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Prenatal Head Circumference in Autism Spectrum Disorder

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Research has documented a phenomenon of early brain overgrowth in autism spectrum disorder (ASD). Although the most significant overgrowth seems to occur within the first few years of life, the exact timeline, clinical correlates, and relation to earlier brain development are not well understood. A growing body of research points to a prenatal origin, yet few studies have examined prenatal head circumference (HC) in children diagnosed with ASD. Previous investigations have been limited to comparing HC measurements at the second trimester, neglecting later points in gestation when critical regulatory and developmental processes may be going awry. In addition, these analyses may be influenced by biased normative data, as has been recently suggested by various research groups. Finally, the connection between prenatal HC and later developmental outcomes has yet to be explored.

The current study sought to expand upon current literature by examining both second and third trimester prenatal HC measurements in children with ASD, as well as the rate of growth between trimesters. Additionally, the current study explored the relation between prenatal HC growth and later symptom severity. Examining HC later in gestation contributes to a more complete understanding of how and when brain growth dysregulation occurs in the development of ASD. Analyses indicated an unanticipated finding of significantly smaller mean standardized HC for ASD participants as compared to normative growth charts. In addition, second and third trimester HC measurements suggested an accelerated rate of neural growth for children who later developed ASD.

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Chapter One: Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by impairments in reciprocal social communication and by the presence of repetitive and stereotyped interests and behaviors (American Psychiatric Association [APA], 2013). The behavioral markers of ASD are typically identified before three years of age, often accompanied by abnormalities in cognitive functioning and sensory processing (Lovaas, Schreibman, Koegel, & Rehm, 1971; Lincoln et al., 1988; Filipek et al., 2000; Liss, Saulnier, Fein, & Kinsbourne, 2006; Landa & Garrett-Mayer, 2006; Landa, Holman, & Garrett-Mayer, 2007; Bolte, Dziobek, & Poustka, 2009; Ozonoff et al., 2010; Jones & Klin, 2013). The term "autism spectrum" describes the disorder's phenotypic diversity and reflects the wide range of abilities within the affected cognitive and behavioral domains. The recently published Diagnostic and Statistical Manual, Fifth Edition (DSM-5; APA, 2013) now refers to one diagnostic category of Autism Spectrum Disorder (ASD) in place of the previous diagnostic terms of Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS) (APA, 2000). This most recent revision of the diagnostic criteria marks a significant departure from previous conceptions of autism diagnosis.

Improvements in diagnostic clarity are crucial as ASD is among the most prevalent and fastest growing developmental disabilities. In 1994, the Diagnostic and Statistical Manual of Mental Disorders estimated autism prevalence rates of 2-5 cases per 10,000 births (APA, 1994). Since then, prevalence rates have increased substantially,

with recent estimates citing as many as one in every 68 children [males: 1:42; females: 1:189] (Autism and Developmental Disabilities Monitoring Network [ADDM], 2014). Changes in diagnostic criteria, improvements in identification, and increased public awareness have all been suggested as contributing explanations for the dramatic increase in prevalence (Fombonne, 2003; 2009; Wing & Potter, 2002). However, research suggests these do not fully account for the growing incidence (Rutter, 2005; Shattuck, 2006; Hertz-Picciotto & Delwiche, 2009) and ASD remains a major health concern.

The increased prevalence has also raised questions surrounding the relative impact of environmental and genetic causes. Prenatal viral exposures, fetal exposure to antidepressants, and antibiotics have all been hypothesized to trigger ASD, but the influence of environmental factors is inconclusive (Fallon, 2005; Kawashima et al., 2000; Libbey, Sweeten, McMahon, & Fujinami, 2005; Miller & Reynolds, 2009; Croen, Grether, Yoshida, Odouli, & Hendrick, 2011; Gardener, Spiegelman, & Buka, 2009; 2011). In addition, the "shared risk hypothesis" raises questions of causal directionality and proposes that fetal complications may occur as a result of the development of ASD in the neonate (Bolton, Murphy, Macdonald, Whitlock, Pickles, & Rutter, 1997). This question of causal directionality is critical and provides further possibility for a prenatal origin of ASD.

Other evidence points to a strong and complex genetic basis (Folstein & Rutter, 1997a,b; Bailey, Le Couteur, Gottesman, Bolton, & Siminoff, 1995; Gillberg & Coleman, 2000; Ritvo, Freeman, Mason-Brothers, Mo, & Ritvo, 1985). The first twin study of ASD found significant concordance differences among monozygotic (36%) and

dizygotic (0%) twin pairs (Folstein & Rutter, 1997a,b). Subsequent studies have cited similar findings, including the largest twin study to date citing 50% to 77% ASD concordance rates among monozygotic twins. Among dizygotic twins, concordance ranged from 31% to 36% (Hallmayer et al., 2011). Of interest, ASD recurrence rates among non-twin siblings are lower than the reported concordance found among fraternal twins in this study, citing 18.7% recurrence of ASD (Ozonoff et al., 2011). Due to the disparity in concordance and recurrence rates, Hallmayer and colleagues suggested that other shared environmental factors, including the prenatal environment, may be at play in about 58% of cases. As concluded by this recent study, and consistent with other neuropathology, the development of ASD is most likely a complex interaction between genetic susceptibility and environmental triggers.

While the etiology of ASD remains unknown, a variety of data support the involvement of number of brain regions in the neuropathology of autism, including the limbic system, corpus callosum, basal ganglia, brainstem, and the cerebellum (Piven et al., 1992; Williams et al., 1980; Gaffney, Kuperman, Tsai, & Minchin, 1988; Hashimoto et al., 1995; Bailey, Phillips, & Rutter, 1996; Courchesne, 1997; Kemper & Bauman, 1998; Rodier et al., 1998; Baron-Cohen et al., 2000; Folstein & Rowen-Sheidley, 2001; Acosta & Pearl, 2004; Palmen et al., 2004; Allen, 2006; Weigel et al., 2010; Uppal et al., 2014). Structural magnetic resonance imaging (MRI) has provided a body of evidence suggesting that some individuals with ASD have an overall abnormally large brain size at some time in development (Filipek et al., 1992; Aylward et al., 1999, 2002; Haznedar et al., 2000; Piven et al., 1995; Courchesne et al., 1999; Rojas et al., 2002; Sparks et al.,

2002; Hardan et al., 2003; Tsatsnanis et al., 2003; Herbert et al., 2003; Kates et al., 2004; for review, see Vaccarino, Grigorenko, Smith & Stevens, 2009; Schumann et al., 2010; for review, see Chen, Jiao, & Herskovits, 2011; Shen et al., 2014). A meta-analysis examining brain size in ASD across the lifespan and concluded that the most significant overgrowth of brain size occurred within the first few years of life, with size differences declining throughout childhood (Redcay & Courchesne, 2005). Studies of children between the ages of two and four years consistently report enlarged cortices (Carper et al., 2002; Sparks et al., 2002; Sparks et al., 2004; Hazlett et al., 2005; Bloss & Courchesne, 2007; Amaral, Schumann, & Nordahl, 2008; Schumann et al., 2010; Amaral et al., 2011; Hazlett et al., 2011; for review, see Carper, Wideman, & Courchesne, 2006). Additionally, a recent investigation of neural connectivity in 2 year olds found significantly decreased local and global efficiency over temporal, parietal and occipital lobes in high-risk infants later classified as ASD (Lewis et al., 2014). Overall, these studies have demonstrated a consistent phenomenon of early brain overgrowth in ASD.

Given these documented increases in cerebral volume, it is not surprising that one of the most consistent findings remains abnormal head circumference (HC). Enlarged HC occurs at an unusually high frequency among children with ASD and their non-ASD family members (Davidovitch et al., 1996; Woodhouse et al., 1996; Lainhart et al., 1997; Stevenson et al., 1997; Fombonne et al., 1999; Fidler et al., 2000), with brain volumes that exceed the population average in an estimated 90% of children with ASD (Courchesne et al., 2001). It is estimated around 20% of children with ASD meet criteria for macrocephaly, diagnosed when the circumference of the head is 2.5 standard

deviations above normal for weight and gender (Lainhart et al., 1997; Fombonne et al., 1999; Miles et al., 2000; Tager-Flusberg & Joseph, 2003). Another study reported that 59% of infants diagnosed with ASD showed a HC increase of 2 standard deviations or more during the first year of life as compared to 6% of normal infants (Courchesne et al., 2003). This increase in HC is thought to be due to accelerated head growth during the first years of life (Aylward et al., 2002; Redcay & Courchesne, 2005; Sparks et al., 2002), and may correlate with severity of symptoms (Courchesne et al., 2003). A recent study of over 7,000 individuals indicated gender, height, weight, genetic ancestry, and ASD status were significant predictors of HC. In addition, larger HC was associated with ASD symptom severity and regression (Chaste et al., 2013).

While a body of research has consistently demonstrated this neurobiological phenomenon in ASD, it is important to note the recent literature that has called to question the impact of different head circumference growth charts on the prevalence of macrocephaly in ASD. In an effort to detect whether several of the reference norms used to define early brain overgrowth in ASD may be biased toward detecting HC overgrowth, Raznahan and colleagues (2013) systematically reviewed all published HC studies in children with ASD and found that comparisons with locally recruited control subjects were significantly less likely to identify early brain overgrowth in ASD than norm-based studies. Another recent study tested the hypothesis that the presence of macrocephaly might vary depending on the specific growth chart used. Future research should continue to assess the potential bias of normative HC data.

Given the utility in HC as an index for underlying brain size in young children (Aylward et al., 2002; Bartholomeusz et al., 2002), identifying the earliest point of overgrowth is key to understanding the etiology of ASD. The exact timeline, clinical correlates, and relation to earlier brain development are not well understood. A growing body of research points to a prenatal origin in the development of ASD. A number of perinatal risk factors have been associated with increased risk of ASD, including parental age, multiple pregnancies, low birth weight, and exposure to medications during pregnancy (Zhang et al., 2010; Gardener, Spiegelman, & Buka, 2011; Croen et al., 2011). Other perinatal factors associated with increased risk for ASD include, but are not limited to, exposure to high levels of air pollution during pregnancy (Volk, Lurmann, Penfold, Hertz-Picciotto, & McConnell, 2013), maternal infection during pregnancy, (Hsiao, McBride, Cho, Mazmanian, & Patterson, 2012), maternal history of rheumatoid arthritis and celiac disease (Atladóttir et al., 2009), obesity (Krakowiak et al., 2012), and maternal diagnosis of asthma or allergies during the second trimester (Croen, Grether, Yoshida, Odouli, & Van de Water, 2005). Additionally, maternal consumption of folic acid around the time of conception has been associated with decreased risk of the development of ASD (Schmidt et al., 2013). Taken together, this body of research not only outlines many risk factors that may increase a child's risk of developing ASD, but it also strongly suggests that the prenatal environment is vulnerable to factors that may influence the development of ASD.

Still, the most direct evidence of prenatal developmental abnormality may come from neurobiological studies. One of the most consistent findings in postmortem studies

of the brains of individuals with ASD is a reduction in Purkinje cells of the cerebellum (Bailey et al., 1998; Fehlow, Bernstein, Tennstedt, & Walther, 1993; Bauman & Kemper, 2005; Kemper & Bauman, 1998; Lee et al., 2002; Ritvo et al., 1986; Vargas et al., 2005; Wegiel, 2004; Williams, Hauser, Purpura, DeLong, & Swisher, 1980). In early descriptions of Purkinje cell (PC) reduction, a lack of gliosis (Kemper & Bauman, 1998; Ritvo et al., 1986; Williams et al., 1980), empty basket cells (Bailey et al., 1988), or retrograde neuron loss in the inferior olive (Kemper & Bauman, 1998) suggested an early prenatal onset of this pathology. More specifically, the preservation of olivary neurons in the presence of markedly reduced numbers of Purkinje cells in ASD suggests that the cause of these processes may occur at or before approximately 30 weeks of gestation (Bauman & Kemper, 1994, Kemper, 2010). Others have suggested a cause at or before 32 weeks gestation (Bailey et al., 1998).

