblings, and across the broad spectrum of children diagnosed with autism, early development, which may initially deviate similarly from typical infancy, becomes more and more broadly heterogeneous in phenotypic presentation over the course of child development. Little is known about the mechanisms driving such variable outcome, though in keeping with models of other unfolding medical and psychiatric disease processes (e.g., Colodro-Conde et al., 2017; Kuo, Chang, Cheng, & Kao, 2017), it can be hypothesized that individuals' functioning at outcome results from the simultaneous action of both disease-promoting and disease-remitting factors. As such, in ASD, the coordinated impact of factors driving atypical development and those driving typical development yield an outcome across a spectrum of clinical affectedness. Particularly, among siblings at high familial risk, some go on to develop atypically, but without the full symptomology of ASD (constituting the BAP). Such heterogeneity of outcomes across children at familial risk for ASD and those who demonstrate early-emerging symptomology highlights an

ideal population for possibly identifying mechanistic self-righting processes that may direct adaptive development.

While it is not yet possible to intervene on disease promoting processes without better understanding their mechanisms of action, treatment research suggests that disease-remitting processes can be artificially introduced through targeted intervention. Thus, the wide variability of clinical outcome is further contributed to by interventions that promote social-communicative skill acquisition among children with ASD and related delays (Kasari et al, 2012; Dawson et al, 2010). The impact that acquiring social-communicative skills has on clinical affectedness in children already diagnosed with ASD suggests mechanistic importance of social-communicative and adaptive skills as perhaps contributing to resilience.

Clear risk, resilience, and protective factors are poorly defined. There are likely as many and as varied routes to the heterogeneity of genetic, familial, and phenotypic presentations as there are heterogeneous outcomes themselves. As reviewed here, genetic mutations likely occur in vastly varied ways in order to yield ASD, and phenotypic presentation is impacted simultaneously by both early developmental insults and by ongoing intervention and other disorder-remitting processes. There remains, however, a paucity of well-defined risk factors for ASD beyond sibling status. Even more relevant to current efforts to understand the unfolding heterogeneity of clinical presentation is the striking lack both of clearly defined or mechanistically understood resilience factors that could serve to ameliorate early atypical development (Szatmari, 2017), and of those factors that may act protectively against later-acting insults to social development. The present dissertation will aim to introduce into autism research a theoretical and methodological framework for the study of risk, resilience, and protection. What has been learned about risk, resilience, and protection in other fields will be utilized and applied to the study of

autism in order to frame important questions and research gaps within present understanding of ASD emergence.

Existing Frameworks for the Study of Risk, Resilience, and Protection

Many areas of study have approached risk classification by focusing on dose-dependent changes that result from variable exposure to insults. Biomedical, public health, and developmental psychology fields of study have made efforts to clearly characterize processes that yield increased likelihood of a disease state at later measurement. To this end, risk and resilience frameworks have been adopted in many diverse areas of research.

Across several disciplines, a foundational concept appears to be that with a variable amount of exposure to a risk factor, a system will experience a resulting variable amount of negative impact. Examples of such dose-dependent risk—whereby graded increase in risk experience appears to yield gradation in the degree of symptomology of the resulting disease state—span multiple disciplines and manners of study (Bowes & Jaffee, 2013; Francis, Young, Meaney, & Insel, 2002; Heim, Shugart, Craighead, & Nemeroff, 2010; Jacquemont, Hagerman, Hagerman, & Leehey, 2007; Meaney & Szyf, 2005; Rutter, 2006a). What is consistent among them is the idea that variable exposure to one factor yields variability in the presence of another.

This concept is applicable in the study of Fragile-X Syndrome, in which inherited intellectual disability results from a mutation leading to insertion of 200 or more repeats of a sequence on a particular gene. Phenotypic differences are evident between those with the full mutation, and individuals for whom fewer repeats are present, constituting the pre-mutation. In these less-affected individuals, phenotypic disability and physical abnormalities are graded, varying in degree with variations in mutation length (Jacquemont et al., 2007). In a more neurobehavioral context, variability in the amount of stress experienced by an animal system yields

variability in activity of the hypothalamic-pituitary-adrenal axis and the stress-responsive release of cortisol in the brain (Bowes & Jaffee, 2013; Meaney & Szyf, 2005). Speaking to similar factors in relation to child development, a large literature exists addressing factors that contribute to less clinically affected outcome in children at risk for later developing psychopathology.

Specifically, child maltreatment (comprising both abuse and neglect) serves as a potent risk factor for the development of maladaptive psychosocial and neurocognitive sequelae (Cicchetti, 2013). In the childhood maltreatment literature, similarly to the previously noted Fragile X pre-mutation, apparent psychosocial consequences of abuse or neglect tend to result in a graded manner, varying in severity with variations in the extent or intensity of the risk experience. Relative increase in the number of trauma experiences endured by a child, as well as increase in the intensity and frequency of trauma experiences increases that child's risk for depressive symptoms (Bifulco, Brown, & Adler, 1991; Heim et al., 2010; Mcquaid, Pedrelli, McCahill, & Stein, 2001; Rutter et al., 1975). Animal models demonstrate graded outcome varying both with the intensity of a risk experience (i.e., maternal neglect; Meaney & Szyf, 2005) and with the temporal factors of a risk experience (Ackerman, Hofer, & Weiner, 1975; de Kloet & Oitzl, 2003).

Factors that protect against the impact of insults to the system—inherent in the child's experience, regardless of risk exposure—and resilience factors that drive pro-typical developmental processes in the face of risk-purveying insults can be considered on every level of a child's developmental experience. From a neurobiological perspective, neural plasticity in early childhood may serve a protective role in response to physical insult to the brain (Johnston, 2009), and the growth of new neural connections that promote regained function would constitute a mechanism of resilience in an individual with a traumatic brain injury. From a psychosocial perspective, reciprocal friendships and personality characteristics may serve protective roles in

response to psychosocial abuse or maltreatment (Kim-Cohen, 2007), while the introduction of cognitive and affective coping skills learned in therapy may serve to drive resilience. Notably, in many of the reviewed studies, resilient outcome was not related to less severe maltreatment, proposing separate and theoretically dissociable roles in determining outcome for the intensity of a risk factor, and any subsequent resilience mechanisms at play (Cicchetti, 2013). It is certainly the case that ASD is *not* associated with risk derived from maltreatment. However, applying a similar framework for understanding risk and resilience across disciplines allows for a novel consideration of the heterogeneity of developmental outcomes of children at familial or genetic risk for ASD.

The Present Framework

In keeping with the foundational concept of dose dependence applied across literatures, Kraemer et al (1997) define terms in the study of risk, across psychologically-informed disciplines so as to assign uniform meaning to the study of risk. The codification of risk and resilience introduced by Kraemer et al is well-suited to approaching longstanding questions in the ASD field. The present dissertation presents a framework distilled from definitions presented by Kraemer et al in concert with definitions of resilience and protection across the extant developmental psychopathology literature. Within this framework, a risk factor is defined as a *measurable* characteristic or experience of an individual that *precedes* the outcome of interest and can be *used* to divide a population into two groups (i.e., high and low risk). The factor can have variable levels of *potency* (e.g., the extent to which it meaningfully differentiates risk groups). Elements of risk factors (e.g., potency; time of onset; variability) are considered as means of specifying their relationships with outcomes of interest. The present framework defines resilience as adaptive development despite the presence of experiences that constitute quantified risk for

psychopathology or developmental difficulties, (e.g., marked symptom presentation or increased risk for atypical development; Cicchetti, 2013; Kraemer et al., 1997; Rutter, 2006b), and a resilience factor as one whose onset marks a demonstrated shift toward improved outcome. The present framework defines protection as pro-typical development due to factors characteristic of an individual that intercept and modify the impact of a risk factor on a developing system, and a protective factor as one such characteristic factor that appears to interrupt the translation of risk into maladaptive outcome.

Current knowledge of ASD includes proposed factors of risk, resilience, and protection that have not been well studied within such an all-encompassing framework. Thus, what is currently understood about the etiology and emergence of ASD may not fit well within this framework that considers each risk, resilience, and protection from a common lens. Risk factors proposed in the extant ASD literature include, among others, genetic markers, pre- and perinatal factors, and status as a younger sibling of a child with autism. The enormity of genotypic and phenotypic heterogeneity, however, illuminates the challenge in identifying factors of measurable, experienced risk, as it reflects similarly broad heterogeneity in the measurable early driving factors of ASD development. Regarding the requirement that risk be measurable, genetic factors, as reviewed above, are not yet measurable enough or broadly applicable enough to consider them a quantifiable marker of risk across the autism spectrum. Further, as reviewed, the field does not yet have the concrete understanding of the path from risk experience to outcome necessary in order to consider such heterogeneous genetic insults to be truly predictive markers. Regarding the requirement that risk be experienced, sibling status, as reviewed above, suggests a higher probability of ASD, though does not indicate the individual child's actual mutation presence or

experienced developmental anomaly. As such, it is the *measurable* and *experienced* risk factors that are difficult to identify amid the remaining unknowns in the field.

Resilience factors proposed in the extant literature are few, are minimally researched, and are conflated in the literature with the concept of protection (Szatmari, 2017). Potential factors that could be considered as promoting resilience, as defined by the present framework, could include the onset of exposure to typical peers, early intervention that introduces new social developmental skills and opportunities, or the acquisition of new social-communication skills. It is challenging to test the validity of these as mechanisms of 'resilience,' however, in isolation. In approaching these understudied potential self-righting mechanisms, it is crucial to be able to differentiate between what could be termed *resilience* and what would be better characterized as uninterrupted *typical development*. Development that appears 'resilient' would be defined as typical, if it did not occur in the presence of an insult to typical trajectories of social development. Thus, to define self-righting mechanisms or otherwise typical development-promoting processes as resilience mechanisms, they must be differently active in the presence of insults to the inherent typical developmental trajectory. Within the proposed conceptual framework of risk, resilience, and protection, any factor tested as a resilience mechanism must be tested in the presence of measurable, experienced risk.

Protection factors proposed in the literature have largely been focused on clarifying the consistently present and fairly unexplained skewed sex ratio in ASD, whereby approximately 4 males are diagnosed with ASD for every 1 female. Several hypotheses exist conceptualizing a potential Female Protective Effect, whereby something inherent in 'femaleness' serves to protect against the development of ASD (Gockley et al., 2015). These hypotheses, which range from questions of diagnostic ascertainment (Constantino & Charman, 2012; Dworzynski, Ronald,

Bolton, & Happé, 2012) to those of differences in the genetic burden (Jacquemont et al., 2014), have been difficult to address, given the small number of females with ASD to be studied. While protective factors, being characteristic of the individual, are perhaps easier to define relative to risk and resilience, given the degree to which they may actually protect against the disorder, they are difficult to study with methodological accuracy.

Current Studies

The limitations presented here highlight the value of applying to ASD a conceptual framework that defines each construct of risk, resilience, and protection in relation to the others. This framework stands to afford the opportunity to frame important questions in the study of autism within a straightforward and common language. Such a framework approach provides an opportunity to capitalize on the inherent heterogeneity in ASD clinical presentation and development to conceptualize risk as a constellation of cumulative vulnerabilities, and to identify factors that effectively fulfill the qualities of each a risk, resilience, and protection factor.

The present chapter of this dissertation has presented the ongoing challenge of substantial unknowns in the field of ASD, which are wrapped up in the vast heterogeneity of both genetic and phenotypic presentation. Here, a framework has been introduced to more clearly and stringently define, quantify, and contextualize risk, resilience, and protection. The second chapter of this dissertation will review a series of biological and behavioral factors that have been studied in ASD, but have been studied only in isolation, and outside a defined framework of risk, resilience, and protection. The second chapter will present the application of this framework of stringent definitional and quantitative criteria to the reviewed biological and behavioral factors to determine whether any of these can be considered risk factors. The third chapter will introduce a novel biomarker of infant experience and will explore the concept of quantifying the degree of an

individual infant's atypical social experience via an eye-tracking-based measure of social engagement. This novel marker of an infant's own atypical social experience and exposure will be evaluated within the risk, resilience, and protection framework to assess whether it could qualify as a measurable, experienced risk factor for ASD. The risk, resilience, and protection framework will further be applied in the presence of measurable, experienced risk, to evaluate the degree to which a series of reviewed potential markers can be considered resilience mechanisms. Lastly, the fourth chapter of this dissertation will discuss future implications of the present work for investigations of protective factors for ASD.

The following studies utilize a sample of 198 infants. Data was collected as part of a largescale longitudinal study of ASD in infancy, and the sample includes male and female infants with and without older siblings previously diagnosed with ASD. Thus, the following studies aim to validate the use of the aforementioned framework in order to better define factors indicative of risk, resilience, and protection within a population that is enriched both for ASD and for a range of clinical outcomes and levels of developmental delay and clinical affectedness seen across the autism spectrum and across inherent variability in typically developing populations.

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Examining Concepts and Quantifications of Risk in the Study of Autism Spectrum Disorder

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Abstract

Autism Spectrum Disorder (ASD) is one of the most highly heritable of all psychiatric disorders, and presents phenotypically with enormous heterogeneity of social, cognitive, adaptive, and behavioral symptoms and levels of functioning. With early diagnosis and early intervention, prognoses are improved, yet clinicians and researchers are limited by a lack of measurable risk factors that can predict atypical development. While ASD likely has a genetic etiology, the state of the field limits the ability to prospectively identify genetic risk for the disorder on an individual level. The current study introduced a framework for approaching the identification and quantification of risk, resilience, and protective factors in infancy. Participants were 198 infants assessed longitudinally and six potential risk factors proposed in the ASD literature were evaluated. The risk, resilience, and protection framework was applied to pre- and perinatal factors (e.g., gestational age; birthweight), characteristic factors (e.g., infant sex; presence of an older sibling with ASD), and developmental factors (e.g., cognitive development; adaptive functioning) to assess their usability and potency as risk factors for atypical social development across three levels of resolution (e.g., a diagnostic threshold; nuanced categorical diagnoses; level of clinical affectedness). Results revealed four factors (infant sex, sibling status, cognitive development, and adaptive functioning) as usable risk factors for ASD, and variable potency among factors, in accordance with each of the three levels of outcome resolution. Differing usability and potency among factors at each outcome resolution highlight the complexity of predicting atypical development from a single marker of risk and the need for a risk factor for ASD that is individually measurable, usable, and potent.