Further support for a prenatal onset of cerebellar pathology comes from a recent direct examination of in vivo prenatal cerebellar development. Through a retrospective investigation of prenatal ultrasound records, Allen and colleagues (manuscript in preparation) found that second trimester fetal measurements of the cerebellum were significantly smaller for children who later developed ASD when compared to a normative sample. This study represents the first direct evidence for abnormal prenatal brain development in ASD. Given the cerebellum's widespread anatomical connections to all major brain regions, and its large afferent: efferent ratio (40:1) (Allen, McColl, Barnard, Ringe, Fleckenstein, & Cullum, 2005), early aberrant cerebellar development

will likely have a significant impact on other brain systems known to be affected in ASD, such as the prefrontal and parietal cortices.

Indeed, through postmortem tissue analysis, a recent study found that male children with ASD had 67% more neurons in the prefrontal cortex than control subjects (Courchesne et al., 2011). This critical finding is the first direct confirmation of the theory that an excess of neurons could be an underlying cause of brain overgrowth in children with ASD—possibly due to dysregulation of neuron proliferation, apoptosis (i.e. cell death), or both (Courchesne et al., 2001). Because all cortical neurons are generated prenatally (Samuelsen et al., 2003; Uylings et al., 2005; Bhardwaj et al., 2006; Larsen et al., 2006; Gohlke, Griffith, & Faustman, 2007; Rabinowicz, de Courten-Myers, Petetot, Xi, & de los Reyes, 1996), this pathological increase in neurons strongly indicates prenatal causes. The vast majority of neurons are created between weeks 10 and 20 of gestation, resulting in a normative overabundance of neurons by as much as 100% (Gohlke, Griffith, & Faustman, 2007). However, during the third trimester of pregnancy and early life of an infant, about half of those neurons are removed in a process referred to as pruning or apoptosis (programmed cell death) (Kanold, 2009). As proposed by Courchesne and colleagues (2011), failure of that key early developmental process would create a pathological overabundance of cortical neurons, and could lead to overall larger head volume.

As previously mentioned, increased HC is among the most consistently reported neurobiological differences among children with ASD. Although the precise time when brain overgrowth begins is uncertain, first signs of this abnormal neural development are

reported to begin as early as 9 to 18 months of age (Courchesne, Carper, & Askshoomoff, 2003; Dawson et al., 2007; Schumann et al., 2010). However, given the recent finding that suggests the underlying mechanisms that govern this overgrowth may begin as early as the third trimester in prenatal gestation, differences in HC may be apparent even earlier in development. While a number of studies have reported normal HC at birth among children later diagnosed with ASD (for review, see Redcay & Courchesne, 2005), others have reported decreased HC (Courchesne et al., 2003), and still others have found increased HC at birth (Gillberg & de Souza, 2002). Given the heterogeneity of ASD and the use of different clinical HC norms, varied results are not surprising. It is also possible that neurobiological variability represents distinct etiologies, or variations among the developmental timeline of ASD. Nevertheless, the pooled prevalence of macrocephaly at birth in these studies is double the population rate (Hobbs et al., 2007). A critical line of investigation is to explore the relationship between prenatal HC and the development of ASD.

Addressing such research questions has begun, as two previous studies have examined mean prenatal HC in children diagnosed with ASD through the use of prenatal ultrasound (Hobbs et al., 2007; Whitehouse, Hickey, Stanley, Newnham, & Pennell, 2011). Hobbs and colleagues (2007) completed the first retrospective investigation of fetal head and body size during midgestation in children later diagnosed with ASD. Second trimester fetal ultrasounds were collected for 45 children with ASD and compared to 222 control subjects through case-control comparisons. Subjects were matched on gestational age, calculated from the standard composite of fetal biometry

(Hadlock, Deter, Harrist, & Park, 1984), and year of ultrasound. Sixty percent of the ASD sample underwent diagnostic confirmation using the gold standard assessments (i.e. Autism Diagnostic Observation Schedule-Generic; Lord et al., 2000; Lord, Rutter, DiLavore, & Risi, 2008. Control data was obtained from medical records and developmental exclusionary criteria were not included. HC, biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) (see Appendix B) measurements were extracted from ultrasound records and standardized. Results indicated the ASD group did not differ significantly from control subjects on any of the individual standardized fetal measures. Trends toward increased discrepancy between standardized BPD and HC measures suggested a tendency for brain width to grow at an increased rate relative to the brain as a whole. Unique limitations to this study included a relatively small sample size for anthropometric studies and lack of confirmation that control children were indeed typically developing.

A more recent study completed an ambitious prospective study of fetal HC by collecting HC and BPD measurements from over 2,000 children around 18 weeks gestation and shortly after birth. During follow-up visits, parents were asked whether their child had ever received a diagnosis of ASD by a health professional. Out of the original sample, 14 children reported ASD diagnosis and had ultrasound data available. Each child with ASD was matched with four control children according to a number of factors. Other developmental or learning disabilities were ruled out for control subjects. In this case, fetal head measurements were standardized independent of head biometry (e.g., HC and BPD), using only FL reference data (Hadlock et al., 1982, 1984). Birth

measurements were standardized using gender-stratified reference data. Independent samples *t*-tests found no difference between cases and controls at second trimester or at birth. Examination of 'difference scores', which quantified head circumference relative to body size, revealed that a small number of children with ASD had a disproportionately large head size at the second trimester (n = 5) and birth (n = 2) assessments, although statistically significant group differences were not reached. Unique limitations to this study included lacking diagnostic confirmation using 'gold standard' observational tools and a small ASD sample.

Although both studies found no significant mean differences in prenatal head measurements among children with and without ASD, each study offered methodological limitations and encouraged further investigation into prenatal HC in ASD. One area that remains to be explored is HC in later gestation. Second trimester ultrasound remains the standard of care for fetal anatomical evaluation (Salomon et al., 2010), and thus typically provides the largest sample of ultrasounds in retrospective designs. Given that the normative process of neurogenesis is quite different from second trimester to third trimester, it is critical to look at HC size during the third trimester, as well as the rate of growth from second to third trimester. If an excess of cortical neurons is indeed the underlying mechanism driving the overgrowth seen in many children with ASD, examining the point at which pruning of these neurons is expected to occur may provide the earliest evidence for this growth dysregulation.

Another important addition to this line of investigation is to expand the ways in which the relationship between HC and ASD is explored. First, both previous studies

examining prenatal HC in ASD have compared mean HC between ASD and control groups of children. Although rates of macrocephaly are higher in children with ASD, large HC is not present in every child with ASD. Given the heterogeneity of ASD, and that the etiology remains unknown, comparing group means between ASD and control samples may not be the most appropriate way to initially address the questions surrounding the relationship between prenatal brain development and the diagnosis of ASD. Rather, exploring whether the probability of developing ASD is associated with larger prenatal HC through is of interest, and may provide greater clinical utility. Second, the recent controversy surrounding the use of national normative growth charts for HC provides an impetus to explore new ways to assess HC size in ASD. Comparisons to local community controls and analysis of raw measurements may provide sound ways to address these concerns.

Finally, although postnatal HC has been correlated to symptom severity and other diagnostic variables, this connection has yet to be explored using prenatal HC. Although skill acquisition and symptom severity is undoubtedly affected by early developmental factors such as age of diagnosis, early intervention, and early developmental experiences, it is reasonable to predict that symptom severity may be positively correlated to the earliest detected differences in brain development. Accordingly, understanding the mechanisms governing such early neuropathology is essential to making gains in the areas of etiology, early detection, and early intervention.

Given the hypothesis that the underlying biological cause of brain overgrowth in children with ASD is an excess of neurons, the current study did not expect differences in

neuronal growth to be apparent during the second trimester. It was however hypothesized that differences in fetal HC will be detected when dysregulation of neuronal pruning begins in the third trimester, and thus the rate of HC growth from second to third trimester is expected to differ and predict the development of ASD. Finally, a more dysregulated rate of growth (e.g. faster) is expected to predict greater symptom severity at the time of diagnosis.

Chapter Two: Method

Data were collected as part of a larger, ongoing NIH-funded study through the Kennedy Krieger Institute Center for Autism and Related Disorders (CARD) and The Johns Hopkins University School of Medicine. Recruitment sources for this project include ASD advocacy groups, conferences, schools, physicians' offices, and playgroups. This study was approved by the Institutional Review Boards at The Johns Hopkins University School of Medicine and the University of Texas at Austin. All families gave written informed consent for their participation.

Participants

Participants are part of a longitudinal study investigating early markers of ASD. Infants at high and low risk for ASD participated, including siblings of children with idiopathic autism (AU sibs) and low risk (LR) controls having no known family history of ASD. Participants in either group were ineligible if they met any of the following criteria: family's first language being other than English, low birth weight (<1500 g), severe birth trauma, head injury, prenatal illicit drug or excessive alcohol exposure, or severe birth defects. In addition, in order to ensure independence of sampling, pairs of siblings were not included due to the high correlation of head size among family members.

In addition to regular assessments tracking cognitive, behavioral, and diagnostic information, a subset of families consented to retrospective collection of prenatal medical records. Within this sample, smaller subsets of families had prenatal ultrasound records from the second trimester (N=67), from the third trimester (N=28), from both the second

and third trimesters (N=25). Finally, a subset of the larger sample had symptom severity scores from age of diagnosis in addition to fetal ultrasounds at both time points (N=20). The subsamples correspond to the four main hypotheses being addressed within the current study. Results will be organized and presented accordingly.

Measures

Primary dependent variables were from the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, DiLavore, & Risi, 1999) and the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003). From the total sample, diagnostic outcome and severity was determined using the ADOS for 46 out of 50 (92%) participants. The remaining 4 participants received diagnostic screening through parental report using the SCQ. Of note, all participants enrolled as AU sibs were administered the ADOS and the full diagnostic battery. Of the four participants whose diagnostic outcome was determined by the SCQ, all were enrolled as LR controls.

Autism Diagnostic Observation Schedule. The Autism Diagnostic Observation Schedule-Generic (ADOS-G; Lord, et al., 1999) is a semi-structured, play-based assessment with standardized administration and scoring used to help diagnose ASD. Diagnostic algorithms with thresholds set for 'autism' and 'broader autism spectrum/PDD' were generated from a prior operationalization of DSM-IV/ICD-10 criteria, factor analyses, and ROG curves. The scoring algorithm permits diagnostic classification when individual Social and Communication domain scores, as well as the Social + Communication domain scores, meet instrument thresholds. Inter-rater reliability ranges from .82 to .93 for algorithm totals for stereotyped behaviors and

restricted interests, social interaction, communication, and communication-social interaction. Inter-rater agreement in diagnostic classification for autism versus non-spectrum comparisons, based on the ADOS-G algorithm, range from 90% to 100% across the four developmental modules (Lord et al., 2000). Research staff at CARD established ≥ 80% inter-rater reliability in administering the ADOS-G.

The ADOS-G has four different modules designed to best fit the expressive language skills of the individual child in order to make judgments about social and communication abilities independent from the effects of level of language delay. Module 1 (minimal to no language, N=18) and Module 2 (non-echoed phrase speech, N=32) were administered.

Diagnostic Outcome. Outcome diagnosis of ASD was made at 36 months (M = 36.2 months, SD = 2.3) because diagnosis at that age is reliable (Bauman & Kemper, 2003; Courchesne, Carper, & Akshoomoff, 2003; Mandell, Maytali, Novak, & Zubritsky, 2005; Mandell, et al., 2010; Rosenberg, Landa, Law, Stuart, Law, 2011) and still relatively early in terms of early identification of ASD. The authors stress the importance of using the ADOS in conjunction with a developmental history, corroborating information from other sources, and the use of clinical judgment (Lord et al, 2008, Lord & Bishop, 2010). Accordingly, research staff administered a battery of developmental, cognitive, and behavioral assessments as part of the protocol for the larger longitudinal study. Parents also completed a series of questionnaires, including adaptive behavior measures, regarding their child's development. Thus, ADOS scores in conjunction with additional measures and clinical judgment determined diagnostic outcome by master's- or

doctoral-level clinical research examiners who had experience with young children with autism.

Symptom Severity. Symptom severity was quantified using Calibrated Severity Scores (CSS) calculated from ADOS total raw scores (Gotham, Pickles, & Lord, 2008). Although ADOS raw totals are commonly used as a measure of ASD severity, the ADOS is a diagnostic tool and was not specifically designed to facilitate longitudinal and cross-sectional comparisons of data. Effects of age and language level on raw scores have also been cited (Joseph Tager-Flusberg, & Lord, 2002; Gotham et al., 2007). CSS based on ADOS raw totals offers a method of quantifying ASD severity with relative independence from individual characteristics such as age and verbal IQ. Raw ADOS totals are mapped onto a 10-point severity metric. Severity scores 1–3 were set so as to represent the distribution of raw scores receiving a non-spectrum ADOS classification. Severity scores from 4–5 represented ASD classification; severity scores from 6–10 represented raw totals receiving an autism classification. The use of CSS has since been validated with other independent samples (de Bilt et al., 2011; Shumay et al., 2012), and thus was employed in the current study as standardized measure of symptom severity.

Social Communication Questionnaire. The SCQ is a 40-item caregiver-report questionnaire that screens for autism spectrum disorders. The instrument is based on items from the revised version of the Autism Diagnostic Interview (ADI-R; Lord, Rutter, & Le Couteur, 1994) and the diagnostic criteria outlined by the DSM-IV (APA, 1994). Particular items were selected for their discriminant diagnostic validity (Rutter, Bailey, & Lord, 2003). The SCQ is valid for children over 4 years of age (with a mental age of 2)

and results in a total score (range 0-39). A cutoff score of 15 points or greater is indicative of a possible ASD (sensitivity 0.85; specificity 0.75; Berumen, Rutter, Lord, Pickles, & Bailey, 1999).

Current and Lifetime versions of the SCQ are available. The Lifetime form focuses on behaviors observed during any point in a child's life, along with certain behaviors that may have been observed between the child's fourth and fifth birthdays. This version should be used for diagnostic screening. The Current form focuses on behaviors observed during the past three months and should be used to track changes over time. Of the four participants in the current study who were screened using the SCQ, all were administered the Lifetime version.

Fetal biometry. Primary independent variables were from fetal ultrasound measurements taken during the second and third trimesters of gestation. According to the American Institute of Ultrasound in Medicine (AIUM) Practice Guideline for the Performance of Obstetric Ultrasound Examinations (2013), a standard obstetric sonogram in the second or third trimester includes an evaluation of fetal presentation, amniotic fluid volume, cardiac activity, placental position, fetal biometry, and fetal number. With modern equipment, 3- to 5-MHz abdominal transducers allow sufficient penetration in most patients while providing adequate resolution. After the first trimester, a variety of sonographic measurements can be used to estimate gestational age, including head circumference (HC), biparietal diameter (BPD), femoral diaphysis length (FL), and abdominal circumference (AC) (AIUM, 2013; see Appendix B). These four

measurements are routinely used to calculate a composite indicating estimated gestational age (Hadlock, Deter, Harrist, & Park, 1982; 1984).

Inter-rater reliability for fetal ultrasound measurements is known to be high and the measurements are considered quite accurate (intraclass correlation coefficients .994 – .996) (Perni et al., 2004). The accuracy and high reliability are related to standardized ultrasound planes, simple anatomic landmarks, and standard methods of measurement. The HC is measured at the level of the thalami and cavum septi pellucidi or columns of the fornix, around the outer perimeter of the calvarium. The measurement is taken from the outer edge of the proximal skull to the inner edge of the distal skull and is not affected by head shape. The FL is most accurately measured with the beam of ultrasound being perpendicular to the shaft, excluding the distal femoral epiphysis (AIUM, 2013). Fetal HC correlates strongly with and is used to assess fetal brain size ($r = .97$, $p < .001$) (Endres & Cohen, 2001).

Study Design

Prenatal medical records were collected retrospectively. Mothers of AU sibs and LR controls were asked for permission to obtain their pregnancy and obstetrical records. Informed consent was obtained along with the contact information for their medical providers. From each ultrasound record, the following information was abstracted: maternal age, estimated gestational age, and fetal biometry (HC, BPD, FL, AC).

This information was collected for each prenatal ultrasound conducted during second and third trimesters, when available. The second trimester '18-22 week scan' remains the standard of care for fetal anatomical evaluation (Salomon et al., 2010), in

part due to anatomical clarity. Ultrasounds records were considered to be within the second trimester if obtained during 14 and 26 weeks of gestation. If obtained during 27-39 weeks gestation, records were considered to be within the third trimester. Notably, if more than one ultrasound examination occurred during a trimester, the measurements from the examination that took place most closely to the median gestational week for that trimester (second trimester median = week 20; third trimester median = week 33) were included in the analysis. As part of the current study, the average number of ultrasounds obtained per participant was calculated for each diagnostic group.

Analyses

The primary purposes of this study were (1a) to examine the predictive relation between standardized second trimester prenatal HC and the development of ASD, (1b) to examine the predictive relation between standardized second trimester prenatal HC and ASD symptom severity, (2a) to examine the predictive relationship between standardized third trimester prenatal HC and the development of ASD, (2b) examine the predictive relation between standardized third trimester prenatal HC and ASD symptom severity, (3a) to examine the predictive relation between standardized HC rate of growth from second to third trimester and the development of ASD, (3b) to examine the predictive relation between raw score HC rate of growth from second to third trimester and the development of ASD, (4a) to examine the predictive relation between standardized prenatal HC growth and ASD symptom severity, and (4b) to examine the predictive relation between raw prenatal HC growth and ASD symptom severity. Binary logistic and simple regression analyses were used to test these primary research questions.

Statistical Package for the Social Sciences: Version 22 was used to analyze data (SPSS; IBM, 2013). Post hoc power analyses were conducted using G*POWER software to compute the achieved power for the binary logistic and simple regression analyses.

Descriptive analyses. Descriptive statistics were computed for predictor and outcome variable for each of the analyses. Frequencies and percentages were calculated for dichotomous and categorical variables (diagnostic outcome, gender). Basic descriptive statistics were calculated and analyzed for each continuous variable (maternal age, gestational age, HC, number of ultrasounds obtained, symptom severity), including means, standard deviations, and minimum and maximum values. Although HC outliers were present, extreme values of HC are of particular interest to the research question, and thus were not discarded from the sample. Race and SES data were not available, but the Kennedy Krieger Institute patient population is predominantly Caucasian and middle class.

Preliminary analyses.

Gestational Age Conversion. Prior to conducting the primary research analyses, preliminary analyses were necessary to convert available raw score measurements. As previously mentioned, ultrasonographic gestational age is typically calculated from a composite of HC, BPD, AC, and FL (Hadlock et al., 1982, 1984). However, given the prediction of fetal HC differences in ASD, it was critical that the gestational age used in the analyses be independent of any head measurement. As a single measurement, femur length has been shown to be highly predictive of gestational age (r^2 = 97.3%) (Hadlock et al., 1982). Utilizing the reference values from Hadlock and colleagues (1982; see

Appendix C), composite gestational ages were converted to gestational ages based on femur length only ($_{FL}$GA), prior to analyses.

Fetal Measurement Standardization. Next, in order to control for variation in gestational age at second and third trimester time points, raw fetal HC measurements were converted to standardized z-scores using conventional methods: (participant's measurement - reference data mean at participant's gestational age)/reference data standard deviation at participant's gestational age, again utilizing the Hadlock reference values, and the converted gestational age based on femur length: (fetal HC - μ_{FL}GA / ∂_{FL}GA). Standardized z-scores were used to test hypotheses 1, 2, 3a, and 4a.

Raw Score Rate of Growth Calculation. Due to recent literature citing potential bias and inaccuracy of utilizing standardized measures based on normative growth curves, the current study conducted analyses using raw score fetal measurements when appropriate. As such, a raw score rate of growth (Raw_ROG) variable was computed to capture HC growth from second to third trimester. Difference in raw score from second to third trimester was divided by difference in $_{FL}$GA from second to third trimester: (HC_3rdTri – HC_2ndTri)/ (flGA_3rdTri – flGA_2ndTri). Raw_ROG was used to test hypotheses 3b and 4b.

Prevalence of Macrocephaly. Standardized HC data was also used to calculate prevalence estimates for large HC ($z > 1.28$, $> 90^{th}$ percentile) and macrocephaly ($z > 1.88$, $> 97^{th}$ percentile) in both the ASD and control samples at second and third trimester time points. Prevalence rates were compared to the expected population rates of 10% for large HC and 3% for macrocephaly.

Tests of Research Questions.

Hypothesis 1a. Standardized second trimester fetal HC measures will not predict diagnostic outcome. Logistic regression analysis examined the predictive utility of second trimester zHC in predicting diagnostic outcome (ASD or nonASD). The vast majority of neurons are created during 10-20 weeks of gestation, a time period that aligns with the beginning of the second trimester. Given that the underlying biological cause of brain overgrowth in children with ASD is thought to be due to an excess of neurons, which is hypothesized to be a result of a lack of pruning later in gestation, the current hypothesis did not predict differences in neuronal growth to be apparent at the prenatal time point in which these neurons are first being produced.

Hypothesis 1b. Standardized second trimester fetal HC measures will not predict ASD symptom severity. Simple regression analysis examined the predictive utility of second trimester zHC in predicting symptom severity (ADOS CSS scores). Differences in neuronal growth are not predicted to be apparent at the second trimester time point, and thus any relation between growth dysregulation and later symptom development is not predicted at this time point.

Hypothesis 2a. Standardized third trimester fetal HC measures will predict diagnostic outcome. Logistic regression analysis examined the predictive utility of third trimester zHC in predicting diagnostic outcome (ASD or nonASD). Since the normative process of pruning, or programmed cell death begins during the third trimester, and given the hypothesis that the underlying cause of excessive neurons in children with ASD is

due to a lack of pruning, it was hypothesized that differences in fetal brain anatomy would first be detected during the third trimester.

Hypothesis 2b. Standardized third trimester fetal HC measures will predict ASD symptom severity. Simple regression analysis examined the predictive utility of third trimester zHC in predicting symptom severity (ADOS CSS scores). It was hypothesized that differences in fetal brain anatomy would first be detected during the third trimester, and thus a relation between growth dysregulation and later symptom development is predicted at this time point.

Hypothesis 3a. Standardized fetal HC rate of growth from second to third trimester will predict diagnostic outcome. Logistic regression analysis examined the predictive utility of the difference in HC z-scores (zHC_ROG) from second to third trimester (zHC_3rdTri - zHC_2ndTri) in predicting diagnostic outcome (ASD or nonASD). While it was hypothesized that differences in fetal brain anatomy will be first be detected during the third trimester (hypothesis 2), third trimester differences are predicted to be driven by accelerated brain growth from the second to third trimester in the ASD sample.