Examining Concepts and Quantifications of Risk in the Study of Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social-communicative functioning and the presence of restricted, repetitive interests and behaviors (American Psychiatric Assocaition, 2013; Kanner, 1943). While it has been well-substantiated that ASD has genetic (Abrahams & Geschwind, 2008; Constantino, Zhang, Frazier, Abbacchi, & Law, 2010; Geschwind, 2011), and likely congenital origins (Kanner, 1943; Willsey et al., 2013), measurable risk factors for ASD remain poorly understood. To better delineate the developmental course by which symptoms emerge in early life, ASD has been the focus of substantial prospective longitudinal research in recent years (Droucker, Curtin, & Vouloumanos, 2013; Leezenbaum, Campbell, Butler, & Iverson, 2014; Mitchell et al., 2006; Schwichtenberg, Young, Sigman, Hutman, & Ozonoff, 2010; Peter Szatmari et al., 2016; Talbott, Nelson, & Tager-Flusberg, 2015; Zwaigenbaum et al., 2007). In the first two years of life, some early markers of the disorder itself (Jones & Klin, 2013), as well as broader signs of social disability (Dundas, Gastgeb, & Strauss, 2012; Elsabbagh et al., 2009; Merin, Young, Ozonoff, & Rogers, 2007; Nichols, Ibañez, Foss-Feig, & Stone, 2014), have been increasingly well-characterized and have led to earlier diagnosis and earlier intervention on the unfolding development of ASD (Dawson et al., 2010; Rogers, 2009; Wetherby et al., 2014). Despite such progress, however, pre-symptomatic factors used to identify risk for the disorder have largely been limited to familial risk factors.

Twin and sibling studies have revealed that ASD is among the most highly heritable of any neuropsychiatric disorder (Constantino et al., 2013; Folstein & Rutter, 1977). Among infant siblings of children already diagnosed with ASD, 1 in 5 will develop ASD themselves, while

another 3 in 10 will develop atypically, with either subthreshold ASD symptoms or with non-ASD developmental delays (Constantino et al., 2010; Ozonoff et al., 2011, 2014; Szatmari et al., 2016). Given the very high heritability of ASD and its likely genetic etiology, an infant's status as a younger sibling of a child diagnosed with ASD has been considered a strong proxy for possible genetic insults and for increased likelihood of ASD outcome. However, although the probability of ASD is clearly markedly higher in infant siblings, 50% of these siblings will nonetheless not have autism or other developmental delays. Thus, while sibling status clearly defines a population with higher probability of ASD and higher probability of atypical development, it does not actually mark the presence of distinct risk experienced by any given child.

Clinical research and clinical practice are both enormously limited by the paucity of clearly defined markers of experienced, measurable risk for social disability and ASD. It is only with the discovery and delineation of specific markers indicating experienced risk for atypical social development that successful efforts can be made to diagnose early, intervene quickly, and treat effectively so as to prevent onset of the full ASD clinical presentation in infants at risk. Streamlining the identification of biological and behavioral factors marking measurable, experienced risk for ASD in infancy has the potential for immediate translational utility toward improving early diagnosis and increasing access to early intervention that may preempt full symptom emergence. In streamlining the classification of risk factors, it is imperative that early markers of risk be identified and defined in measurable form. To this end, the present study will introduce a formalized theoretical and organizational framework for the study of risk, resilience, and protection into the study of ASD, focusing here on a systematic analysis of the quantification and validation of potential risk factors for ASD.

Defining, Identifying, and Quantifying Risk in ASD

The current challenge to more clearly define, characterize, and assess ASD risk in infancy can be helped by other fields of study that have grappled with similar challenges. Here, we adopt a framework for defining risk in developmental science proposed by (Kraemer et al., 1997). In this framework, the requisite attributes of a risk factor are (1) *measurability*—whether or not the factor and the extent to which it has been experienced by an individual can be quantified, (2) *precedence*—the presence of the factor in measurable form prior to the emergence of the outcome of interest, (3) *usability*—the ability for the factor to dichotomize a population into higher and lower risk groups (i.e., the prevalence rate of the outcome in a population is impacted by dividing a sample based on the presence of the risk factor), and (4) *potency*—the extent to which the factor meaningfully predicts outcome. Introducing to ASD research this formalized framework for the study of risk creates opportunities to assess potential risk factors according to common criteria. It also creates opportunities to aggregate risk factors in both the conceptual and methodological senses, thereby enhancing potential for translational and mechanistic insights from a wider range of disparate initial sources. In line with this need, many candidate variables in the study of ASD could serve as quantifiable risk factors but have not necessarily been investigated in ways that consider some, all, or any of the key attributes of risk noted above (Table 1). Candidate variables span characteristic factors (e.g., genetic markers, biological sex, the presence of an older sibling with ASD), pre- and perinatal factors (e.g., gestational age, birthweight), and developmental factors (e.g., emerging cognitive ability, developing adaptive functioning).

Genetic indices (Table 1, a) have ready appeal for their potential to serve as early and impactful factors in identifying children at risk for atypical development (Constantino et al., 2010; State & Šestan, 2012). However, as reviewed in the introductory chapter of this dissertation, rare mutations identified to date can only account for a small percentage of actual ASD cases

(Abrahams & Geschwind, 2008; Krumm et al., 2014; State & Šestan, 2012). In addition, mutations of major effect are rare and their effects on outcome still variable (Iossifov et al., 2012; O'Roak et al., 2011), so that practical implementation of screening for these markers faces many challenges (O'Roak & State, 2008). In addition, beyond rare variants, the larger portion of genetic liability for ASD appears to be accounted for by common variants (Gaugler et al., 2014; Klei et al., 2012). As the paths from common variants to risk of actual ASD presentation are as yet unknown and unquantified, the ability to use measures of common genetic variation to characterize level of risk experienced by any given child remains substantially limited (Gaugler et al., 2014; Iossifov et al., 2014; Krumm et al., 2014; State & Šestan, 2012; Yu et al., 2013). Thus, paradoxically, despite ASD's notably high heritability, genetic indices in their current state fail the risk factor requirement of measurability: despite all we've learned about the genetics of ASD and the strong genetic basis of ASD, prospective genetic risk for ASD cannot actually be quantified. Without measurability, genetic factors, in the current state of the field, cannot be tested within the present framework of risk, resilience, and protection, and cannot be considered usable as markers of risk for ASD.

Pre- and perinatal factors (Table 1, b, c) have also been cited as risk factors for ASD (Kuban et al., 2016; Ochiai et al., 2015; Verhaeghe et al., 2015), though this finding is inconsistent (Linsell et al., 2016). Problematically, it is difficult to separate these factors' contributions to ASD from their potential contributions to other developmental delays or other neurological disorders of infancy and early childhood (Schieve et al., 2016). However, the potentially nuanced vulnerabilities that these factors may impart should not be discarded. Set in context within the present framework, identification and aggregation of each incremental increase in risk experience could inform the overall predictive utility of risk identification. Identifying aggregated risk factors

that propagate atypical development may provide new paths for intervention that could serve to mitigate risk experience and atypical developmental sequelae.

An uneven sex ratio in ASD, whereby upwards of 4 males are diagnosed with ASD for every 1 female (Christensen, Baio, Braun, et al., 2016), has been one of the most consistent and one of the most poorly understood findings in autism. The sex of an infant (Table 1, d) can contribute in calculating the probability of an ASD outcome. However, the extent to which biological sex serves as a factor indicative of meaningful experienced risk for ASD outcome is unclear, and likely dependent on the context in which risk is being assessed.

An infant's status as the sibling of a child already diagnosed with ASD (Table 1, e)—suggesting increased probability of ASD, similarly to biological sex—has been the most prominent and widely utilized factor for measuring risk of ASD outcome (Zwaigenbaum et al., 2007). This factor is measurable, precedent to ASD diagnosis, and has been utilized in myriad prospective studies of ASD to enrich infant samples for likelihood of ASD outcome (Jones & Klin, 2013; Mitchell et al., 2006; Zwaigenbaum et al., 2007). Although increased probability of ASD outcome is clearly present, the heterogeneous nature of genetic insults associated with ASD as well as the frequency of contributing *de novo* events (Iossifov et al., 2014; Krumm et al., 2014) should not be overlooked, as these factors mitigate the validity of sibling status as a risk factor for any specific child (i.e., if ASD in an older sibling is associated with a *de novo* event, sibling status would not impart the same level of risk). In addition, sibling status does not capture the full array of genetic variability contributing to ASD, nor does it necessarily index insults to the typical developmental trajectory actually experienced by an individual child. While infant siblings are, as a whole, at increased probabilistic risk for ASD, they also experience a higher probability (relative to the general population) of a broad range of atypical developmental profiles that are not specific to

ASD. Further, it is not clear as yet exactly what differentiates infant siblings who develop with subthreshold symptoms constituting the Broader Autism Phenotype (BAP), from those who develop with non-ASD developmental delays (DD), from those who appear to develop typically. Thus, while both sex and—to a greater degree—sibling status may define a child's probability of being diagnosed with ASD, neither factor is necessarily informative about the child's actual experienced risk for atypical development.

Measures of cognitive and adaptive ability and performance (Table 1, f, g) are another domain of behavior that have been considered as potential risk indicators. These measures are enormously helpful in characterizing and diagnosing ASD in children, as scores on measures of intellectual ability and adaptive skill tend to fall into profiles common to ASD (Klin et al., 2007; Oliveras-Rentas, Kenworthy, Roberson, Martin, & Wallace, 2012; Ventola et al., 2011). These measures characterize the developmental and social functioning of a child, thus indexing the ways in which the child might be interacting with his or her social environment, and the social and communicative learning opportunities afforded by such interactions. While these factors are easily assessed by standardized clinical assessment measures, thus fitting the measurability criterion, the relevant deficits in areas of cognitive and adaptive functioning characteristic of ASD emerge together with the unfolding of the disorder, and, in fact, make up part of the syndromic definition of the disorder itself. Therefore, in considering ASD from a developmental neuroscience perspective, hence assuming congenital atypicality, these factors fail to demonstrate precedence to ASD and instead develop alongside it. However, considering ASD from a psychometric and diagnostic perspective, the emergence of cognitive and adaptive skills in measurable form (Mullen, 1995; Sparrow, Balla, & Cicchetti, 1984) well precedes the age at which ascertainment of a diagnosis is feasible or most likely (Christensen, Baio, Braun, et al., 2016). These measures of

adaptive and cognitive functioning have real virtue, as well, in acting as proxies for the environment the child is shaping by his or her own skills and behaviors that then may constrain that child's learning opportunities.

The Present Study

The present study employs a framework for conceptualizing and quantifying risk, resilience, and protection to evaluate a series of potential markers of risk identified in the autism literature for their viability as risk factors for ASD. In introducing the use of this formal framework to streamline the identification of risk factors, there is value in applying it to factors whose impact and utility in ASD is already generally known. This provides the opportunity to both validate the framework prior to its use with novel markers, and to embrace the nuance it lends to evaluating individual factors so as to glean useful information about the risk-conferring nature of each factor discussed here.

In addressing the questions of usability and potency of each of these factors, outcome is defined at three levels of resolution, indicative of differing contexts in which risk assessment may be important: dichotomized (outcome defined by clinical diagnostic threshold for ASD, split into two outcome groups of ASD and non-ASD infants), categorical (continues to be defined by clinical thresholds, but includes the more nuanced outcome groups of ASD, DD and BAP, and TD), and dimensional (a continuous outcome measure of symptom severity). The dichotomous level of resolution mirrors the high bar of clinical diagnostic threshold, splitting the sample into those who meet diagnostic criteria as clinically affected and those who do not. The categorical level of resolution identifies more nuanced clinical groupings identifying potential levels of clinical affectedness for which a factor might be more or less usable or potent. Lastly, the dimensional level of resolution identifies risk indices that may play out meaningfully in a

dimensional way, and might provide opportunities for broader, population-based implementation of universal interventions and protections against sub-threshold risk experience. In defining outcome across several levels of diagnostic resolution, the present approach embraces the gradation of social disability and social-communicative atypicality at outcome.

Methods

Participants

Participants in this study were 198 infants (122 male) followed longitudinally. The sample was enriched for ASD with 100 younger siblings of a child with ASD (65 male). Remaining participants were infants identified as younger siblings with no 1st, 2nd, or 3rd degree relatives with ASD. Infants were assessed longitudinally as part of a larger study at 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, and 24 months of age, and with confirmatory diagnostic characterization at 36 months. Across these time points, clinical assessment measures (detailed below) were administered.

Direct Clinical Assessment

The *Mullen Scales of Early Learning* (*Mullen*; Mullen, 1995) were administered at 24 months of age to obtain a standardized measure of general cognitive developmental functioning. The *Mullen* is an integrated measure assessing an infant's cognitive and motor abilities across five scales—Gross Motor, Visual Reception, Fine Motor, Expressive Language, and Receptive Language—yielding a T-score (M = 50, SD = 10) and a normed age equivalent for each scale, along with a full-scale Early Learning Composite score.