Hypothesis 3b. Raw score fetal HC rate of growth from second to third trimester will predict diagnostic outcome. Logistic regression analysis examined the predictive utility of the difference in HC raw score (Raw_ROG) from second to third trimester (HC_3rdTri – HC_2ndTri)/ (flGA_3rdTri – flGA_2ndTri) in predicting diagnostic outcome (ASD or nonASD). Given the potential limitations of using standardized HC scores in investigating brain development in ASD, this exploratory hypothesis examined

whether raw measurements better capture the predicted accelerated brain growth from the second to third trimester, particularly in the ASD sample.

Hypothesis 4a. Standardized fetal HC rate of growth from second to third trimester will predict symptom severity. Simple regression analysis examined the relation between zHC_ROG and ADOS calibrated severity scores. It was hypothesized that a greater rate of growth will predict more severe scores.

Hypothesis 4b. Raw score fetal HC rate of growth from second to third trimester will predict symptom severity. Simple regression analysis examined the relation between Raw_ROG and ADOS calibrated severity scores. It was hypothesized that a greater rate of growth will predict more severe scores.

Exploratory Analyses

Post hoc exploratory analyses were performed to examining mean zHC. Standardized HC measurements at second and third trimester were compared to both normative population averages, and between ASD and typically developing (TD) groups. Independent samples *t* tests were conducted with and without Bonferroni correction for these comparisons.

Chapter Three: Results

Total Sample

Diagnostic Outcome. From the total sample of participants enrolled in the study as AU sibs ($N = 41$) and LR controls ($N = 26$), twenty out of sixty-seven (30%, 16 males, 4 females) went on to develop ASD. Nineteen out of the 41 (46%) AU sibs developed ASD, and one out of 26 LR controls (.04%) developed ASD. As compared to recent estimates, citing recurrence rates among siblings around 18.7% (Ozonoff et al., 2011), the current study sample found a recurrence rate of 46%. The prospective nature of diagnostic data collection, and the present sample size may have influenced results. Overall, this rate of recurrence supports the strong heritability of ASD. Subsamples of ASD and TD outcomes for each of the main research analyses will be outlined in subsequent sections.

Prevalance of Macrocephaly.

Second Trimester. Utilizing the most widely used reference data (Hadlock et at., 1982, 1984), standardized HC measurements revealed 2 out of 67 (.03%, both LR/TD) participants with large head, and 1 out of 67 participants with macrocephaly (.01%, AU sib/TD) at the second trimester. Conversely, 6 out of 67 (.08%) were microcephalic at the second trimester. Of these six participants, 3 were AU sibs, 3 were LR controls, and 2 (1 sib, 1 control) went on to develop ASD. Of note, the participant with the most extreme zHC (-4.71) was the single LR control who went on to develop ASD.

Third Trimester. Of the twenty-eight participants with third trimester HC measurements, 2 out of 28 (.07%, both AU sib/ 1 ASD, 1 TD) participants had large

heads. Another 2 out of 28 (.07%, both LR/TD) were macrocephalic. Of note, none of these participants were considered large head or macrocephalic at the second trimester. In this sample, 1 out of 28 (.04%, LR/TD) was microcephalic at the third trimester, and was also microcephalic at the second trimester.

Number of Fetal Ultrasounds. The average number of fetal ultrasounds obtained was significantly different between ASD and TD groups ($p = .022$). The ASD group obtained an average of 2.05 fetal ultrasounds, while the TD group obtained an average of 1.45 fetal ultrasounds. While a vast majority of participants from both groups obtained less than 3 fetal ultrasounds, three outliers from the ASD group obtained 4, 5, and 6 fetal ultrasounds. It is unclear why additional fetal ultrasounds were obtained for these participants. Insurance coverage, fetal complications, and physician preference all could influence this outcome. Additionally, although subsequent ultrasounds may have been obtained due to fetal concerns or complications, it is still unclear to researchers whether neonatal complications may actually be caused by the hypothesized dysregulated prenatal development of ASD. For these reasons, participants were not excluded for having multiple fetal ultrasounds.

Hypothesis 1a: Second Trimester HC as a Predictor of Diagnostic Outcome

Demographics. Demographic characteristics for the ASD and TD participants with second trimester HC measurements are displayed in Table 1.1. While a majority of the sample from both groups was male, the ratio of males to females in the ASD group mirrored the estimated 4:1 ratio in the ASD population. The ratio of males to females was more balanced in the TD sample. In terms of other demographic and clinical variables,

there were no significant differences between groups on gestational age, adjusted FL gestational age, or maternal age.

Table 1.1.

Participant Characteristics – Hypothesis 1a, Second Trimester Outcome Prediction

Characteristic	ASD	TD	Group Difference
N	20	47	
Male (%)	16 (80%)	28 (60%)	
Gestational Age (weeks), Mean (SD)	19.91 (1.5)	19.67 (1.6)	.580
FL Gestational Age (weeks), Mean (SD)	20.16 (1.6)	19.79 (1.7)	.428
Maternal Age (years), Mean (SD)	33.60 (4.2)	33.34 (3.3)	.791

Diagnostic Outcome Prediction. Binary logistic regression was performed on diagnosis as outcome and second trimester standardized fetal HC (zHC_2ndTri) as the predictor variable. A test of the model with the predictor variable against a constant-only model was not statistically significant, χ^2 (1, N = 67) = 2.37, p = .123, indicating that zHC_2ndTri cannot reliably predict which children will develop ASD (see Figure 1). Post hoc power analysis revealed that for a two-tailed logistic regression analysis, with 67 participants, a normal distribution, an alpha level of .05, and an odds ratio (effect size) of .662, the level of achieved power was 0.31 with a critical z at -1.96.

Figure 1. Logistic Regression of 2nd Trimester HC on Diagnostic Outcome

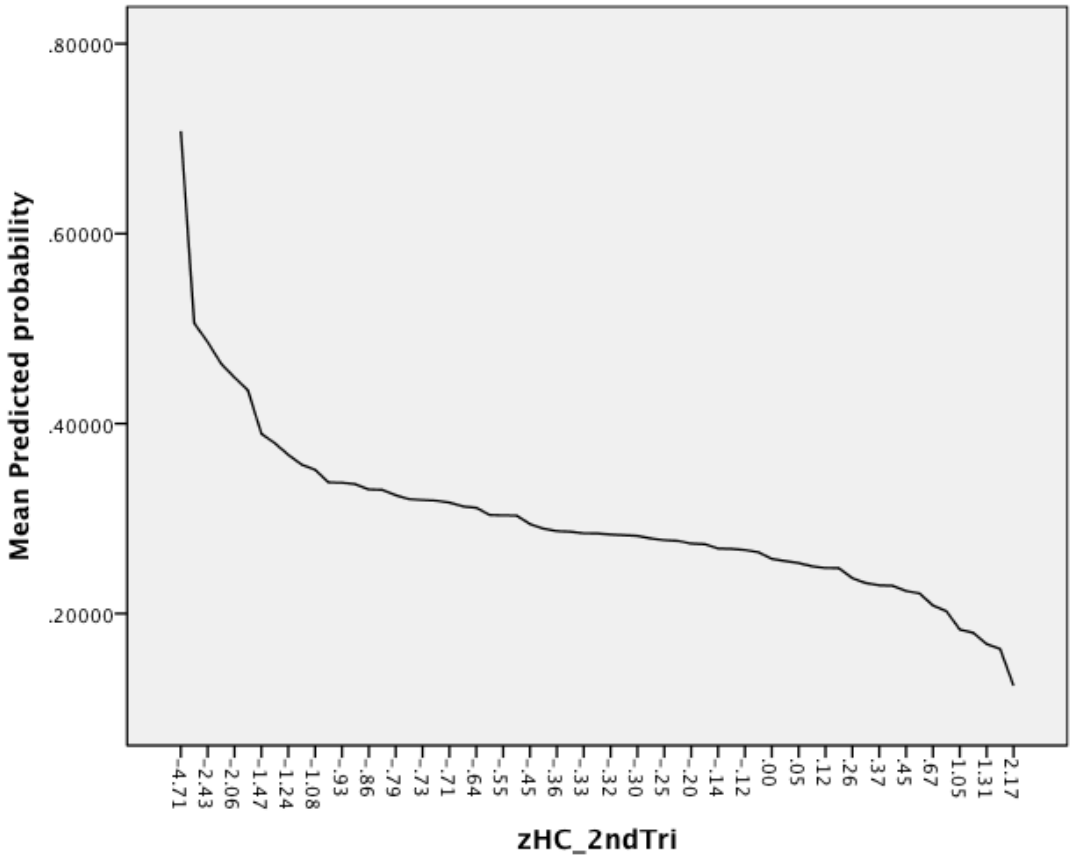

Figure 1. Mean predicted probability of ASD outcome for each zHC at second trimester.

Hypothesis 1b: Second Trimester HC as a Predictor of Symptom Severity

Demographics. Demographic characteristics for the ASD and TD participants with both second trimester HC measurements and ADOS CSS are displayed in Table 1.2. While a majority of the sample from both groups was male, the ratio of males in the ASD group mirrored the 4:1 ratio population estimates. The ratio of males to females was more balanced in the TD sample. In terms of other demographic and clinical variables, there were no significant differences between groups on gestational age, adjusted FL

gestational age, maternal age, or ADOS age of administration. While a majority of the ASD sample was administered Module 1, a majority of the TD sample was administered Module 2, reflecting a difference in verbal communication skills between the two groups of participants.

Table 1.2.

Participant Characteristics – Hypothesis 1b, Second Trimester Severity Prediction

Characteristic	ASD	TD	Group Difference
N	20	27	
Male (%)	16 (80%)	13 (48%)	
Gestational Age (weeks), Mean (SD)	19.91 (1.5)	19.67 (1.6)	.580
FL Gestational Age (weeks), Mean (SD)	20.16 (1.6)	19.79 (1.7)	.428
Maternal Age (years), Mean (SD)	33.60 (4.2)	33.34 (3.3)	.791
ADOS Age (months), Mean (SD)	36.14 (3.3)	36.21 (1.5)	.927
Module 1	14	4	
Module 2	6	23	

Symptom Severity Prediction. Simple regression analysis explored the relation between second trimester standardized fetal HC (zHC_2ndTri) and severity of social and communication difficulties at 36 months (ADOS CSS). Results indicated zHC_2ndTri did not significantly correlate with symptom severity ($F(45) = .540$, $p = .47$) for the total sample. However, a simple correlation analysis between zHC_2ndTri and ADOS CSS within the ASD sample revealed a strong positive relation ($r = .472$, $p < .05$) (see Figure 2). In other words, the larger the HC at second trimester, the more likely for ADOS CSS to be more severe. Post hoc power analysis revealed that for a two-tailed simple

regression analysis, with 47 participants, a normal distribution, an alpha level of .05, and an effect size of .29, the level of achieved power was 0.95 with a critical *F* at 4.06.