The *Vineland Adaptive Behavior Scales* (*Vineland-II*; Sparrow et al., 1984) were administered at 24 months of age to obtain standardized measures of adaptive behavior in four domains: communication, daily living skills, socialization and motor skills. Scores on the *Vineland-II* inform on a child's ability to translate cognitive ability into functional skills that allow

him or her to navigate the world. In ASD, a common and diagnostically informative adaptive profile consists of relative weakness in communication and socialization skills, in some cases alongside relative strengths in daily living skills (Klin et al., 2007). Standardized scores provided include Age Equivalents for subdomains (reported in the current study).

The *Autism Diagnostic Observation Schedule* (*ADOS-2*; Lord, Rutter, DiLavore, & Risi, 1999) was administered at 24 months of age, and again at 36 months as part of the confirmatory diagnostic characterization. The *ADOS-2* is a semi-structured play session in which a child's naturalistic play behavior and response to social and communicative opportunities are scored according to an algorithm comprised of nuanced diagnostic markers of ASD. The *ADOS-2* is a well-validated tool for ASD diagnosis, widely used in conjunction with parent interviews, adaptive behavior measures and impressions of experienced clinicians to make up the 'gold standard' for ASD diagnosis (Volkmar, Chawarska, & Klin, 2005). For assessment of social disability, the present study will employ the *ADOS-2* symptom severity score.

A maternal health history questionnaire covering details about factors of a child's birth (e.g. birthweight, gestational age, parity of the child within the family system) was completed by parents at the time of enrolling in the study. Notably, premature birth or clinically low birthweight were exclusionary to participation in the study, thus the range of these two factors in the sample is restricted.

Levels of Resolution and Participant Characteristics

The *usability* and *potency* of potential risk factors to be tested were assessed in regards to their ability to predict clinical developmental outcome at three levels of resolution: (A) dichotomized, collapsing clinical characterization into two groups: individuals with ASD diagnoses, and those without ASD diagnoses; (B) categorical, separating out a third group made

up of those infants who do not receive ASD diagnoses, but who are determined not to be typically developing; (C) dimensional, measuring symptom severity continuously across the full sample. Participant characterization, as regards diagnostic outcome and clinical measures within the sample is reported in Table 2.

Results

Usability

Usability analyses were carried out across each of the three levels of outcome resolution. A *p*-value threshold of 0.05 was used to determine whether a factor "passed" or "failed" the test of usability within a given level of outcome resolution (statistical results summarized in Table 3). Those potential risk factors measured continuously (e.g., gestational age, birth weight, cognitive ability, adaptive ability) were tested for their ability to predict dichotomized outcome using a Receiver Operating Characteristic (ROC) curve, an indicator of the sensitivity and specificity of a predictor to an outcome. Those potential risk factors measured categorically (e.g., biological sex, sibling status) were tested for their ability to predict dichotomized outcome using a Cohen's kappa statistic. By these measures, three factors "passed" the test of usability, statistically differentiating the sample into dichotomized outcome groups of ASD and non-ASD: *sibling status, cognitive ability, and adaptive ability*.

Continuously measured factors were tested for their ability to predict 3 categorical outcomes using ordinal regression. Categorically measured factors were tested for their ability to predict 3 categorical outcomes via one-way between groups Analysis of Variance (ANOVA). By these measures, four factors "passed" the test of usability, statistically differentiating the sample among three outcome groups of ASD, BAP/DD, and TD: *sex, sibling status, cognitive ability, and adaptive ability*.

Continuously measured factors were tested for their association with an individual's level of clinical affectedness or ASD symptomology using nonparametric Spearman's correlation. Categorically measured factors were tested for the degree to which levels of symptomology vary dependent on group membership as defined by sex or sibling status using independent samples t-tests. By these measures, three factors "passed" the test of usability across a dimensional measure of symptomology: *sibling status, cognitive ability, and adaptive ability*. The results of these usability analyses are distilled in Figure 1, which depicts factor usability at each level of resolution.

Potency

Potency analyses were carried out across each of the three levels of resolution. Irrespective of whether a factor "passed" or "failed" the test of usability according to a *p*-value threshold, potency was assessed via measurement of the effect size of the usability statistic for each factor. Effect size for each factor at each level of resolution was evaluated based on Cohen's conventions for qualitative descriptions of effect size: small, medium, and large (Cohen, 1992). Effect size for those factors tested for usability with ROC curves in the dichotomized level of resolution was indicated by the relative area under the ROC curve (AUC). For those factors tested with a kappa classifier, the magnitude of the kappa statistic indicates effect size, according to Fleiss's guidelines (Fleiss, 1981).

Effect size statistics are presented in Table 4, and distilled visually according to a gradient corresponding to the relative effect size from small to large, across factors and levels of resolution (Figure 2). By these measures, sibling status is a potent predictor of categorical diagnostic outcome and of dimensional ASD symptom severity, though does not differentiate clearly between those who go on to meet full diagnostic criteria for ASD, and those who do not. Of note, BAP was

defined as a collection of subthreshold symptoms present in the siblings of children with ASD (Constantino et al., 2010; Ozonoff et al., 2014), and the categorical outcome level of resolution is likely of particular relevance when splitting a sample based on its potential to fit the BAP, by virtue of sibling status. In contrast, the cognitive and adaptive abilities of infants were especially potent as predictors of full ASD criteria, and of categorical diagnostic outcomes, but were relatively poor predictors of dimensional symptom severity, as measured by the ADOS. While the sex of the child passed the test of usability as a predictor of categorical clinical outcome, it demonstrates only moderate potency.

Discussion

The present study identified factors in the extant ASD literature that have been hypothesized to contribute risk for the development of ASD later in childhood. A framework was appropriated from other fields of study in efforts to elucidate the impact of each of six individual factors on the developmental course, and to characterize these in the study of risk and resilience in ASD. Results of the present study suggest the value of applying a framework that encompasses an array of risk markers, thus maintaining the nuance and dimensionality inherent in the complex clinical outcome itself. The present study also highlights the problem that underlies current attempts to identify mechanisms of resilience, given the degree to which each of currently utilized measures of risk are flawed across levels of resolution.

Framework Approach to Risk Characterization

With the goal of introducing a common language from which to address the limitations of current approaches to studying risk and resilience in ASD, a singular framework was employed (Kraemer et al., 1997). This framework was distilled, for the purpose of this study, to assess four qualities of a risk factor: measurability, precedence, usability, and potency. The potential risk

factors identified in the literature (and then formally tested in this study) fulfill the requirements of both measurability and precedence (with the exception of cognitive and adaptive development, which differs in its fulfillment of the precedence requirement when viewed from either a developmental or a diagnostic viewpoint). Thus, these factors were tested for their usability and potency in predicting ASD and atypical social development in infancy and toddlerhood.

Across three levels of resolution of clinical outcome, perinatal factors (e.g., gestational age and birth weight) were demonstrated to fail the test of usability, suggesting that these do not meaningfully predict outcome as unitary risk factors. Across three levels of outcome resolution, the presence of an older sibling with ASD and the cognitive and adaptive abilities of the child in infancy and toddlerhood were demonstrated to successfully pass the test of usability, predicting an individual's diagnostic status (using dichotomized and more nuanced clinical categories) as well as level of clinical affectedness. The sex of the child uniquely predicted clinical outcome when the more nuanced clinical categories were used to define the outcome, but was not successful as a unitary measure of risk when differentiating ASD from non-ASD status, in accordance with diagnostic thresholds, nor in predicting the level of clinical affectedness of a given child.

While perinatal factors do not indicate risk for ASD individually by the test of usability, failing to meaningfully separate the sample by any measure of outcome, gestational age of an individual has greater potency as a predictor that a child meets full diagnostic criteria for ASD, while it is relatively meaningless at more nuanced levels of outcome resolution. The presence of an older sibling with ASD—the most widely utilized risk factor in prospective, longitudinal studies of ASD developmental pathways—demonstrates greatest potency as a predictor of clinical status, taking BAP and DD into account, or as a predictor of a child's symptomatic affectedness, regardless of diagnostic status. This finding is consistent with how BAP is defined: as a

subthreshold phenotypic presentation in first degree relatives of an ASD proband (Dawson et al., 2002; Ozonoff et al., 2014). In contrast, a child's adaptive behavior, while lacking precedence according to a developmental science—as opposed to clinically diagnostic—view of ASD, has greatest potency as a predictor of diagnostic categorization but relatively low-potency as a predictor of symptom severity.

Clinically speaking, the demarcation of each of these two factors as potent at different levels of outcome resolution has important diagnostic implications. While sibling status indicates increased probability of ASD and usably predicts social disability across all three levels of outcome resolution, it is most clinically and diagnostically relevant for the identification of children with symptoms that are subthreshold but impactful. In monitoring and screening infant siblings of children with autism, clinicians must be alert to the quandary that subthreshold symptomology presents for differential diagnosis. Adaptive behavior, on the other hand, may not lend substantial clinical value when true functional impairment is not present. It should be taken fully into account, however, in clarifying the diagnostic picture for a child with substantial symptomatology.

This framework and the resulting tests of usability and potency indicate that individual risk factors are usable and potent in accordance with the context of the clinical outcome that one is attempting to predict. As such, the prevailing practice of studying risk factors in isolation yields dismissal of factors that do not demonstrate the potency necessary to meaningfully—and independently—predict the development of ASD. For instance, perinatal factors, such as gestational age and birth weight, have been investigated as risk factors for ASD and other atypical neurodevelopmental events as singular risk factors or as factors within a cluster of perinatal conditions. Relative to sibling status, perinatal factors have rarely been considered to impart

meaningful specific risk for ASD, and thus prospective ASD studies identify sibling status as the sole risk factor of relevance and, in fact, often define low birthweight and prematurity as exclusionary factors for study enrollment. While the goal of prospective longitudinal studies is generally to enrich a sample for the likelihood of ASD outcomes, and thus the most potent risk factor is selected, such attempts to maintain "clean" samples may disguise the value of less-penetrant risk factors, limiting the field's ability to truly identify characteristics of risk experienced by a child.

The contrast presented here between which factors may be "usable" and which may be "potent," as risk indicators for distinct measures of outcome highlights this problem. It is likely that risk for ASD is most meaningful when considered as a constellation of vulnerabilities, mirroring the likely multi-genic nature of the disorder's congenital etiology. Considering risk-experience as a multi-factor web of interconnected vulnerabilities for ASD is valuable in effort to better characterize and screen children at risk, as it implies that individual risk factors are likely most impactful in coordinated effort with others. Further, in addressing the aggregated risk experiences likely to promote atypical development, the specific constellations of present risk factors in given individuals may highlight subtypes of atypical development or of ASD, that could carve nature at its joints, within a spectrum that has been fairly resistant to successful subtyping.

Applying a singular system and framework according to which factor is assessed is the first step in conceptualizing risk for ASD as a constellation of interconnected vulnerabilities, amassing in an individual system to propagate atypical development. As such, the cumulative presence of several characteristic, behavioral, or experiential factors, present to different degrees in each individual, may be most directly predictive of atypical social development.

Framework Implications for Developmental Mechanisms and Intervention Targets

As genetics research continues to progress and to clarify the meaningful impact of measurable genetic markers on ASD and social development, questions remain about the mechanisms by which genetic atypicalities (characteristic of the child, and thus fairly stagnant, barring epigenetic changes) are translated into the behavioral phenotypes identifiable in the ever-unfolding development of social ability across infancy. Thus, the use of a framework of interconnected risk experiences is valuable as a means of better characterizing and screening children at risk, as well as a means of potentially identifying mechanisms of action of individual factors.

If each factor imparting some measurable amount of risk to a child exists within a web of interconnected developmental factors, it is likely that the robust presence of one factor would be necessary, but not sufficient, for the derailment of the system's developmental course. The present framework approach to characterizing risk experienced by a given child has implications for future efforts to clarify the necessary peripheral circumstances under which a particular factor is able to impact unfolding development.

Further, this framework could be utilized to identify the peripheral factors that might be intervened upon in order to ameliorate a potent risk factor's ability to devastate a system. The impact of one vulnerability might be *amplified* by the influence of other present vulnerabilities, or *mitigated* by skilled intervention targeting other present vulnerabilities. In this case, interventions could be designed to target an individual child's constellation of vulnerabilities (i.e., in the case of a premature birth of a male younger sibling of a child with ASD, intervention might aim to bolster his cognitive and adaptive development, and pre- and perinatal growth, targeting remaining avenues for ASD vulnerability). Further informing intervention, this framework could be applied, as well, to identify and clarify mechanisms of *resilience* in the face of experienced

risk for ASD, creating opportunities for interventions designed to target the individual child's constellation of vulnerabilities.

Framework Implications for Moderators or Protective Factors

The application of this framework is also valuable for illuminating the potential interplay of a series of factors in contributing risk and providing avenues for intervention. Perhaps most compelling is the differential usability and potency of individual risk factors demonstrated across different levels of outcome resolution. Specifically, the sex of the child appears to mean different things for clinical outcome at different levels of outcome resolution. The skewed ratio of male to female infants with ASD has only become more dramatic with continued investigation. It is intriguing, therefore, that the sex of an infant is not implicated as a predictor of a child's likelihood to meet the full diagnostic threshold for ASD, nor as a predictor of the child's symptom severity, but emerges as a usable factor predictive of a child's likelihood to fit within more nuanced clinical categories.

In keeping with literature suggesting a requisite "higher genetic burden" in female children with ASD—such that a female must amass greater amounts of genetic risk in order for ASD to develop (Robinson, Lichtenstein, Anckarsäter, Happé, & Ronald, 2013; Werling & Geschwind, 2013)—these usability results suggest that "femaleness" may underlie the distinction between ASD and subthreshold atypical development. The idea of a "higher burden" in females is consistent with implications of the framework presented here, whereby an aggregated approach to assessing risk experience for each individual child is suggested to improve risk detection. Females may require not only a "higher genetic burden," but a "higher risk factor burden" as well. The existence of sex differences in typical social development in infancy, whereby females are largely precocious in their earliest social-communicative milestones relative to males (Bavin et al., 2008;

Fenson et al., 1994, 2006; Özçalişkan & Goldin-Meadow, 2010), further supports the possibility that there is something inherently "protective" about the female sex of a child, against risk experiences otherwise aggregated to promote ASD.