Figure 2. Linear Regression of 2nd Trimester HC on Symptom Severity

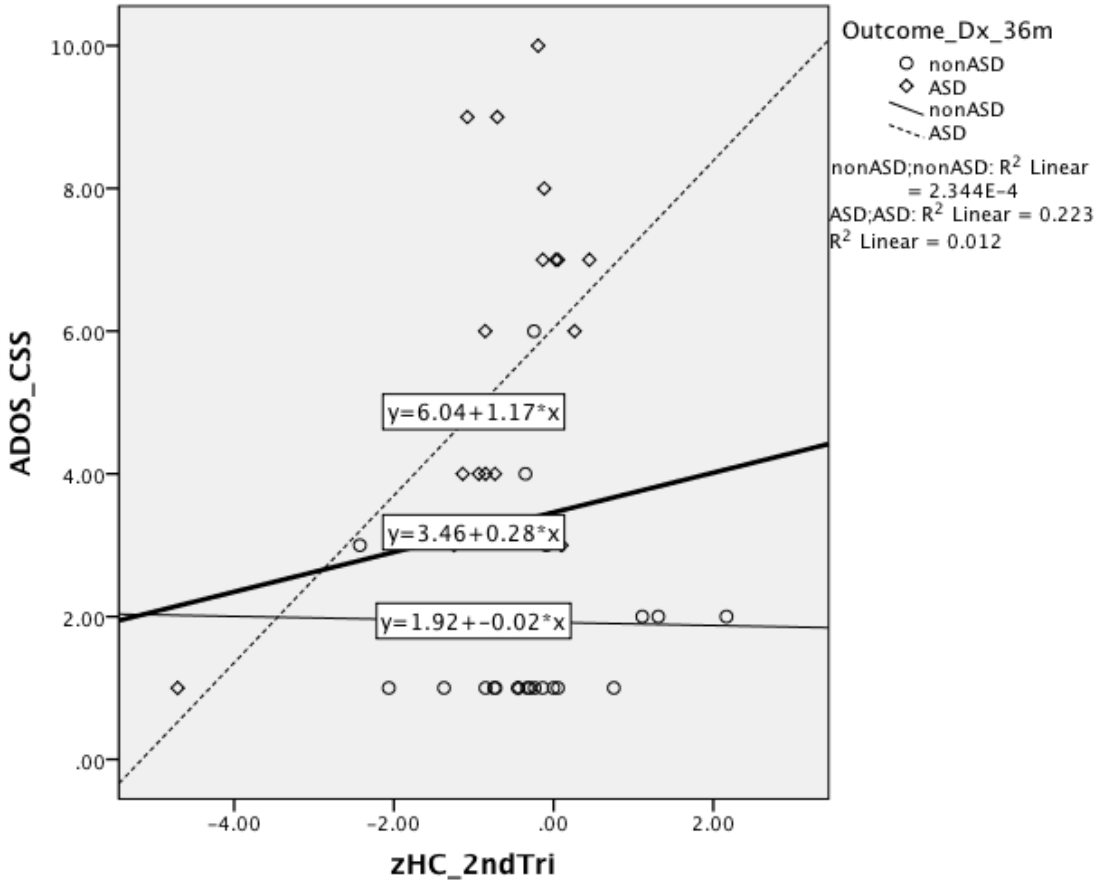

Figure 2. Plot showing the relationship between ADOS CSS scores and zHC at second trimester for the total sample, TD controls (nonASD), and ASD sample.

Hypothesis 2a: Third Trimester HC as a Predictor of Diagnostic Outcome

Demographics. Demographic characteristics for the ASD and TD participants with third trimester HC measurements are displayed in Table 2.1. In this sample, there

were no females with ASD, and an overall small number of participants with ASD who had obtained third trimester fetal ultrasounds (N=7). The TD group was three times more likely to have a third trimester ultrasound, and this sample was about one third female. In terms of other demographic and clinical variables, there were no significant differences between groups on gestational age, adjusted FL gestational age, or maternal age.

Table 2.1

Participant Characteristics – Hypothesis 2a, Third Trimester Outcome Prediction

Characteristic	ASD	TD	Group Difference
N	7	21	
Male (%)	7 (100%)	13 (62%)	
Gestational Age (weeks), Mean (SD)	32.44 (3.5)	33.42 (3.2)	.501
FL Gestational Age (weeks), Mean (SD)	32.02 (3.8)	33.02 (3.3)	.517
Maternal Age (years), Mean (SD)	33.00 (4.8)	34.38 (3.8)	.449

Diagnostic Outcome Prediction. Binary logistic regression was performed on diagnosis as outcome and the predictor variable of third trimester standardized fetal HC (zHC_3rdTri). A test of the model with the predictor variable against a constant-only model was not statistically significant, χ^2 (1, N = 28) = .018, p = .893, indicating that zHC_3rdTri cannot reliably predict which children will develop ASD (see Figure 3). Post hoc power analysis revealed that for a two-tailed logistic regression analysis, with 28 participants, a normal distribution, an alpha level of .05, and an odds ratio (effect size) of 1.057, the level of achieved power was 0.05 with a critical z at 1.96. The very low achieved power was likely affected by small sample size.

Figure 3. Logistic Regression of 3rd Trimester HC on Diagnostic Outcome

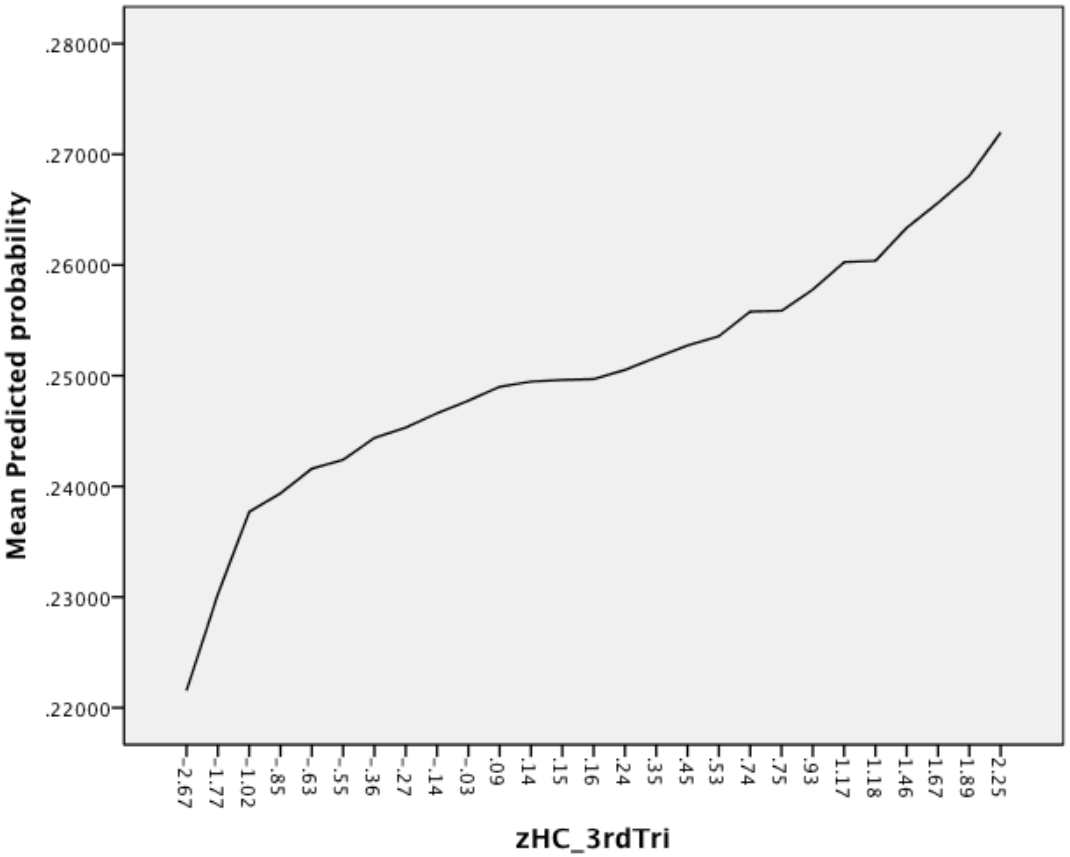

Figure 3. Mean predicted probability of ASD outcome for each zHC at third trimester.

Hypothesis 2b: Third Trimester HC as a Predictor of Symptom Severity

Demographics. Demographic characteristics for the ASD and TD participants with both third trimester HC measurements and ADOS CSS are displayed in Table 2.2. Again, all participants in the ASD sample were male, and this was a relatively small sample (N=7). The ratio of males to females was more balanced in the TD sample. In terms of other demographic and clinical variables, there were no significant differences between groups on gestational age, adjusted FL gestational age, maternal age, or ADOS

age of administration. Again, a majority of the ASD sample was administered Module 1, while a majority of the TD sample was administered Module 2, reflecting the difference in verbal communication skills between the two groups of participants.

Table 2.2

Participant Characteristics – Hypothesis 2b, Third Trimester Severity Prediction

Characteristic	ASD	TD	Group Difference
N	7	16	
Male (%)	7 (100%)	9 (56%)	
Gestational Age (weeks), Mean (SD)	32.44 (3.5)	33.67 (3.6)	.453
FL Gestational Age (weeks), Mean (SD)	32.02 (3.8)	33.36 (3.5)	.422
Maternal Age (years), Mean (SD)	33.00 (4.8)	34.31 (2.8)	.419
ADOS Age (months), Mean (SD)	35.64 (2.5)	36.72 (1.2)	.172
Module 1	6	2	
Module 2	1	14	

Symptom Severity. Simple regression analysis explored the relation between third trimester standardized fetal HC (zHC_3rdTri) and severity of social and communication difficulties at 36 months (ADOS CSS). Results indicated zHC_3rdTri did not significantly correlate with symptom severity for the total sample ($F(21) = .131$, $p = .721$), suggesting that zHC_3rdTri cannot reliably predict later severity of social and communication deficits (see Figure 4). Post hoc power analysis revealed that for a two-tailed simple regression analysis, with 23 participants, a normal distribution, an alpha level of .05, and an effect size of .02, the level of achieved power was 0.09 with a critical *F* at 4.32. Again, small sample size likely affected the level of achieved power.

Figure 4. Linear Regression of 3rd Trimester HC on Symptom Severity

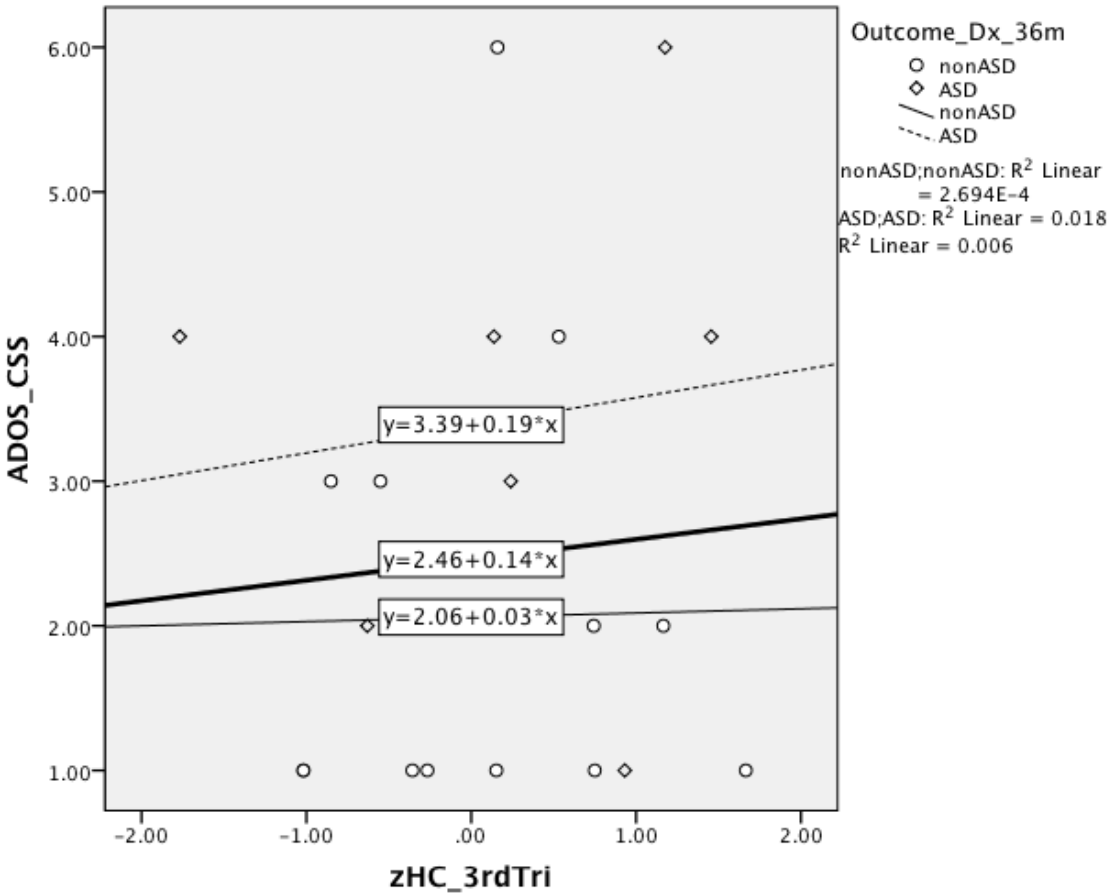

Figure 4. Plot showing the relationship between ADOS CSS scores and zHC at second trimester for the total sample, TD controls (nonASD), and ASD sample.

Hypotheses 3a & 3b: Rate of Growth as a Predictor of Diagnostic Outcome

Demographics. Demographic and clinical characteristics for the ASD and TD participants with both second and third trimester HC measurements are displayed in Table 3. This sample of participants was almost identical to the sample with third trimester data, minus three participants with outcome diagnoses of TD. Those three participants had ultrasound records from the third trimester only (no second trimester

ultrasound), and thus were not part of the rate of growth analysis. In this sample, again seven males comprised the ASD group, and there were no females with ASD. Again, approximately one third of the TD group was female. There were no significant differences between groups on gestational age, adjusted FL gestational age, or maternal age at both time points. For the rate of growth analysis, it was also important to ensure that both groups were equal on the amount of time between second and third trimester fetal ultrasounds. ASD and TD groups did not differ in the amount of time between fetal ultrasounds.

Table 3

Participant Characteristics – Hypotheses 3a & 3b, Rate of Growth/Diagnostic Outcome

Characteristic	ASD	TD	Group Difference
N	7	13	
Male (%)	7 (100%)	6 (50%)	
GA 2ndTri (weeks), Mean (SD)	19.47 (1.8)	19.81 (1.5)	.636
FL GA 2ndTri (weeks), Mean (SD)	19.83 (1.9)	19.99 (1.5)	.827
MA 2ndTri (years), Mean (SD)	32.71 (4.9)	34.06 (3.9)	.480
GA 3rdTri (weeks), Mean (SD)	32.44 (3.5)	33.41 (3.0)	.497
FL GA (weeks), Mean (SD)	32.02 (3.9)	33.02 (3.2)	.518
MA 3rdTri (years), Mean (SD)	33.00 (4.9)	34.28 (4.0)	.510
GA Difference (weeks), Mean (SD)	12.18 (4.4)	13.02 (1.7)	.227

Diagnostic Outcome.

Standardized Rate of Growth. Binary logistic regression was performed on diagnosis as outcome and the predictor variable standardized fetal HC rate of growth (zHC_ROG) from second to third trimester (zHC_3rdTri – zHC_2ndTri). The original

hypothesis was for an abnormally large fetal HC at the third trimester for the ASD group. Thus, the original hypothesis predicted an increased rate of growth for the ASD group, relative to normative growth charts, and relative to the TD group. Therefore, the analysis used a more targeted one-tailed binary logistic regression. A test of the model with the predictor variable against a constant-only model was statistically significant, χ^2 (1, N = 25) = 3.025, p = .041, indicating that zHC_ROG can reliably predict which children will develop ASD (see Figure 5). Post hoc power analysis revealed that for a one-tailed logistic regression analysis, with 25 participants, a normal distribution, an alpha level of .05, and an odds ratio (effect size) of 2.484, the level of achieved power was 0.52 with a critical z at 1.64.

Figure 5. Logistic Regression of zHC Rate of Growth on Diagnostic Outcome

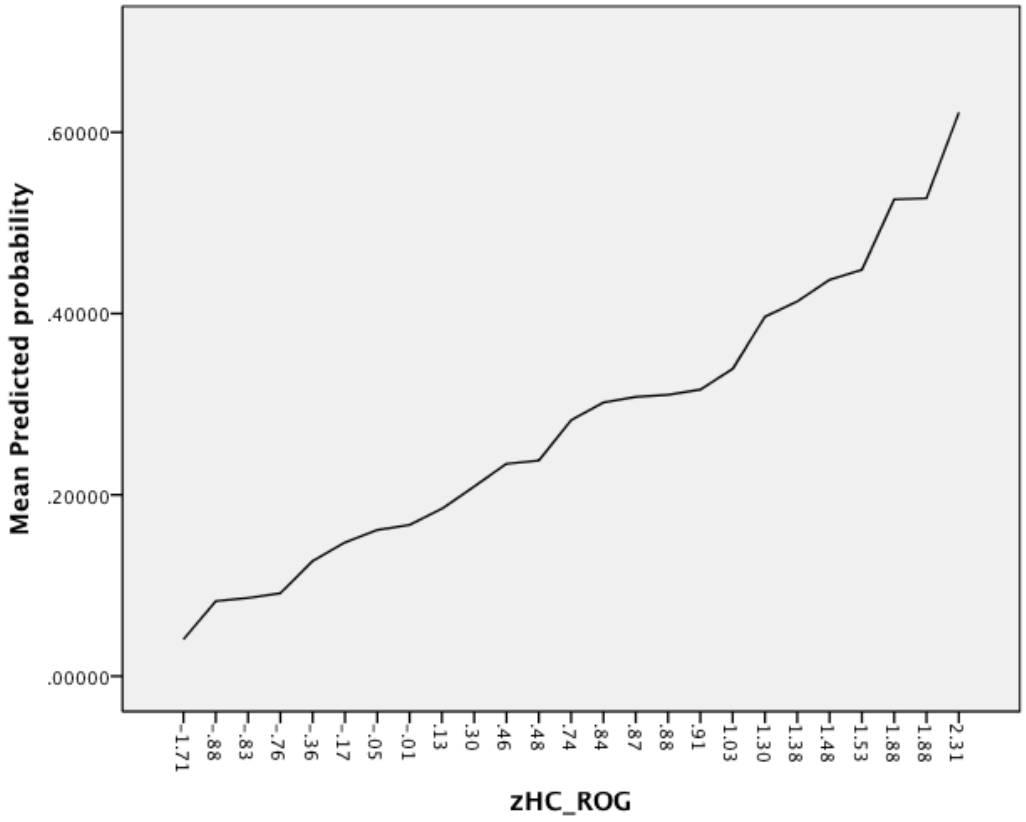

Figure 5. Mean predicted probability of ASD outcome for each zHC_ROG per participant.

Raw Score Rate of Growth. In an effort to explore fetal HC in ASD through an analysis independent of potential bias from normative growth charts, a raw score rate of growth (Raw_ROG) was calculated to capture HC growth in millimeters from second to third trimester. While zHC_ROG was already shown to reliably predict diagnostic outcome, this exploratory analysis aimed to provide further evidence for the phenomenon of fetal brain overgrowth, and provide evidence for an additional methodology. Again, a

one-tailed binary logistic regression was conducted. A test of the model with the predictor variable against a constant-only model was statistically significant, χ^2 (1, N = 25) = 3.428, p = .032, indicating that Raw_ROG can also reliably predict which children will develop ASD (see Figure 6). Post hoc power analysis revealed that for a one-tailed logistic regression analysis, with 25 participants, a normal distribution, an alpha level of .05, and an odds ratio (effect size) of 2.071, the level of achieved power was 0.41 with a critical z at 1.64.

Figure 6. Logistic Regression of Raw HC Rate of Growth on Diagnostic Outcome

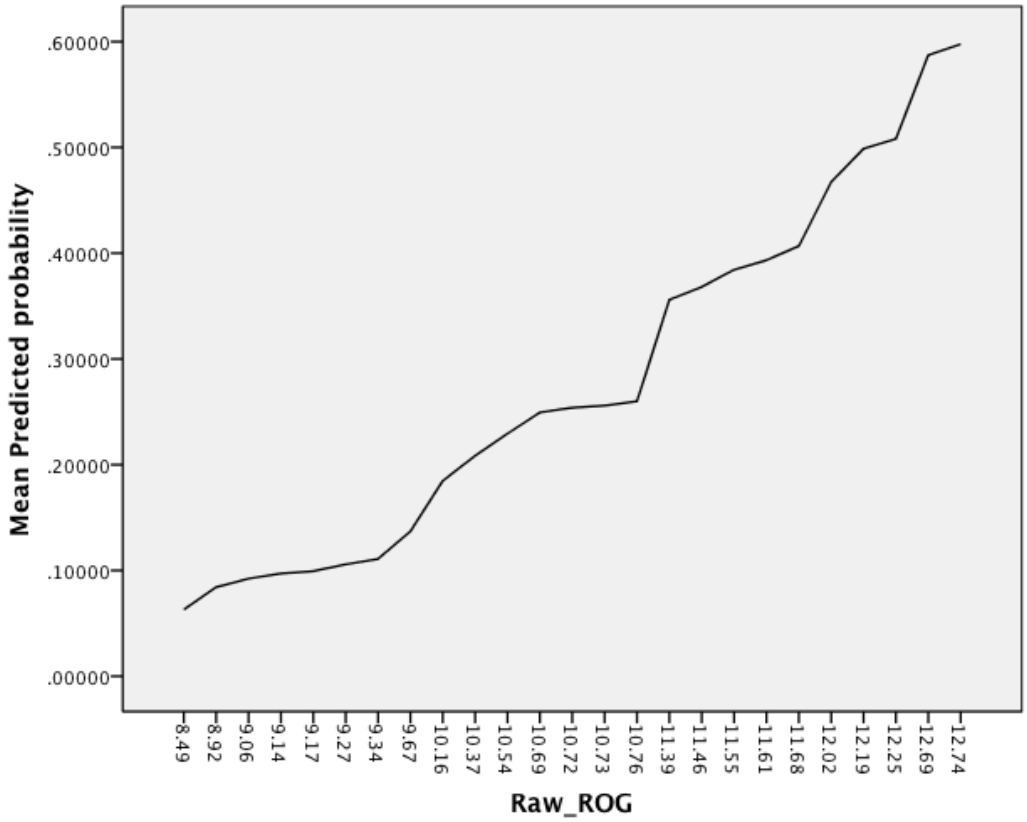

Figure 6. Mean predicted probability of ASD outcome for each Raw_ROG per participant.

Hypotheses 4a & 4b: HC Rate of Growth as a Predictor of Symptom Severity

Demographics. The final hypotheses resulted in the smallest sample of participants for whom data were available from both fetal ultrasound time points and ADOS symptom severity scores (N=20). Demographic and clinical variable for these twenty participants are displayed in Table 4. Again, all ASD participants were male, while the TD group had a more even sample of males and females. Again, the groups remained equal on the amount of weeks between second and third trimester fetal ultrasounds. While a majority of the ASD sample was administered Module 1, a majority of the TD sample was administered Module 2. Age of administration was not significantly different.

Table 4

Participant Characteristics – Hypotheses 4a & 4b, Rate of Growth/Symptom Severity

Characteristic	ASD	TD	Group Difference
N	7	13	
Male (%)	7 (100%)	8 (62%)	
GA Difference (weeks), Mean (SD)	12.18 (4.4)	13.63 (3.1)	.405
Module 1	6	2	
Module 2	1	11	
ADOS Age (months), Mean (SD)	35.64 (2.4)	36.48 (1.0)	.294

Symptom Severity. Data from 36 month ADOS Calculated Severity Scores were used to explore the relation between prenatal HC growth rate and severity of social and communication difficulties, as the magnitude of growth dysregulation was hypothesized to be correlated to developmental outcomes. This question was explored using both the

standardized rate of HC growth (zHC_ROG) and the rate of growth calculated from raw score measurements (i.e. independent of normative data) (Raw_ROG).