Future Directions

In examining extant factors in the ASD literature for their adherence to this framework, it is clear that as of yet, the field is missing a truly quantifiable factor that measures an individual child's experience and exposure, with precedence to ASD outcome, so as to be usable and potent in constituting risk for ASD or atypical social development. As such, future efforts in longitudinal study of infant development should aim to identify factors that meet these criteria. Existing measures of a child's early social-communicative experience (e.g., an infant's social-visual engagement) may serve as robust behavioral markers of a child's cumulative risk experience. Infants who go on to develop ASD already demonstrate atypical social-visual engagement trajectories from the first months of life (Jones & Klin, 2013). This infant-determined social experience has been hypothesized to perpetuate the derailment of a developmental system away from typical social development. Such a behavioral marker provides a rare opportunity to utilize a child's own experience in early infancy to signify the missed opportunities for social learning that might amass over the course of many months of atypical social-visual engagement.

While heritability has been capitalized on mostly as a means for enriching samples of study, and as an indicator of increased risk for ASD, it cannot be ignored that the presence of an older sibling does not, in fact, provide any meaningful information about the actual, experienced presence of genetic mutation determinant of likely ASD development in an individual child. Thus, while sibling status prevails as an important factor, the presence of which requires alert clinical screening in light of the high heritability of ASD, its utility in the present endeavor to better

characterize mechanisms of risk, resilience, and protection is quite low. In future efforts to capitalize on what is known about risk, in order to pursue questions about mechanisms of resilience, it is critical that risk be measured in its experienced form. Proxies for risk experience (such as sibling status, which denotes higher likelihood—but not measurable presence—of a genetic anomaly associated with ASD) will not be meaningful in identifying the self-righting processes that lead children who have enhanced risk for ASD to develop with subthreshold atypicalities. In moving toward capitalizing on the variability of risk experience for atypical development towards better characterizing resilience, it is critical that the ambiguity of current definitions and assignment of risk be addressed.

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Appendix A: Manuscript 1 Tables

Table 1. Potential factors indicative of risk for atypical development

Factor	Measurability	Precedence	Usability	Potency
a. Genetic Vulnerability	N	Y	--	--
b. Premature Birth	Y	Y	~	~
c. Low Birth-weight	Y	Y	~	~
d. Sex	Y	Y	Y	~
e. Sibling Status	Y	Y	Y	~
f. Cognitive Abilities	Y	Y/N	Y	~
g. Adaptive Abilities	Y	Y/N	Y	~

Note: Y = fulfills this requirement; N = does not fulfill this requirement, ~ = may fulfill this requirement, dependent on context.
Table represents general hypotheses.

Table 2. Participant Characteristics

	A. Dichotomized (ASD vs. Non-ASD)	B. Categorical (ASD, BAP/DD, TD)	C. Dimensional (ADOS-2[i])
N (N of males)	**ASD:** 31 (23) **Non-ASD:** 167 (99)	**ASD:** 31 (23) **BAP/DD:** 27 (23) **TD:** 140 (76)	**Full Sample:** 198 (122)
Mean Gestational Age[ii] (SD)	**ASD:** 38.62 (1.46) **Non-ASD:** 38.91 (1.47)	**ASD:** 38.62 (1.46) **BAP/DD:** 38.89 (0.86) **TD:** 38.92 (1.57)	**Full Sample:** 38.87 (1.47)
Mean Birth Weight[iii] (SD)	**ASD:** 122.30 (18.91) **Non-ASD:** 123.17 (17.79)	**ASD:** 122.30 (18.91) **BAP/DD:** 123.36 (14.61) **TD:** 123.13 (18.37)	**Full Sample:** 123.03 (17.92)
Mean Cognitive Ability[iv] (SD)	**ASD:** 49.67 (15.06) **Non-ASD:** 57.64 (11.76)	**ASD:** 49.67 (15.06) **BAP/DD:** 53.38 (10.90) **TD:** 58.47 (11.78)	**Full Sample:** 56.37 (12.64)
Mean Adaptive Ability[v] (SD)	**ASD:** 14.97 (3.91) **Non-ASD:** 21.77 (6.36)	**ASD:** 14.97 (3.91) **BAP/DD:** 17.04 (4.13) **TD:** 22.76 (6.30)	**Full Sample:** 20.63 (6.53)

[i] *ADOS-2* Symptom Severity Score, assessed at 24 months of age
[ii] Gestational age measured in weeks
[iii] Birth weight measured in ounces
[iv] *Mullen* Visual Reception Domain T-score, assessed at 24 months of age
[v] *Vineland-II* Interpersonal Subdomain Age Equivalent, assessed at 24 months of age
Note: Participant characteristics presented in each of three levels of outcome resolution. The dichotomized level of resolution splits outcome groups by diagnostic threshold, the categorical level of resolution divides outcome groups among 3 clinical categories, and the dimensional level of resolution depicts outcome as a continuous measure of symptom severity.

Table 3. Usability results

	A. Dichotomized (ASD vs Non-ASD)	B. Categorical (ASD, DD/BAP, TD)	C. Dimensional (Full Sample)
Gestational Age	ROC curve $p = 0.221$	$\beta = -0.096$, $p = 0.361$	$\rho = -0.005$, $p = 0.947$
Birth Weight	ROC curve $p = 0.634$	$\beta = -0.001$, $p = 0.871$	$\rho = 0.055$, $p = 0.4617$
Sex	$\kappa = 0.068$, $p = 0.117$	$F = 7.866$, $p = 0.006$	$t = -1.399$, $p = 0.164$
Sibling Status	$\kappa = 0.232$, $p < 0.001$	$F = 61.002$, $p < 0.001$	$t = -5.258$, $p < 0.001$
Cognitive Ability	ROC curve $p = 0.003$	$\beta = -0.143$, $p < 0.001$	$\rho = -0.196$, $p = 0.007$
Adaptive Ability	ROC curve $p < 0.001$	$\beta = -0.287$, $p < 0.001$	$\rho = -0.329$, $p < 0.001$

Table 4. Potency results

	A. Dichotomized (ASD vs Non-ASD)	B. Categorical (ASD, DD/BAP, TD)	C. Dimensional (Full Sample)
Gestational Age	AUC = 0.570	Pseudo $R^2 = 0.005$	$\rho = -0.005$
Birth Weight	AUC = 0.527	Pseudo $R^2 < 0.001$	$\rho = 0.055$
Sex	$\kappa = 0.068$	$\eta^2 = 0.039$	Cohen's d = 0.211
Sibling Status	$\kappa = 0.232$	$\eta^2 = 0.242$	Cohen's d = 0.783
Cognitive Ability	AUC = 0.670	Pseudo $R^2 = 0.106$	$\rho = -0.196$
Adaptive Ability	AUC = 0.833	Pseudo $R^2 = 0.358$	$\rho = -0.329$

Appendix B: Manuscript 1 Figures

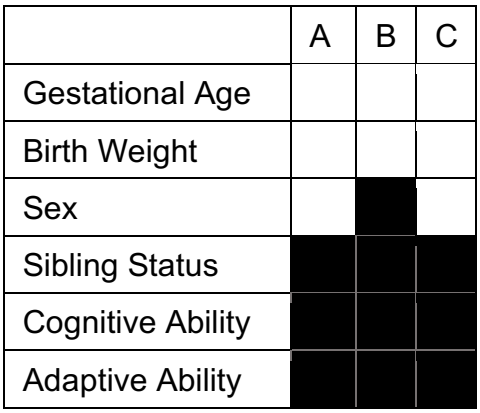

Figure 1. Usability across three levels of resolution.
Visual representation of usability statistics presented in Table 3. Cells that are filled in indicate risk factors that are usable, by meeting p-value threshold for significance, at given levels of outcome resolution: A. Dichotomized, B. Categorical, C. Dimensional.

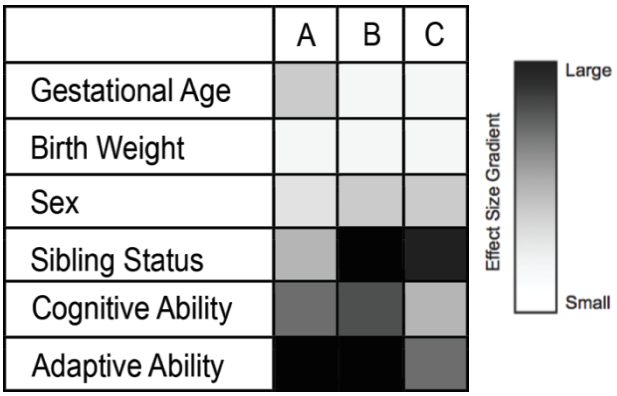

Figure 2. Potency across three levels of resolution.
Visual representation of potency results presented in Table 4. Cells shaded to represent magnitude of effect size, using qualitative descriptors in accordance with Cohen's conventions, and demonstrated in shading gradient key. Potency is presented at three levels of outcome resolution: A. Dichotomized, B. Categorical, C. Dimensional.

Examining Concepts and Quantifications of Resilience in the Study of Autism Spectrum Disorder

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Abstract

There is enormous heterogeneity in clinical presentation, adaptive functioning, and symptom severity among toddlers and young children with Autism Spectrum Disorder (ASD). As of yet, however, strikingly little has been understood regarding mechanisms that drive such variability, and particularly little regarding mechanisms of resilience that may allow some infants demonstrating risk for atypical development to develop with reduced levels of clinical impairment and subthreshold symptomology. In part, this has been impossible to study cleanly due to a paucity of measurable, usable, and potent risk factors defined in the ASD literature. Without such a risk factor identified, processes that promote adaptive social development cannot be considered mechanisms of resilience, and may simply be components of typical development. The present study applies a risk, resilience, and protection framework to a novel measure that quantifies infant social-visual engagement in the first 2-6 months of life to evaluate its potential as a usable, potent risk factor. The current study employs this risk factor to elucidate mechanisms of typical social-communicative development that fulfill the aforementioned framework's definition of a resilience factor. Results suggest that social-visual engagement with faces in the first 2-6 months of life is an appropriate risk factor for ASD and atypical social development in male infants. Further, results indicate that receptive language and communication skills developed in the first year of life meet the framework criteria for a mechanism of resilience in the presence of measurable, experienced risk for ASD.

Examining Concepts and Quantifications of Resilience in the Study of Autism Spectrum Disorder

The enormous heterogeneity of clinical presentation of children with Autism Spectrum Disorder (ASD), defined by varying degrees of deficit in social-communication and restricted and repetitive interests and behaviors (American Psychiatric Association, 2013), presents a critical opportunity to understand the full range of the spectrum of ASD symptomology across development. It is in doing so that mechanisms driving more typical social and communicative development may emerge, which can be capitalized upon to inform intervention development. Treatment research has indicated that, with intensive, empirically-driven, and early-enacted intervention, some young children demonstrating early emerging symptoms of ASD can achieve what have been termed 'optimal outcomes,' whereby adaptive and cognitive functioning enter the average range, and the child no longer meets DSM-IV or DSM-5 criteria for ASD (Helt et al., 2008; Orinstein et al., 2015). This suggests that certain processes targeted in intervention are capable of ameliorating, to varying degrees, the derailment of social communicative development in some children. It leaves in question, however, the mechanisms that appear to more naturally account for less-atypical development in some children with diagnoses across the ASD spectrum and the possibility that some processes of typical development are conserved in—and supporting the development of—those children who go on to develop with less functional social impairment than others. The present study aims to quantify these mechanisms of typical development that promote resilience, appearing to drive reduced severity of social disability in children who have demonstrated early atypicalities suggestive of risk for ASD in infancy.

Variability of Clinical Affectedness

Children with ASD represent the full range of cognitive functioning (Christensen, Baio, Braun, et al., 2016), despite a common core deficit in social-communicative and social-adaptive functioning—the hallmark of an ASD diagnosis. Profiles of strength and weakness in specific neurocognitive and executive functioning skills are heterogeneous among children with ASD (Cantio et al., 2016; Munson, Faja, Meltzoff, Abbott, & Dawson, 2010). Such variability in intellectual ability is predictive of variability in adult outcomes, with full scale intellectual quotient (FSIQ) scores above 70 (the clinically recognized cutoff for intellectual disability; American Psychiatric Assocaition, 2013) associated with greater adaptive functioning and independence in adulthood (Howlin, Goode, Hutton, & Rutter, 2004). Intellectual ability, however, does not appear to impact variability in the more fundamental social-cognitive deficits and atypical social processing central to ASD (Burger-Caplan et al., 2016).

Some of the core social deficits of ASD can be captured by the construct of adaptive behavior. Profiles of adaptive functioning generally seen in children with ASD are complex, and can be parsed into areas of functioning that are either closely tied to FSIQ or areas that are independent of FSIQ but still deficient in ASD. The socialization and receptive communication domains of adaptive behavior are often most impacted by social disability, regardless of intellectual functioning. Motor skills, adaptive daily living skills, and expressive communicative skills are less impacted by ASD-specific deficits, instead remaining variable in accordance with developmental delay and cognitive ability (Klin et al., 2007, 1992; Ventola et al., 2011). In individuals with ASD without intellectual disability, adaptive and cognitive functioning become even more dissociated from each other across development (Kanne et al., 2011; Klin et al., 2007).

Further contributing to heterogeneity in presentation of ASD, the presence, type, and impact of restricted interests and repetitive behaviors (RRB's) is widely variable and can have significant

implications for a child's level of adaptive functioning (Bishop et al., 2013; S. H. Kim & Lord, 2010; Richler, Huerta, Bishop, & Lord, 2010; Rodriguez & Thompson, 2015). While dimensions of broad cognitive, adaptive, and behavioral functioning contribute to the phenotypic heterogeneity in ASD, this can be further delineated by variability among individuals in their development of skills important for cognitive and adaptive functioning.

It has long been substantiated that the prognosis—in terms of adaptive development and functional outcome—of a child with ASD is improved with the development of language, relative to children who are nonverbal (Howlin, 2003; Howlin et al., 2004). Early communicative and verbal abilities in children with ASD serve as some of the best predictors of language and cognitive outcomes later in childhood (Anderson et al., 2007; Charman, 2003; Mundy, Sigman, & Kasari, 1990; Szatmari, Bryson, Boyle, Streiner, & Duku, 2003; Venter, Lord, & Schopler, 1992). Acquisition of first words by 24 months of age has been proposed as a benchmark indicative of improved prognosis (Mayo, Chlebowski, Fein, & Eigsti, 2013). Functional language development by 5 years of age has been associated with higher levels of residential independence and higher social outcome ratings in adulthood relative to peers who do not achieve functional language by this age (Howlin et al., 2004).

Variability of Social-Communication in Typical Development

There is substantial variability in cognitive, adaptive, and social-communicative abilities among typically developing (TD) children as well. This variability, however, exists within the limits of normative development, whereby there is an expected progression that TD children generally follow. The first two years of life comprise one of the most dynamic periods of learning, growth and development across the lifetime. Variability exists in the age of acquisition of social-communicative and developmental milestones accrued within these first two years. There is

general consistency, however, in the progression from early skills—indicative of social preference and social engagement—towards later-emerging, more concrete social skills, such as verbal communication and social-pragmatics among TD infants.

Early-emerging in this trajectory of social-communicative development is infant preference for faces in particular and social stimuli in general (Farroni et al., 2005; Farroni, Menon, & Johnson, 2006). Preferential attention to particular stimuli serves to constrain the infant's environmental experience, likely attuning perceptual systems to more social input (Johnson et al., 2005; Jones & Klin, 2013). Infants demonstrate similar preference for social auditory stimuli, preferring human to non-human speech sounds (Rossano, Carpenter, & Tomasello, 2012). The constraint of preferred inputs to the developing infant's experience confines his or her learning environment. Thus, to some degree, the infant self-selects—likely based on such congenital preferences and intrinsic drives toward social stimuli—the learning environment out of which he or she will develop.

It follows that the acquisition of skills that increase contingent social interaction might serve to build up social-communicative learning opportunities in the environment of the infant. Upon the emergence of the social smile—in typical infancy, developing around 2 months of life (Anisfeld, 1982; Emde & Harmon, 1972)—caregivers have been found to increase their contingent smiling with the infant (Lavelli & Fogel, 2013; Messinger & Fogel, 2007; Ruvolo, Messinger, & Movellan, 2015). In this way, an infant's acquisition of a social-communicative milestone directly solicits an increase in the amount of contingent social and communicative exposure from which the infant can learn. Other milestones acquired over the course of infant development, including joint attention developing between 6 and 12 months (Bakeman & Adamson, 1984; Charman, 2003), motor skills that increase an infant's range of movement and physical independence

(LeBarton & Iverson, 2013; Nickel, Thatcher, Keller, Wozniak, & Iverson, 2013), and deictic gestures including pointing and referential looking (Gliga & Csibra, 2009; Huttunen, Pine, Thurnham, & Khan, 2013; Iverson & Goldin-Meadow, 2005) similarly engage adults, thereby inciting the presence of increased social-communicative stimuli.

Pre-linguistic use of gesture, in particular, has been demonstrated to both precede and facilitate the development of spoken language (Bavin et al., 2008; Harding & Golinkoff, 1979; Iverson & Goldin-Meadow, 2005; Rowe & Goldin-meadow, 2009). Use of gesture for a given referent tends to precede use of words for the same referent (Iverson & Goldin-Meadow, 2005). Gesture use in conjunction with emerging language skill appears to facilitate language development, whereby early use of *supplementary* gesture-word combinations—in which the gesture and word do not necessarily refer to the same object—predicts earlier transition to two-word speech (Iverson & Goldin-Meadow, 2005). The supplementary nature of these gesture-word pairings suggests that the use of gesture in combination with speech plays a facilitative role in speech production, regardless of gesture-word congruence. Gesture also predicts early comprehension of language (Bavin et al., 2008), and a long-term association between early gesture and language development is evident beyond the initial transition from gesture to speech (Rowe, Ozçalişkan, & Goldin-Meadow, 2008). Gestures used in infancy are also strongly associated with adaptive communicative ability—the functional use of language and communicative skill (Luyster, Qiu, Lopez, & Lord, 2007; Venter et al., 1992). Further, preverbal communicative behavior predicts and likely enables the production of spoken language (Fenson et al., 1994; Fernald & Marchman, 2012).

Prior to the emergence of child behaviors accessible to the naked eye, infants' preference for socially meaningful stimuli, and their visual engagement with the social world can be measured

using eye-tracking as a tool to quantify an infant's social experience. Social-visual engagement appears to follow trajectories across infancy and toddlerhood that differ between TD infants and those who are diagnosed with ASD later in development (Jones & Klin, 2013; Klin, Jones, Schultz, Volkmar, & Cohen, 2002). Stereotyped looking patterns among TD infants are robust, and appear to delineate typical developmental progressions. TD infants demonstrate markedly increasing visual attention to the eyes in early months (Jones & Klin, 2013), shifting toward increased visual attention to the mouth in the middle of the second year of life (Jones & Klin, 2013; Lewkowicz, 1996) during a period important for language learning and vocabulary acquisition (Nazzi & Bertoncini, 2003). Sex-specific differences in typical development are evident, whereby females are precocious in many communicative (Eriksson et al., 2012; Özçalişkan & Goldin-Meadow, 2010), social (Osofsky & O'Connell, 1977), and neurological (Plante, Schmithorst, Holland, & Byars, 2006; Shaywitz et al., 1995) aspects of development. Further, such female precociousness is present in TD trajectories of social-visual engagement, with females demonstrating an earlier shift toward visual attention on the mouth, commensurate with female precociousness in vocabulary acquisition (Burger-Caplan, 2014). Such shifts immediately preceding the acquisition of skills important for social-communication suggests that social-visual engagement, too, may serve as a child-driven behavior that indexes and promotes the child's further exposure to stimuli important for development. When this system is attuned to socially meaningful visual experiences, it follows that it may promote further exposure to social stimuli across development.

Variability of Skill Acquisition in Autism

In keeping with the premise that the development of discrete, developmentally progressing skills increases an infant's opportunities for—and successful engagement with—social-

communicative learning, interventions that aim to increase a child's arsenal of acquired social-communicative skills are expected to improve developmental outcomes. Thus, targeted intervention that promotes skill acquisition may be most successful in reducing symptomology. This has been demonstrated in particular among interventions that target language acquisition and discrete expressive and receptive communication and symbolic play skills (Dawson et al., 2010; Kasari, Gulsrud, Freeman, Paperella, & Hellemann, 2012; Koegel, Koegel, & Surratt, 1992). Environmental enrichment appears to have similar effects both in rodent models, whereby the presence of toys and tools that increase opportunities for behavioral learning promote improved skill development in a dose-dependent manner, and in human infants, whereby relatively greater eye contact and social contingency from caregivers may have implications for later development (Jacquemont et al., 2007; Meek, Lemery-Chalfant, Jahromi, & Valiente, 2013).

The recognition that earlier intervention appears to impact developmental outcome (Bailey et al., 1995; Volkmar, Lord, Bailey, Schultz, & Klin, 2004) has initiated a trend toward attempting to study diagnostic markers for ASD earlier and earlier in development. In keeping with this initiative, early diagnostic research shifted to focus on prospectively studying a population enriched for the likely presence of ASD: the infant siblings of children with autism (Zwaigenbaum et al., 2007). As the study of ASD and emerging symptoms has focused on children at younger ages, a new segment of the heterogeneity has become apparent in what has come to be known as the broader autism phenotype (BAP), defined by sub-threshold ASD symptoms present in siblings of children with ASD who do not meet full diagnostic criteria for the disorder (Volkmar et al., 2005). Building on the population heterogeneity demonstrated by the emergence of autism symptoms in children who do not formally meet criteria for the disorder, the Social Responsiveness

Scale, a broad measure of atypical social symptomatology, has been used to suggest a whole-population spectrum of autism symptoms (Constantino & Todd, 2003).

Little is known about what might account for this reduced clinical affectedness in some children, and the mechanisms driving such variable outcomes in siblings at high familial risk. The heterogeneity of outcomes among these children, and among those who demonstrate early-emerging symptomology, serves to define an ideal population in which to identify mechanistic self-righting processes that may direct adaptive development. The fact that skill acquisition, when initiated through targeted intervention, contributes to this wide variability in clinical outcome suggests mechanistic importance of social-communicative and adaptive skills as perhaps contributing to resilience: adaptive development in the face of marked symptom presentation or experienced risk preceding outcome.

Identifying an At-Risk Population

While infant siblings have been well-supported as an enriched population for ASD by virtue of having a higher likelihood of developing with ASD (Constantino et al., 2010; Ozonoff et al., 2011; Zwaigenbaum et al., 2007), and thus have been studied with the aim of prospectively identifying markers for eventual ASD diagnosis, this is an impractical population for the study of mechanisms of *resilience*, if risk for ASD is defined solely by sibling status. To identify a process as resilient requires the quantified existence of *risk* in the child, prior to resilient outcome (Kraemer et al, 1997). The designation of sibling status as a marker of *risk* for ASD conflates heritability of genotypic factors with presumed genetic insult among all siblings. This illuminates the contrast between probabilistic risk and measured risk experience. In identifying mechanisms of resilience, the latter is required, and remains, as of yet, quite limited in ASD, as outlined in Chapters 1 and 2.

Consistent with the notion that early milestones of social-communicative development facilitate the development of later-emerging skills, and that these early milestones increase the social-communicative exposure and environmental opportunity for an infant, it would follow that identifying chronological variability in the onset of these skills might index variability in later developmental trajectories. In the earliest-emerging indices of a child's developmental experience, such variability contributes to the infant's self-selected learning environment and socially contingent opportunities.

As such, an early marker of an infant's lived experience may denote that infant's experienced vulnerability toward reduced social-communicative input, and may reflect, behaviorally, biological vulnerabilities that were congenital, predating that infant's first exposures to the social world. These two vulnerabilities appear to be well-captured by an infant's social-visual engagement, studied primarily in cohorts of infant siblings using eye-tracking in the context of naturalistic social stimuli (Jones & Klin, 2013; Navab, Gillespie-Lynch, Johnson, Sigman, & Hutman, 2012). Using participant samples enriched for ASD by including infant siblings as a familial risk group, these studies have demonstrated marked differences in the developmental trajectories of this variable between infants who are typically developing, and those who develop ASD. Social-visual engagement demonstrates diagnostic utility, appearing different between TD infants and those who are characterized with ASD at the diagnostic time-point, and even earlier in infancy and toddlerhood (Jones & Klin, 2013; Klin et al., 2002).

This indicator is not solely relevant for its diagnostic potential. It can also inform scientific understanding of typical developmental progressions. TD infants demonstrate markedly increasing visual attention to the eyes in early months (Jones & Klin, 2013), with a brief shift toward increased visual attention to the mouth in the middle of the second year of life (Jones &

Klin, 2013; Lewkowicz, 1996) during a period important for language learning and vocabulary acquisition (Nazzi & Bertoncini, 2003), before returning to a trend toward visual attention to the eyes continuing into childhood.

Differences in typical benchmarks, across measures of social-visual engagement, as well as developmental milestones, and in the developmental timing of these may point toward risk-defining experiences, or toward developmental progressions denoting resilience. The present study capitalizes on social-visual engagement as a measurable and precedent index of an infant's lived experience, testing it as a potential early marker of experienced risk. The identification of a measurable, precedent marker of experienced risk will allow for further consideration of the processes of typical development that promote more-typical development as mechanisms of resilience.

Methods

Participants

Participants in this study were 198 infants (122 male) followed longitudinally. The sample was enriched for ASD with 100 younger siblings of a child with ASD (65 male). Remaining participants were infants identified as younger siblings with no 1st, 2nd, or 3rd degree relatives with ASD. Infants were assessed longitudinally as part of a larger study at 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, and 24 months of age, and with confirmatory diagnostic characterization at 36 months. Across these time points, clinical assessment measures (detailed below) were administered.

Direct Clinical Assessment

The *Mullen Scales of Early Learning* (*Mullen*; Mullen, 1995) were administered at 24 months of age to obtain a standardized measure of general cognitive functioning. The Mullen is an integrated measure assessing an infant's cognitive and motor abilities across five scales—Gross

Motor, Visual Reception, Fine Motor, Expressive Language, and Receptive Language—yielding a T-score (M = 50, SD = 10) and a normed age equivalent for each scale, along with a full-scale Early Learning Composite score.

The *Vineland Adaptive Behavior Scales* (*Vineland-II*; Sparrow, Balla, & Cicchetti, 1984) were administered at 24 months of age to obtain standardized measures of adaptive behavior in four domains: communication, daily living skills, socialization and motor skills. Scores on the *Vineland-II* inform on a child's ability to translate cognitive ability into functional skills that allow him or her to navigate the world. In ASD, a common and diagnostically informative adaptive profile consists of relative weakness in communication and socialization skills, in some cases alongside relative strengths in daily living skills (Klin et al., 2007). Standardized scores provided include Age Equivalents for subdomains (reported in the current study).