Standardized Rate of Growth. One-tailed simple regression analysis explored the relation between zHC_ROG and severity of social and communication difficulties at 36 months (ADOS CSS). Results indicated zHC_ROG did not significantly correlate with symptom severity for the total sample ($F(19) = 1.370$, $p = .129$). Visual analysis suggests that the relation between zHC_ROG and ADOS CSS differs between diagnostic groups. Follow up simple correlation analyses between zHC_ROG and ADOS CSS within each group revealed a non-significant, weak negative correlation for the ASD group ($r = -.230$, $p = .619$) and a non-significant, moderately positive correlation for the TD group ($r = .318$, $p = .290$) (see Figure 7). Post hoc power analysis revealed that for a one-tailed simple regression analysis, with 23 participants, a normal distribution, an alpha level of .05, and an effect size of .071, the level of achieved power was 0.23 with a critical F at 4.32. Again, small sample size likely affected the level of achieved power. Current results should be interpreted with caution, but suggest the need for further research in this area.

Figure 7. Linear Regression of zHC Rate of Growth on Symptom Severity

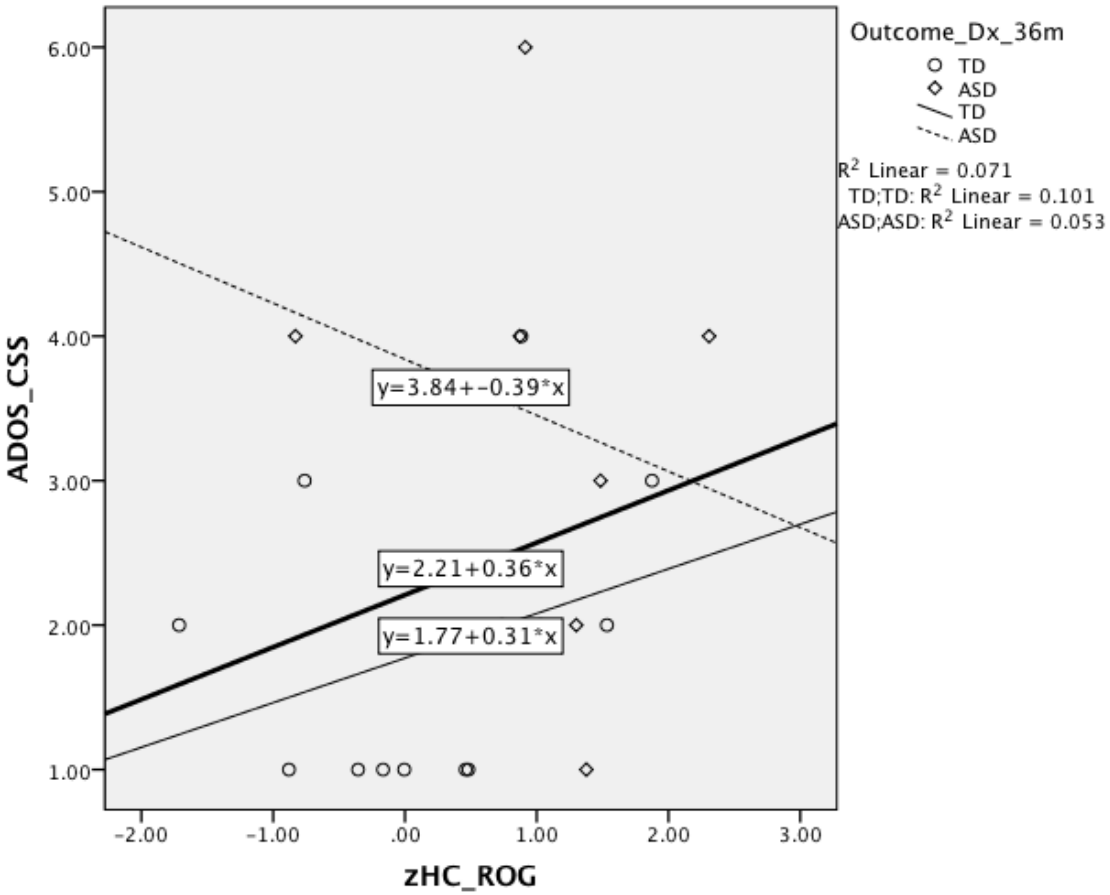

Figure 7. Plot showing the relationship between ADOS CSS scores and zHC_ROG for the total sample, TD controls, and ASD sample.

Raw Score Rate of Growth. One-tailed simple regression analysis explored the relation between Raw_ROG and severity of social and communication difficulties at 36 months (ADOS CSS). Results indicated Raw_ROG did not significantly correlate with symptom severity ($F(19) = 2.191$, $p = .078$), suggesting that Raw_ROG may have some utility in predicting later severity of social and communication deficits. Follow up simple correlation analyses between Raw_ROG and ADOS CSS within each group revealed non-significant results for both the ASD ($r = -.230$, $p = .619$) and TD ($r = .318$, $p = .290$)

(see Figure 8). Post hoc power analysis revealed that for a one-tailed simple regression analysis, with 23 participants, a normal distribution, an alpha level of .05, and an effect size of .109, the level of achieved power was 0.33 with a critical *F* at 4.32. Again, small sample size likely affected the level of achieved power.

Figure 8. Linear Regression of Raw HC Rate of Growth on Symptom Severity

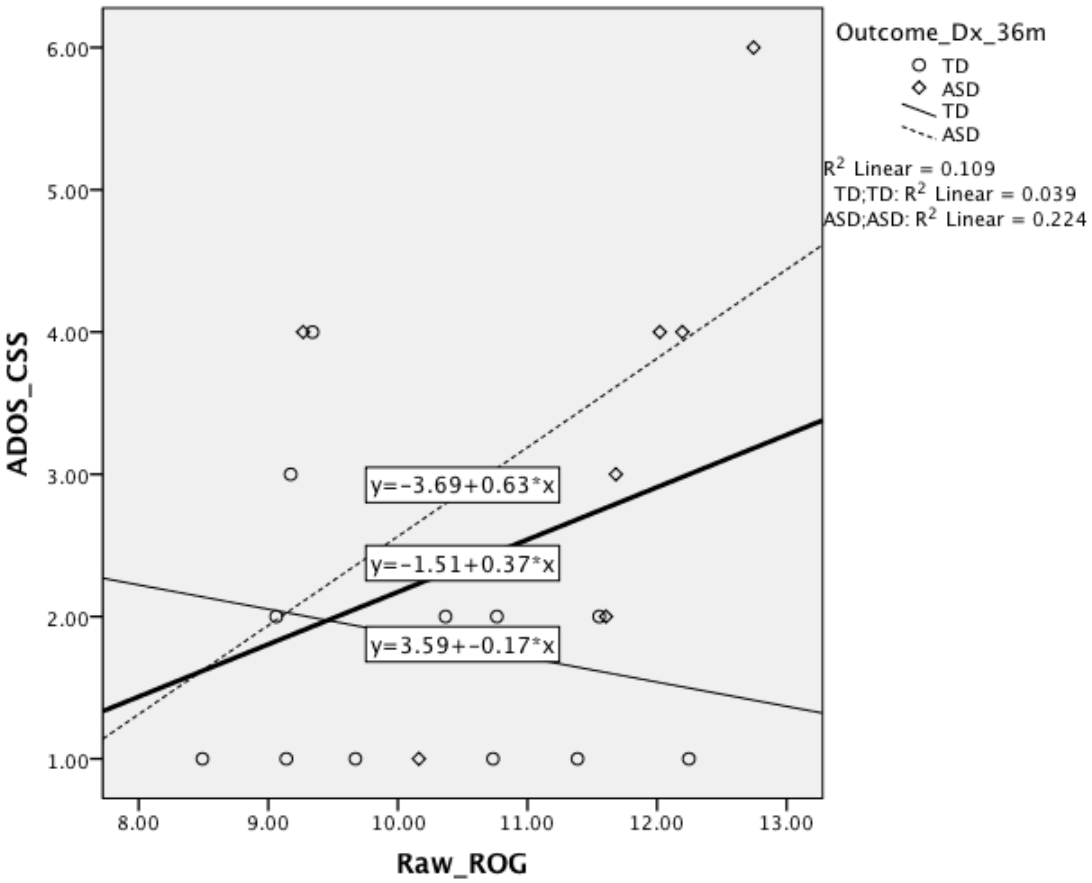

Figure 8. Plot showing the relationship between ADOS CSS scores and Raw_ROG for the total sample, TD controls and ASD sample.

Summary of Main Hypotheses

Standardized fetal HC measurements from second and third trimester were analyzed for their ability to predict the development of ASD and later symptom severity. While neither of these two cross-sectional analyses reliably predicted the development of ASD, the rate of HC growth from second to third trimester significantly predicted later diagnostic outcome and group membership. Table 5 summarizes these results. Specifically, children who later went on to develop ASD had an accelerated rate of HC growth from second to third trimester. Moreover, this effect was captured using both standardized measurements of fetal HC as well as raw score differences in head growth. Finally, none of the fetal HC measurements or growth measurements correlated to later symptom severity (see Table 6). Small sample sizes and low achieved power warrant further exploration.

Table 5.

Logistic Regression Predicting Diagnostic Outcome

Predictor	N	β	Wald χ^2	*p*	Odds Ratio
zHC_2ndTri	67	-.413	2.206	.137	.662
zHC_3rdTri	28	.055	0.018	.893	1.057
zHC_ROG	25	.910	2.459	.041‡**	2.484
Raw_ROG	25	.728	2.845	.032‡**	2.071

‡One-tailed analysis
** $p < .05$

Table 6.

Linear Regression Predicting Symptom Severity

Predictor	N	*F*	R	*p*	R Square
zHC_2ndTri	47	.540	.109	.466	.012
zHC_3rdTri	23	.131	.079	.721	.006
zHC_ROG	20	1.370	.266	.129‡	.071
Raw_ROG	20	2.191	.329	.078‡*	.109

‡One-tailed analysis
*$p < .10$

Exploratory Analyses

Second Trimester. When second trimester zHC of ASD and TD control participants were compared with each other and to a normative sample, follow up analyses revealed an unanticipated trend toward significantly smaller mean zHC for both ASD ($t(19) = -2.892$, $p = .009$) and TD control ($t(46) = -2.196$, $p < .05$) participants, as compared to normative growth charts. Although mean zHC did not significantly differ between the two groups ($t(65) = -1.559$, $p = .12$), with Bonferroni correction applied to control for familywise error, only the mean zHC for the ASD group was statistically smaller when compared to the normative sample. The distribution of the zHC_2ndTri for the ASD data showed the effect was possibly driven by one outlier with a particularly low zHC_2ndTri (-4.71). However, follow up analysis with the removal of this outlier suggested this was still a significant finding ($t(18) = -3.621$, $p = .002$). The zHC_2ndTri for the TD control data was evenly distributed.