The *Autism Diagnostic Observation Schedule* (*ADOS-2*; Lord, Rutter, DiLavore, & Risi, 1999) was administered at 24 months of age, and again at 36 months as part of the confirmatory diagnostic characterization. The *ADOS-2* is a semi-structured play session in which a child's naturalistic play behavior and response to social and communicative opportunities are scored according to an algorithm comprised of nuanced diagnostic markers of ASD. The *ADOS-2* is a well-validated tool for ASD diagnosis, widely used in conjunction with parent interviews, adaptive behavior measures and impressions of experienced clinicians to make up the 'gold standard' for ASD diagnosis (Volkmar et al., 2005). For assessment of social disability, the present study will employ the *ADOS-2* symptom severity score.

Clinical Characterization

Clinical characterization at outcome was performed by expert clinicians in the area of ASD diagnosis at 24 months for infants about whom there was no clinical concern, and at 36 months for

infants demonstrating any atypicality requiring confirmatory characterization later in development. Infants were characterized based on 'gold standard' ASD diagnostic procedures, consisting of expert clinician best estimate based on parent interviews, ADOS administration and observations, developmental assessment, adaptive behavior measures, and clinical impression. Infants were diagnostically characterized as meeting diagnostic criteria for Autism Spectrum Disorder (ASD), as exhibiting subthreshold symptoms of ASD consistent with the Broader Autism Phenotype (BAP), as exhibiting developmental delays not consistent with symptoms of ASD (DD), or as typically developing (TD). For the purpose of characterizing infants with atypical development that does not meet criteria for ASD, BAP and DD infants comprise a single category in further analyses.

Eye-tracking Data Acquisition

Eye-tracking data was collected every month from 2 to 6 months of age, with infants resting in a reclined bassinet car seat, while positioned to watch pre-recorded video stimuli on a display monitor. Visual scanning was measured with eye-tracking equipment (ISCAN). Analysis of eye movements and coding of fixation data were performed with software written in MATLAB. Specific features of the ISCAN equipment and analysis software were as detailed in Jones & Klin, 2013.

Regions of interest were traced onto each frame to identify whether a child was looking at Eyes, Mouth, Body, or Object regions of a scene. In order to capture social-visual engagement with social stimuli, encompassing children whose social-visual engagement shift to mouth-looking may be precocious, Eye and Mouth regions were collapsed into a Face region. Principle analysis by conditional expectation (PACE; Yao, Muller, & Wang, 2005) was employed to generate smoothed curves of face-looking across the first 2-6 months for each individual. To

appropriately select which PACE curve best fits each individual's percent face-looking data across early development, individual curves were fit with each a TD benchmark and an ASD benchmark. Face-looking values from the best fit were utilized for continued analyses.

Stimuli Children were shown video scenes of a female actor looking directly into the camera and playing the part of a caregiver: entreating the viewing infant by engaging in infant-directed speech and age-appropriate routines. The actors were filmed in naturalistic settings that emulated the real-world environment of a child's room, with pictures, shelves of toys, and stuffed animals. At each data-collection session, videos were drawn in pseudo-random order from a pool of 35 in total.

Results

Social visual engagement in the first six months of life is proposed as a marker well-suited to behaviorally measuring a child's lived experience and to providing a quantifiable index of experienced risk that is needed in order to elucidate mechanisms of resilience. Prior to assessing the usability and potency of this measure as a risk factor for ASD, the trajectories of social visual engagement across this early period of development were plotted in TD infants, constituting a typical benchmark against which atypical development can be compared. Sex-specific differences have been well-substantiated in several processes of typical development in infancy (Alexander & Wilcox, 2012; Morisset, 1995). As such, male and female infants were examined separately, so as to assess the existence of such sex-specific differences in TD trajectories of social-visual engagement.

Social Visual Engagement in Infancy Indexes Risk for ASD in Toddlerhood

Functional Principle Components Analysis (FPCA) was carried out in a subset of the current participants to determine the presence of sex-specific differences in clean TD and

ASD samples prior to further analyses. This yielded curves delineating trajectories of fixation time on the eyes and on the face across the first 2-6 months in males and females who were characterized as TD at outcome (Figure 1, a, c, e, g). FPCA curves were subsequently generated to delineate 2-6 month eye- and face-looking trajectories in a sample of females and males who were characterized as meeting diagnostic criteria for ASD at outcome (Figure 1, b, d, f, h). In males, the developmental trajectories of social-visual engagement with faces across 2 and 6 months of life differ between infants who are identified as TD at outcome and those infants identified as developing with ASD at outcome (Figure 1, a-d). Conversely, in females, these trajectories do not differ between TD and ASD outcome groups (Figure 1, e-h).

To further assess this factor for its usability and potency as a risk factor, in accordance with the framework employed in Chapter Two of this dissertation (Kraemer et al,1997), trajectories between these two extremes of TD and ASD diagnostic categories, not yet including those infants whose atypicalities or developmental delays do not constitute a full ASD diagnosis, must suggest predictive utility. Thus, in its present form, social-visual engagement—specifically quantified here as an individual's rate of change in face-looking in the first 2-6 months—has promise as a marker of risk in males, but not in females. Subsequent analyses of relative risk and resilience within the male subset of the present infant sample follow, in which the presently-defined construct of atypical social-visual attention to the faces of others in early infancy *does* quantifiably index a difference between early development in infants characterized as having TD and ASD outcomes in toddlerhood.

The specific usability and potency of this measure was tested at the dichotomous level of outcome resolution, testing this factor's ability to predict ASD in its full, diagnosable form, amid a male sample with a range of clinical developmental outcomes. Usability was tested by

identifying this continuous factor's relative sensitivity and specificity in identifying individuals whose 2-6 month data predict full clinical diagnoses of ASD in toddlerhood. A receiver operating characteristic (ROC) curve was generated (Figure 2, $p=0.003$). The point of inflection of this curve was then used as a cut-off value splitting the sample into those with relatively low experienced risk (LER) for ASD and those with relatively high experienced risk (HER) for ASD. This categorical marker of risk, splitting the sample into LER and HER groups, based on ROC inflection point, passes the test of usability ($\kappa=0.255$, $p<0.001$) and retains adequate potency as a risk factor as measured by the magnitude of the calculated Kappa statistic, which marks its effect size, according to Fleiss's guidelines (1981). Characteristics of infants in each risk group are presented in Table 1.

Early Emerging Communicative Skill Promotes More Normative Social-Communication in Toddlerhood

Findings from longitudinal infant research have supported the notion that in infant communicative development, some early developmental processes serve to enhance and further the development of later emerging, related processes (e.g., gesture production enhancing vocabulary and language acquisition; Iverson & Goldin-Meadow, 2005; Rowe & Goldin-meadow, 2009; Rowe et al., 2008). In such cases, the early development of a skill does not solely promote increased later proficiency in the early-acquired skill (e.g., continued growth). Rather, skill development in one domain promotes development in a related skill domain that relies in part on, but is not directly derived from the early-acquired skill. This approach to linking early acquired skills with the development of related—but not directly derivative—later-acquired skills has not been applied in the context of a sample of infants with varied clinical outcomes. Thus, in effort to elucidate mechanisms promoting typical development within a low-risk

sample, the question is posed: what are the early developing skill domains that predict social-developmental outcomes, in the context of *expected typical development*?

Pearson correlations were calculated between early developmental skill levels at 12 months of age and later measures of social-communicative development and clinical affectedness in areas of functioning relevant to ASD, assessed at 24 months of age. Developmental quotients (DQ) were used to estimate the extent of a child's developmental age as measured, relative to their chronological age. In this way, a DQ indicates whether a child is acquiring developmental skills early or late, compared to expected development at their chronological age. Neither receptive nor expressive DQ's differed between LER and HER infants at 12 months, $t(118)=1.097$, $p=0.275$, $t(118)=1.184$, $p=0.239$.

By these measures, early-achieved language and communication development and skill acquisition predict adaptive functioning and social communication one year later across the LER sample. Specifically, early expressive language, as measured by 12 month Mullen Expressive Language DQ, is correlated with greater interpersonal adaptive skill, as measured by the Vineland, one year later, $r(54)=0.476$, $p<0.001$ (Figure 3a). Early receptive language, as measured by 12 month Mullen Receptive Language DQ, is not correlated with interpersonal adaptive skill $r(54)=0.212$, $p=0.118$ (Figure 3c). Early receptive and expressive language and communicative development are each correlated with reduced ASD symptomatology, as measured by ADOS social affect score, one year later, $r(58)=-0.400$, $p=0.002$; $r(58)=-0.302$, $p=0.019$ (Figure 3e, g).

A main effect of both receptive ($F(2, 58)=3.526$, $p=0.036$) and expressive ($F(2, 58)=8.359$, $p=0.001$) language DQ at 12 months on clinical outcome in toddlerhood suggests that the relative chronology of LER infants' language and communicative development predicts their

clinical outcome among TD, BAP/DD, and ASD diagnostic categories, one year later. These findings suggest early emerging developmental skills that appear to predict and facilitate related but distinct domains of later development in a sample absent of experienced risk. In approaching resilience mechanisms promoting more typical development, the question is reprised: what are the early developing skill domains that predict social-developmental outcomes, in the context of early-experienced quantifiable *risk for atypical development*?

Among HER infants demonstrating quantified risk for atypical development and ASD, earlier receptive and expressive language and communicative development are associated with greater interpersonal adaptive abilities one year later, $r(48)=0.441$, $p=0.001$; $r(48)=0.586$, $p<0.001$ (Figure 3b, d), while only earlier acquisition of receptive and not expressive language and communicative developmental skill is associated with reduced ASD symptomatology, $r(54)=-0.360$, $p=0.006$; $r(54)=-0.156$, $p=0.252$ (Figure 3f, h). Similarly, among HER infants, earlier receptive and not expressive language and communicative developmental skill is predictive of clinical outcome $F(2, 56)=6.758$, $p=0.002$, $F(2, 56)=2.394$, $p=0.101$.

In the absence of experienced risk, early acquisition of language and communicative skills predict clinical processes developing one year later, across one of the most dynamic periods of development. Specifically, earlier language and communicative development predicts developmental domains that are not directly derived from language and communication, including interpersonal adaptive skill, ASD symptomatology, and clinical diagnosis. In the presence of experienced risk, however, the impact of earlier development on later clinical and social-communicative skill is largely limited to receptive language and communication skills. While both receptive and expressive DQ's predict later adaptive functioning, only receptive language DQ predicts later symptom presentation and diagnostic outcomes in HER infants. This

difference in the relative contribution of receptive and expressive language and communicative development to each LER and HER infants is present despite no statistical difference in DQ's between risk groups.

Discussion

The present study set out to address the paucity of understanding about what promotes resilient or less atypical development in some infants and toddlers. The vast majority of research on subthreshold ASD remains limited to discrete study of the BAP (Elsabbagh et al., 2009; Nichols et al., 2014), thus the present study sought to capture the variability across the broad range of developmental trajectories and heterogeneous clinical and sub-clinical outcomes. This heterogeneity has presented a core problem in the field, whereby concern has been raised that despite strict exclusion criteria aimed at subtyping and thoughtful measurement across the autism spectrum, ASD research has collapsed heterogeneity into a 'single autism' that is less informative on the myriad pathways of ASD symptom emergence. While attempts have been made to parse this heterogeneity into more nuanced clinical profiles based on outcome measures of social disability and cognitive functioning in school-age (Rice et al., 2012), heterogeneity of early, pre-diagnostic development has not been as thoroughly explored.

In fact, it may be this early heterogeneity that is most relevant for understanding how development of ASD and of social-communicative atypicality emerges, as it amplifies over the course of development into the broad range of variability in areas of skill and deficit noted clinically, even by toddlerhood. What allows for some children's developmental pathway to deviate from the more extreme presence or absence of ASD, and to yield subthreshold clinical symptoms? Is there a self-righting mechanism that mitigates a constellation of genetic, perinatal, and behavioral vulnerabilities for ASD, reducing clinical symptomology? The present study is

built on the premise that elements of TD may be conserved in some infants, serving as mechanisms of resilience in the presence of early risk or early atypicality, and facilitating this homeostatic move in the direction of more-normative development.

To approach these questions about resilience without claiming general typical development as resilient, it is critical that potentially mechanistic processes only be interpreted as such in cases in which they are differently active in the presence of experienced risk (Kraemer et al., 1997). Using a series of requirements of an experienced risk factor applied to the study of ASD in infancy under the framework of risk, resilience, and protection introduced in this dissertation, this study sought out to validate the usability and potency of a quantifiable marker of an infant's lived experience. Social visual engagement with faces was found to be a viable risk factor, providing a base from which to highlight the presence of resilience mechanisms in infants at quantified, relatively high experienced risk.

In the absence of experienced risk, the relationship between early expressive language and communication development at 12 months of life and interpersonal adaptive functioning at two years of life highlights the role that the early attainment of expressive communication skills has in promoting typical social adaptive development. This association is not maintained across communication domains, as early receptive language and communication development at 12 months is not correlated with later adaptive functioning in LER infants. By contrast, while earlier development of both expressive and receptive language appears to promote interpersonal adaptive skills in the presence of experienced risk for atypical development and ASD, only early receptive language and communication development at 12 months of life is associated with ASD symptoms at two years of life. This relationship highlights the role that early attainment of

receptive communication skills may have in promoting reduced ASD symptomology in infants already demonstrating early deviations from TD.

Separating language and communication development into receptive and expressive components, the relative contribution of each to later developmental and clinical gains is different between high and low risk groups. This, despite no global difference in expressive and receptive language and communication skills between risk groups. Early receptive language and communication development appears to be differently active in infants at high experienced risk for ASD, serving to mitigate early derailment from the typical developmental course. This demonstration of greater resilience with earlier receptive language development supports the hypothesis that the early growth and attainment of developmental milestones in language and communication could be intervened upon in infants at high risk for ASD, to potentially ameliorate the risk experience and its impact on continued development. Specifically, results of the present study indicate that receptive language and communication skills serve as an active developmental ingredient in mitigating the impact of risk on the developing system, and are associated with reduced symptom severity one year later among infants at quantified, experienced risk for ASD. Per the working risk, resilience, and protection framework, the earlier attainment of receptive language and communication skills by 12 months of life is deemed a mechanism of resilience against ASD-promoting risk experience.

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Appendix C: Manuscript 2 Tables

Table 1. Participant Characteristics by Risk Group

		LER	HER
	N (N ASD-sib)	62 (29)	60 (36)
	N Outcome Groups: ASD; BAP/DD; TD	4; 13; 45	19; 10; 31
12 months	Receptive Language DQ	Mean: 95.14 SD: 20.74	Mean: 90.98 SD: 20.83
	Expressive Language DQ	Mean: 92.69 SD: 26.66	Mean: 87.38 SD: 22.16
24 months	Outcome Mullen Visual Reception AE	Mean: 27.17 SD: 4.70	Mean: 25.75 SD: 4.97
	Outcome Vineland Interpersonal AE	Mean: 19.93 SD: 5.12	Mean: 18.88 SD: 6.33
	Outcome ADOS Symptom Severity	Mean: 3.28 SD: 3.28	Mean: 5.02 SD: 4.28

Note: Developmental Quotients (DQ) calculated from *Mullen* Receptive and Expressive Language domain age equivalents, relative to chronological age of assessment.

Appendix D: Manuscript 2 Figures

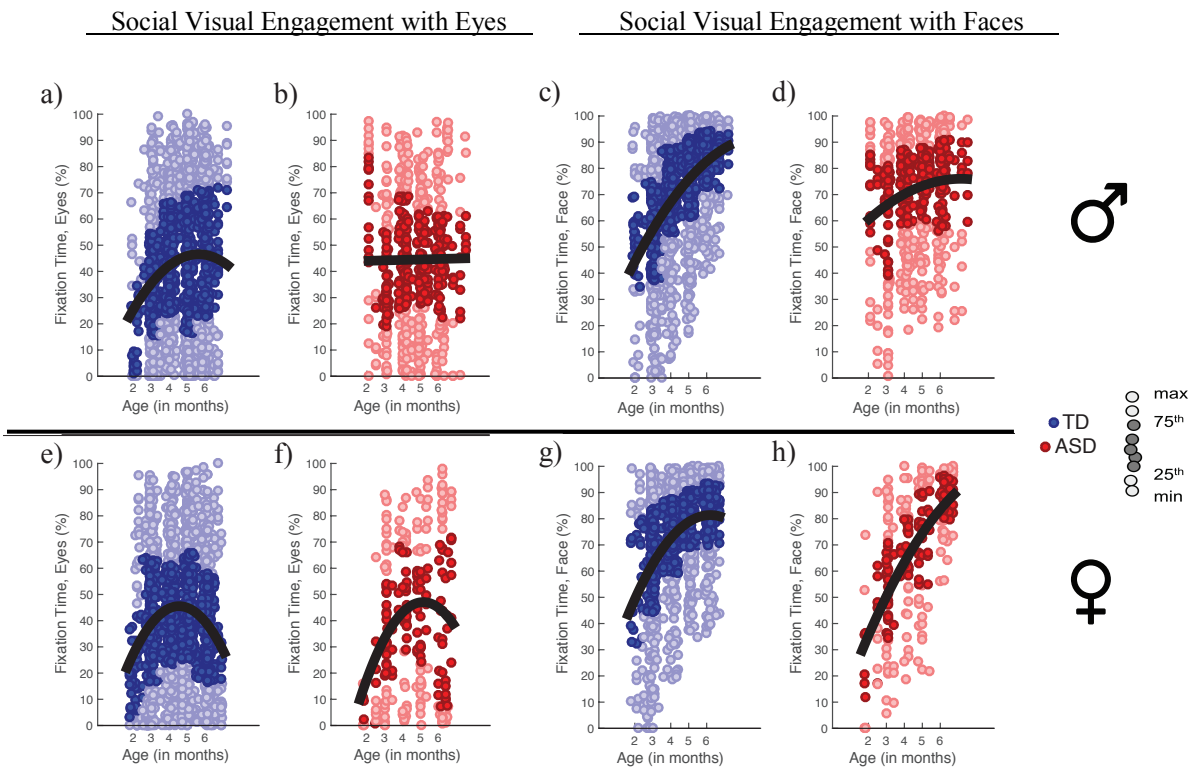

Figure 1. Functional Principle Components Analysis (FPCA) yielded curves delineating 2-6 month eye- and face-looking trajectories in males and females who were characterized as TD at outcome (a, c, e, g), and in males and females who were characterized as meeting diagnostic criteria for ASD at outcome (b, d, f, h). In males, trajectories of social-visual engagement with eyes and with faces across the first 2 to 6 months of life differ between infants later identified as TD and those later diagnosed with ASD at outcome (a-d). Conversely, in females, these trajectories do not differ between TD and ASD outcome groups (e-h).

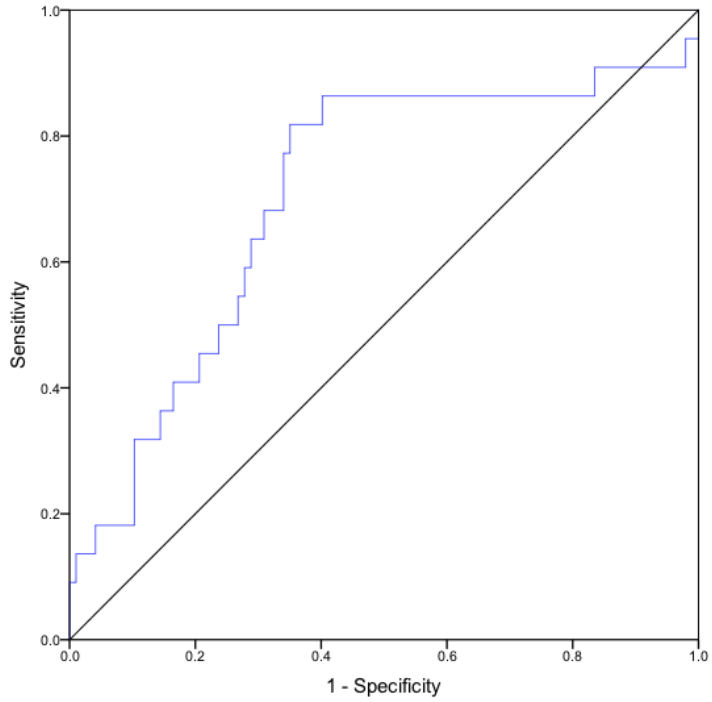

Figure 2. Receiver Operating Characteristic (ROC) curve depicting sensitivity and specificity of social-visual engagement as a measurable marker of risk for ASD, p=0.003. The inflection point of this curve was used to split the sample, based on rate of change of face-looking, into groups at high and low risk for atypical development. This categorical marker of risk, splitting the sample into LER and HER groups passes the test of usability (κ=0.255, p<0.001) and retains adequate potency as a risk factor as measured by the magnitude of the calculated Kappa statistic, which marks its effect size.

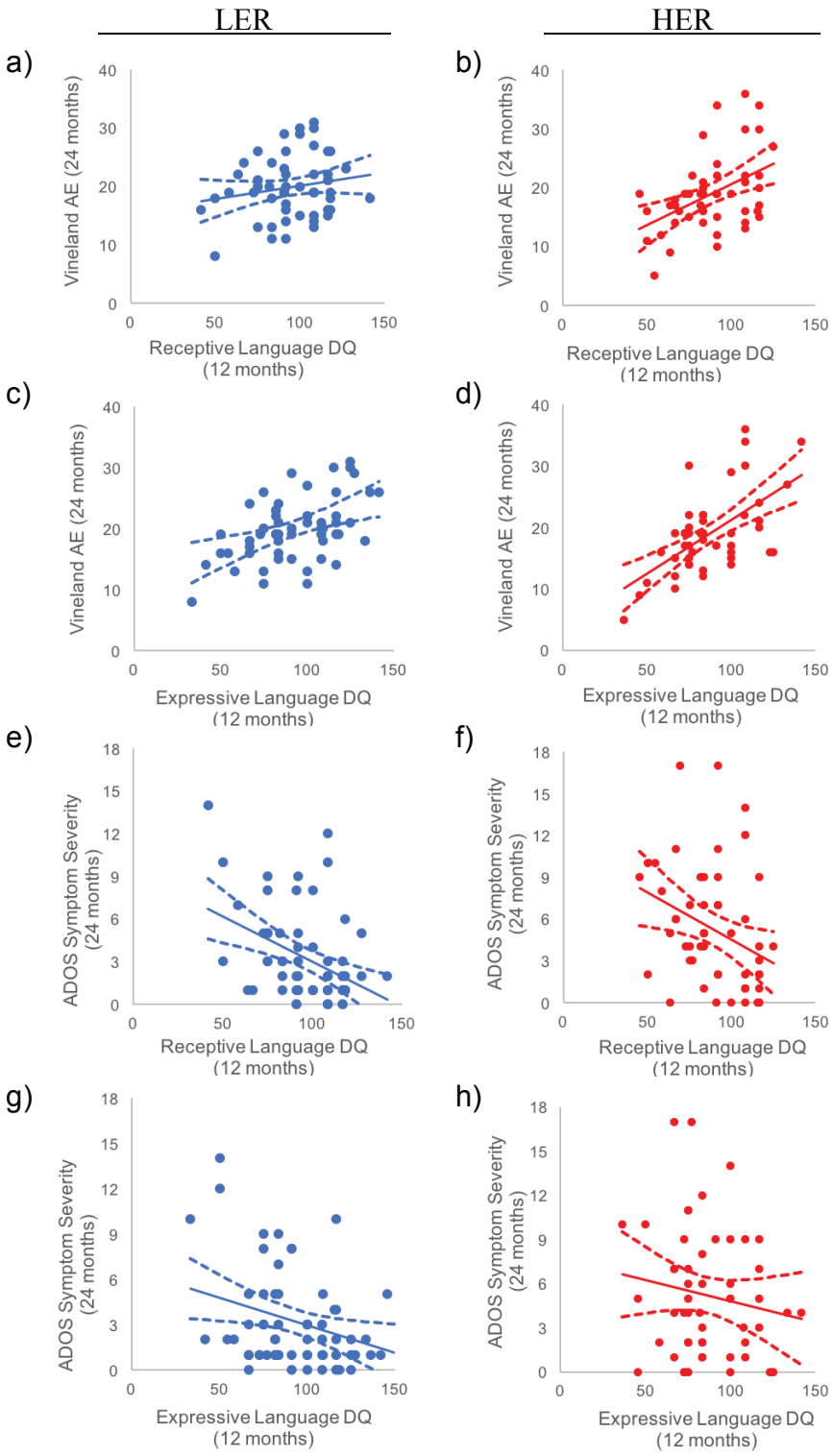

Figure 3. Scatter plots of early language and communication development versus adaptive and clinical outcome. Among infants at LER, early expressive language development is most associated with adaptive outcome, while among infants at HER, early receptive language development is associated with clinical outcome and symptom severity.

Implications of Risk and Resilience for Clarifying Protection

This dissertation introduced a framework for conceptualizing, identifying, and quantifying risk, resilience, and protection factors into the study of Autism Spectrum Disorder (ASD). This framework was applied to a series of proposed markers of risk in the existing literature to assess the potential for each to predict social disability at three levels of diagnostic resolution. This framework was applied to a novel marker of an infant's social experience and engagement with social stimuli, finding social-visual engagement as quantified via eye-tracking to be a usable and sufficiently potent predictor of social disability to serve as a measurable, experienced risk factor. Among infants thus deemed 'at risk' by this measure, this dissertation explored social-developmental mechanisms of resilience, applying the risk, resilience, and protection framework to elucidate factors of social communicative development that may serve, upon onset, to mitigate risk experience. A central finding of these studies was that, among children with experienced risk for ASD, the earlier development of some aspects of social communication, relative to peers, appeared to ameliorate quantified risk for atypical development, and to yield less socially impaired outcome. Arrival at this result relied on the clear delineation of the characteristics required of risk factors, and the subsequent selection of one that would be appropriately measurable and precedent to make room for the identification of mechanisms of resilience.

To this end, Chapter Two demonstrated the paucity of risk markers currently described in the extant ASD literature that fulfill the full requirements of a quantifiable risk factor, and highlighted the need for a behaviorally measurable marker of risk for atypical social development in order to test potential mechanisms of resilience to full ASD symptomology. The

results presented in Chapter Three identify social-visual engagement with faces in the first 2-6 months of life as a quantifiable risk factor that precedes and predicts social disability in toddlerhood. Among those infants deemed at risk in the present studies, the subset whose receptive communication develops relatively earlier is less likely to carry ASD diagnoses at outcome. This phenomenon suggests that precociousness in the receptive language domain of social-communication is in some way driving resilient development, despite existing risk-determinant insults to typical development. The conclusion that a subset of infants at risk may be less likely to develop ASD by virtue of developing along an accelerated trajectory calls to mind a parallel phenomenon whereby another subset of the broad population acquires communicative skill relatively earlier than their peers, and subsequently is less likely to develop ASD—females.

Females are far less likely than their male counterparts to develop ASD, and in fact, the disproportionate ratio of males to females diagnosed with the disorder remains the most consistent sex-related finding in ASD research. Prevalence studies across time and across cultures have reported a male-biased sex ratio with approximately 4 affected males for every 1 affected female (e.g., Christensen et al., 2016; Kim et al., 2011; Wing, 1981).

Several hypotheses have been proposed to explain the discrepancy in diagnostic frequency. One hypothesis hinges on the fact that it is, by definition, a *diagnostic* discrepancy—applicable specifically to those who have actually been diagnosed—and potentially skewed due to bias against detecting females with ASD using current diagnostic approaches. Because diagnostic measures were developed in predominantly-male samples, they may not have adequate sensitivity to the clinical phenotype of ASD in females. This is in keeping with the social-visual engagement data presented in Chapter Three, whereby this measure served as a predictive marker in males, though did not differ among diagnostic outcomes in females.

Measures of deviation from typical social development that indicate ASD in males may not do so consistently in females.

Other biologically-based hypotheses for the discrepancy in ASD prevalence by sex have suggested either additional risk factors for ASD in males (e.g., increased fetal testosterone exposure) or protective factors against ASD in females (e.g., requiring a higher burden of genetic variations; termed the Female Protective Effect). Consistent with these hypotheses, females with ASD carry a higher heritable mutational load than males and, in *de novo* cases, have higher rates of copy number and single nucleotide variants (Levy et al., 2011; Neale et al., 2012; Sanders et al., 2011).

These existing accounts for the uneven sex ratio in ASD have generally overlooked sex differences in typical development, particularly differences in early developmental trajectories of social and communicative behaviors, deviations from which are associated with ASD pathogenesis (e.g., Jones & Klin, 2013). Consequently, a critical future direction for the presented research is to further identify the potential role that female sex may play in protecting against, or mitigating the impact of, experienced risk for ASD. While social visual engagement, at least as operationalized in the current study, does not appear to successfully index such risk in females, the results should be considered an intriguing demonstration that one such marker of early social development does *not* appear to be disrupted in female infants who nonetheless *do* go on to develop ASD. Delineation of sex-specific differences in typical development further bolster the hypothesis that normative sex differences in early development may serve as a protective process against the development of ASD in females.

Sex Differences in Typical Infancy

Infancy and early childhood comprise the period of greatest brain and behavior change in all postnatal development. From the earliest moments of life, a child demonstrates preferential attention to social stimuli and the ability to make sounds that bring a caregiver near (Farroni et al., 2005; Geangu, Benga, Stahl, & Striano, 2010; Vouloumanos, Hauser, Werker, & Martin, 2010). Thus, infants' attentional and developmental resources are primed from infancy to promote their social and adaptive growth, along with growth in other areas important for development. Early cognitive developmental milestones are largely social and communicative in nature, beginning with the emergence of social smiling (Anisfeld, 1982), on through communicative gesture (Crais, Douglas, & Campbell, 2004; Iverson & Goldin-Meadow, 2005), babbling (Ejiri, 1998; McGillion et al., 2017), and eventually functional speech.

Differences between males and females in the typical course of neonatal and infant development are readily evident in the age of onset of multiple of these developmental milestones. Of note, though, such differences are not uniformly distributed across behavioral domains. Specifically, differences are evident in infants' sensitivity to social responsiveness, in the developmental timelines of multiple cognitive skills, and in the rate of initial vocabulary acquisition (Alexander & Wilcox, 2012; Huttenlocher, Haight, Bryk, Seltzer, & Lyons, 1991), as well as in broader behavioral markers of social development. These sex differences are generally not pronounced in the time-course of motor milestone acquisition, whereby across both gross and fine motor development, the sexes do not substantially differ in infancy or toddlerhood (Beckwith, Cohen, Kopp, Parmelee, & Marcy, 1976; Capute, Shapiro, Palmer, Ross, & Wachtel, 1985; WHO Multicentre Growth Reference Study Group, 2006). Instead, sex-associated differences in timelines of milestone achievement and onset of new behaviors appear to be heightened specifically in the social and communicative domains.

Sex differences in social and communicative development exist from early infancy. As neonates, females demonstrate greater social cry responses than males, exhibiting stronger contingent cry and distress behaviors in response to conspecific infant cry (Sagi & Hoffman, 1976; Simner, 1971). Females are also found to orient to social stimuli, including faces and voices, more readily than males (Osofsky & O'Connell, 1977). Although male and female neonates display similar overall levels of attention to others' eyes (Leeb & Rejskind, 2004), differences emerge across infancy and are most evident in social reciprocity. As infants, females engage in more mutual eye contact than their male peers (Leeb & Rejskind, 2004), suggesting that females' eye contact is more sensitive to social contextual factors (e.g., mutual engagement with the social partner). This female advantage in contingent social response is further established by increased distress among female infants to a still-face paradigm relative to males, wherein the removal of social contingency generates differential responses across sexes (Mayes & Carter, 1990).

As infants develop, social engagement and social contingency increasingly involve gestural and vocal communication. Here, too, females show an advantage in development relative to males, acquiring communication skills at earlier developmental time-points. Prior to the emergence of spoken language, the development of other communicative skills and behaviors, such as gesture, predicts future language acquisition. The types of gestures produced and their co-occurring behaviors are strongly related to a child's subsequent language acquisition and development of more complex language abilities (Brooks & Meltzoff, 2008; Iverson & Goldin-Meadow, 2005). From 7 months of age, sex-specific differences are evident in behavioral indices of hemispheric lateralization, considered important for eventual language development, whereby females demonstrate greater lateralized preference in their hand or arm use during

communicative behavior (Humphrey & Humphrey, 1987). Across the first 16 months, females show a consistent advantage in communicative gesture production (Fenson et al., 1994). This female advantage is slight, but consistently present in measures of early phrase comprehension and vocabulary production (Fenson et al., 2006). By approximately 19 months, females begin producing meaningful gesture-speech combinations, about 3 months earlier than male peers (Özçalişkan & Goldin-Meadow, 2010).

That steady progress of early developmental differences and early female advantage in gestural development leads to a rather notable sex difference in the age when typically-developing females first begin to speak in multi-word phrases: females begin pairing two words at 22 months of age, approximately 3 months earlier than males (Özçalişkan & Goldin-Meadow, 2010). Although 3 months seems a short time in hindsight, this difference is equivalent to approximately 1/8 of the child's entire postnatal life at that time. With that head start, females produce more words overall across the first two years of life (Bavin et al., 2008). This female advantage in word production appears most consistently in the middle of the second year of life (Fenson et al., 2006), aligned with a known period of vocabulary surge (Nazzi & Bertoncini, 2003). Importantly, the communicative development preceding language opens a child to exchanges and interactions that present new opportunities for greater social and communicative exposure and learning: stated simply, the child who produces more words is also more likely to receive more words. In this way, precocious development of nonverbal communicative skill in females has the effect of promoting earlier and greater exposure to social-communicative input, which in turn supports further development of spoken communication.

Collectively, this evidence suggests the accumulative consequences of even small initial differences: sex differences in the neonatal and early infancy period can have large and iterative

influence on how and when infants reach subsequent social-communicative milestones. These later differences may be traceable to the early preferences (reviewed above) for contingent social engagement and seem to indicate an inherent female advantage in typical social development, originating in infancy if not before.

Factors That Potentiate Sex Differences in Typically-Developing Children

After 24 months, the female advantage in early language development is attenuated, resulting in minimal differences on measures of language production once children enter the school-age years (Bornstein, 2004; Huttenlocher et al., 1991). Prior to that age, however, the female advantage in early social development likely reflects both neural and environmental influences. Sex-specific differences in physiological and neural development impact the physical and neural 'readiness' of the developing system for certain abilities and behaviors to be manifest. Similar to a critical period, the 'readiness' of a system to be able to perform skills limits that system's opportunity for development. In the area of visual perception (Baillargeon, 1998) and acuity (Makrides, Neumann, & Gibson, 2001), and of neuroanatomical development (Wisniewski, 1998), sex appears to moderate the developmental time-course, indicative of precocious development in females. These differences across sexes in the earliest developmental progressions of physiological and neuroanatomical maturation are paralleled in differences in hemispheric lateralization between males and females later in development (Cahill, et al, 2004; Wager, et al, 2003; Kansaku, Yamura, & Kitazawa, 2000; Shaywitz, et al, 1995; Berman, Bitan, & Booth, 2008). As in the case of gesture paving the way for language, so, too, might neuroanatomical change pave the way for further development. In line with this assumption, sex-specific differences in the neural development or timing of physiological changes in infants and

children might allow females increased development opportunities, earlier in life, relative to males.

The influence of the social environment may also be particularly relevant for the development of sex differences in social behavior, as a child's social response is co-created within context. There are consistent differences in how caregivers respond to male and female infants. Through the first year of life, most studies report that caregivers communicate more expressively with female infants (Kitamura & Burnham, 2003), show greater sensitivity to female infants' facial expressions (Donovan, Taylor, & Leavitt, 2007), and are more interactive and ask more questions with female infants in contrast to being more directive with male infants (Clearfield & Nelson, 2006). These differences in parental behavior can occur even in the absence of identified sex-based differences in the infant's behavior (Mondschein, Adolph, & Tamis-LeMonda, 2000). As a result of these differences in caregiver behavior, the social environment for female infants may include more opportunities for reciprocal interaction and contingent verbal responding. Although these early differences in social environment have small effect sizes, their cumulative effect over time in early development may contribute to larger differences in later development. For female infants, because parental speech input is associated with increased language development (Huttenlocher et al., 1991; Zimmerman et al., 2009), caregivers' more interactive and contingent style may support females' advantage in early communication development and may serve as an additional process contributing to an advantage in social reciprocity.

Implications for Risk, Resilience, Protection, and Autism

Studies directly examining sex differences in social disability in ASD have produced equivocal results. The primary, most consistently identified difference in clinical phenotype has

been in level of cognitive functioning. Females with ASD have, on average, lower cognitive abilities and are more likely to have comorbid intellectual disability (Christensen, Baio, Braun, et al., 2016). When controlling for cognitive functioning though, most studies report that males and females with ASD have similar levels of social impairment across development (e.g., Frazier, Georgiades, Bishop, & Hardan, 2014; Van Wijngaarden-Cremers et al., 2014). Reported sex differences have tended to mirror trends seen in typical development: males with ASD show more externalizing symptoms, such as aggression and increased repetitive behaviors, whereas females with ASD show more internalizing symptoms, such as anxiety (Mandy et al., 2012; Solomon, Miller, Taylor, Hinshaw, & Carter, 2012).

The parallels between the sex differences that *do* appear among children with autism and sex differences in children who are typically developing hint at a common and sex-specific pattern of social development that may be more impactful than previously thought. As previously reviewed, two main avenues have been hypothesized to explain the dramatic sex ratio in autism: higher genetic loading in females who develop ASD, and difficulty with ASD ascertainment in females. Given data presented in the present dissertation and reviewed here, a third contributing avenue is hypothesized, whereby sex-specific differences in typical development might provide inherent protection against insults to a typical developmental trajectory and against the developmental progression of ASD.

Rather than contradicting the previously discussed hypotheses, this consideration of sex-specific differences in typical development bridges considerations of genetic loading and of ascertainment biases. For female infants with a less burdensome constellation of genetic anomalies, early trajectories of neural development may be less affected and females' typically precocious neuroanatomical and communicative development may be more likely to subserve

greater responsiveness to early developmental opportunities. Coupled with caregivers' more interactive and contingent style with female infants, females would then show an advantage in early social development that may serve as a protective process that alters the manifestation of social disability. Increased opportunities in the social environment and increased context-dependent social reciprocity may help to support early social engagement and early language development, perhaps to the degree that females do not meet criteria for a clinical diagnosis of ASD later in childhood based on current ascertainment practices, thereby contributing to the uneven sex distribution in ASD diagnosis. As an additional consequence, females with an identified diagnosis of ASD are likely to have a greater burden of genetic anomalies, more severely affecting early neural development and, in a developmental cascade, diminishing the typical female advantage in early social development and thus contributing to more severe cognitive impairments among identified females.

Greater understanding of the differences between males and females in the developmental trajectories of typical infants and children may further support the Female Protective Effect in the degree to which female precociousness protects against insults to the typical developmental trajectory (Constantino, 2016). Typically developing females are consistently precocious both in their development of skills central to identified mechanisms of resilience (e.g., language and communication), as well as in normative patterns of social visual engagement in infancy. This advantage in skill acquisition and social visual engagement may be conserved in females, even in the context of early genetic or behavioral risk, bolstering and protecting them against the more complete and impactful knockdown of related developmental systems.

While not at the forefront of the risk and resilience questions laid out in the two papers presented here, a compelling finding presented was the demonstration that specific aspects of early development or early skill acquisition are predictive of the later developmental unfolding of related, but not directly derivative, skills. The finding that a developmental quotient indicative of relatively precocious language and communication development at 12 months of age predicts clinical symptomology on the autism spectrum a full year later represents not a simple linear increase in skill, but rather a demonstration of one element of early development contributing to the developmental readiness for the unfolding of another. This is in line with the sexually dimorphic trajectories of several developmental processes (e.g., gesture, vocabulary acquisition, caregiver behaviors) that coexist and appear to co-create one another across early development, as described above.

Returning to the data presented in the present studies, a key future direction will be to continue the application of the risk, resilience, and protection framework presented throughout this dissertation across several markers of infant-determined naturalistic behavioral engagement with the social world, in aim of defining a construct that might appropriately mark risk in females. As reviewed in Chapter One, a quantified marker of behavioral risk in early female infancy is necessary in order to test the hypothesis that the precocious social-communication development demonstrated in TD female infants is conserved in infants at risk for ASD, thus conferring protection and contributing to the dramatically uneven sex ratio of ASD.

The present studies have begun to address risk and resilience within the framework introduced. The proposed protective role of female sex cannot yet be assessed within the risk, resilience, and protection framework without the clear identification of risk experience. As described earlier in this chapter, it may be that the types of social or communicative behaviors

that are often employed as markers of early difference between typical and ASD development will not differ among diagnostic outcomes in females, as they do in males. In keeping with this possibility, given the existence of females who *do* develop ASD, caution must be taken to discern whether conserved developmental processes are in fact 'protective' effects or 'masking' effects that simply delay ASD identification in females. The impact of female sex as a protective factor, however, can only be approached theoretically once a measurable, experienced risk factor is identified.

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