Figure 9. Mean zHC at Second Trimester for ASD and TD participants

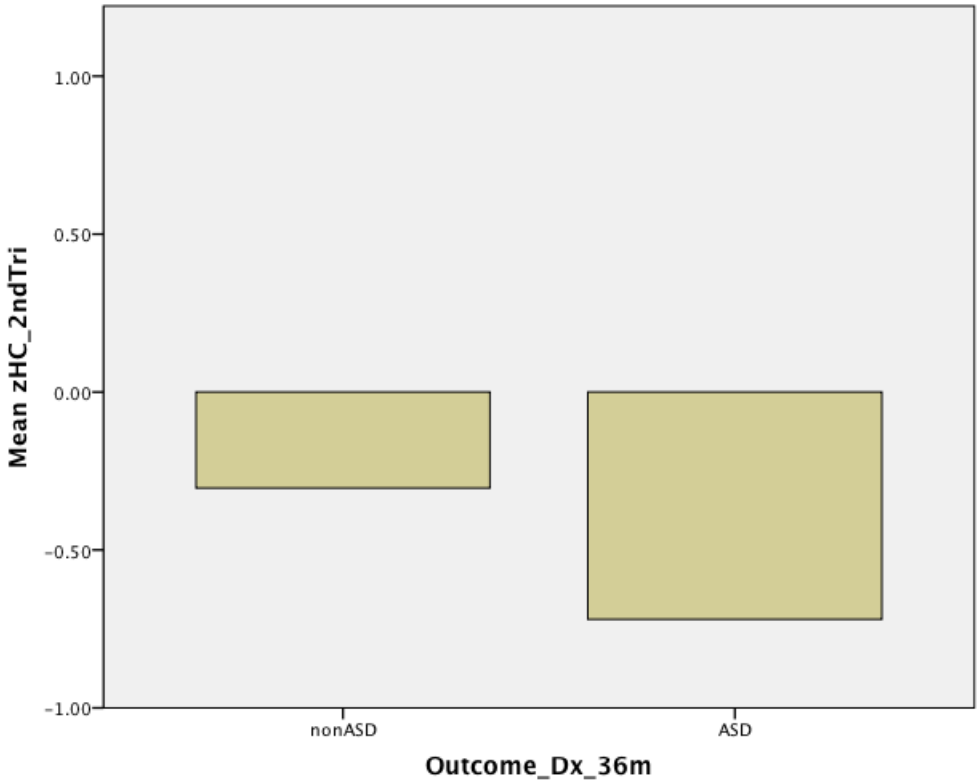

Figure 9. Mean second trimester HC for ASD and TD controls compared to normative sample from Hadlock et al. (1982, 1984).

Third Trimester. ASD and TD control participants were then compared with each other and to a normative sample on the variable of third trimester zHC. Comparing mean zHC at the third trimester for ASD and TD samples revealed that mean zHC is no longer significantly smaller than normal for either the ASD ($t(6) = -.516$, $p = .625$) or TD ($t(20) = .650$, $p < .523$) group. Again, the mean zHC did not significantly differ between the two groups ($t(26) = .130$, $p = .898$). The distribution of the zHC_3rdTri for the ASD and TD control data showed there were no outliers.

Figure 10. Mean zHC at Third Trimester for ASD and TD participants

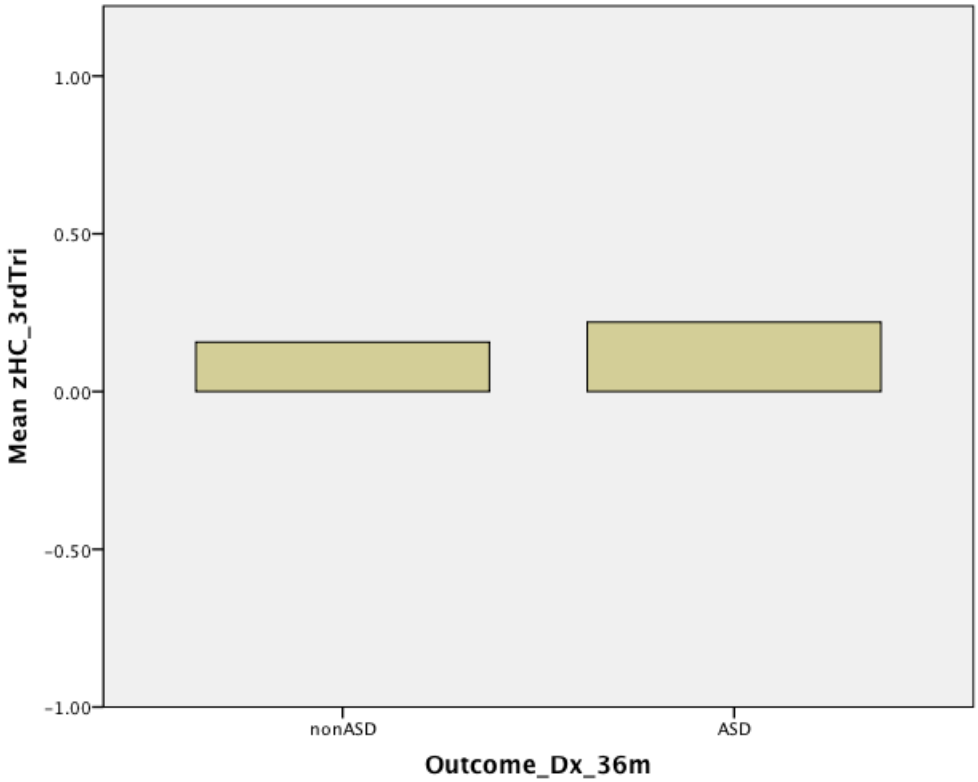

Figure 10. Mean second trimester HC for ASD and TD controls compared to normative sample from Hadlock et al. (1982, 1984).

Chapter Four: Discussion

Importance of Current Study

The current study sought to further explore prenatal neurobiological markers for ASD, expanding upon current literature in several important ways. First, by examining prenatal HC measurements at both second and third trimester time points, this study is the first to explore HC size later in gestation, a critical period during which unique neurodevelopmental processes are occurring. Further, by examining the rate of growth between these two trimesters, this study sought to explore the very nuanced growth changes that can occur during the dynamic process of prenatal neurogenesis, contributing to a more complete understanding of prenatal brain development in ASD.

Next, the present study sought to address concerns surrounding the use of normative growth charts when investigating early brain overgrowth in ASD. Although concerns of bias have been cited for the use of early childhood (i.e., postnatal) growth charts, an analysis of standardized HC measurements was included to explore the utility of this methodology, relative to raw score measures of HC. The use of locally recruited, community-based TD controls was an additional strength of the current study.

Finally, this study explored the relation between prenatal HC and later symptom severity. As part of a larger longitudinal investigation, the current study had unique access to both prenatal medical records and 36-month ADOS CSS data. To the author's knowledge, this study is the first to explore prenatal brain development and later symptom severity in both children with ASD and TD controls who underwent the same

diagnostic battery. As this analysis was somewhat exploratory in nature, both trimester time points and rate of growth between trimesters were explored for correlations with later symptom severity.

Overview of Findings

Second Trimester. Second Trimester HC was hypothesized to not reliably predict later diagnostic outcome or symptom severity. Although these hypotheses were correct, a closer examination of these results tells a different story than originally hypothesized. The current study theorized that early brain overgrowth was due to dysregulated growth (i.e., a lack of pruning, or increased neurogenesis) in children who later develop ASD. Since pruning begins around the third trimester, second trimester HC measurements were not anticipated to predict the development of ASD. Moreover, inherent in this hypothesis were the predictions that mean zHC would not differ significantly from population norms, and mean zHC should not differ between groups. In other words, brain development was anticipated to follow a typical trajectory through the second trimester, as suggested by previous research and current theories of brain overgrowth in ASD.

Follow up analyses revealed an unanticipated finding of significantly smaller mean zHC for ASD participants as compared to normative growth charts. In other words, atypical brain development was identified earlier than anticipated, suggesting a potential trend towards underdevelopment or delayed development of the brain of children who develop ASD. This finding has important implications for both the timing and mechanisms hypothesized to be involved in the development of ASD, both of which will

be discussed in detail in subsequent sections. This finding also has implications for the interpretation of third trimester HC size findings.

Third Trimester. Third trimester zHC was hypothesized to reliably predict later diagnostic outcome and symptom severity. More specifically, it was hypothesized that children with ASD would have larger zHC at the third trimester due to a lack of, or delay in, the normative pruning process present in later fetal development. Again, this hypothesis was not supported. However, third trimester predictions about fetal HC were predicated upon the hypothesis that fetal HC development in ASD was typical at the point of the second trimester. Given that zHC was found to be significantly smaller than normal in our sample of ASD children, snapshots of fetal HC at second and third trimesters may not accurately capture prenatal neural dysregulation in ASD.

Indeed, a follow-up examination of mean zHC at the third trimester that a longitudinal evaluation of fetal HC may provide a clearer picture of fetal HC growth in ASD. Comparing mean zHC at the third trimester for ASD and TD samples revealed that mean zHC is no longer significantly smaller than normal for either the ASD or TD group. Thus, in the current study sample, mean zHC at second and third trimesters suggested an accelerated rate of neural growth for children who later developed ASD. Although this finding indirectly suggested a difference in the rate of fetal brain growth for the ASD and TD controls, a direct examination of this further supported dysregulated prenatal brain growth in children who develop ASD.

Rate of Growth. HC rate of growth analysis revealed that rate of HC growth from second to third trimester significantly predicted later diagnostic outcome and group

membership. Children who later went on to develop ASD had an accelerated rate of HC growth from second to third trimester. Moreover, this effect was captured using both standardized measurements of fetal HC as well as raw score differences in head growth. This preliminary finding suggests the possibility for the earliest detected brain abnormality in ASD, and provides impetus for further investigation into this phenomenon with larger samples. The current study had only seven male participants in the ASD group, making it difficult to conclude this finding is not limited to males who develop ASD. As other findings have suggested certain neurodevelopmental trajectories are unique to males who develop ASD, it is critical to evaluate gender-specific developmental trajectories.

Symptom Severity. In terms of symptom severity, raw score rate of HC growth may have some utility in predicting later severity of social and communication deficits, however sample sizes were small and the power to detect a true finding was very low. Further research with larger sample sizes may better answer the question of whether or not fetal HC growth can predict severity of social and communication deficits. Interestingly, a strong positive relation was found between symptom severity and standardized HC size at the second trimester. In other words, larger HC at second trimester was associated with more severe social and communication deficits as measured by ADOS CSS. This finding is particularly interesting in that standardized HC at the second trimester was found to be significantly below average for the ASD group, and relatively below average even for the TD group. This raises questions regarding the normative data used in this study, which will be discussed later in the limitations section.

As such, HC measurements closer to average at the second trimester may be those that are correlating most highly with later symptom severity. Further evaluation of this finding is critical, as this may reflect separate mechanisms underlying diagnostic outcome and symptom severity.

Earliest Brain Overgrowth. This finding presents the *earliest* observed brain overgrowth in ASD. As previously hypothesized by a body of research, the current findings support a prenatal origin in the development of ASD. Moreover, these findings present direct evidence from data collected during prenatal development. Nevertheless, the current study revealed some unexpected findings in that the origin of dysregulated brain development in ASD may start as early as the second trimester. While it was hypothesized that brain development would be typical at the point of neurogenesis, smaller HC size at second trimester suggests delayed, or reduced fetal neurodevelopment in children who later developed ASD. Although this finding included a relatively larger sample size of children diagnosed with ASD, as compared to previous research in this area, additional analysis with a larger sample of ASD participants is critical to concluding this as the earliest detected brain abnormality in ASD.

Not only does this finding speak to the timing of dysregulated brain development in ASD, but it also has important implications for the underlying mechanisms thought to be at play. Although it was originally hypothesized that brain overgrowth in ASD would be due to a lack of pruning after typical neurogenesis in the second trimester, the current findings suggest that delayed or limited neurogenesis may actually be the catalyst for dysregulated brain development. While previous research has identified rapid brain

overgrowth in the first year of life to precede the observed behavioral deficits of ASD, we have yet to determine what may be driving this overgrowth. The current findings suggest that early brain overgrowth could actually be preceded by even earlier brain undergrowth. While the current study does not negate the hypothesis of inappropriate or reduced synaptic pruning, it does suppose that some sort of altered neuronal signaling in fetal development may be a primary contributing mechanism, which in fact may cause an overcompensation of neural connectivity and growth in early infancy. This proposed trajectory of head growth is depicted in Figure 11 below:

Figure 11. Proposed Longitudinal HC Growth Curves for ASD and TD

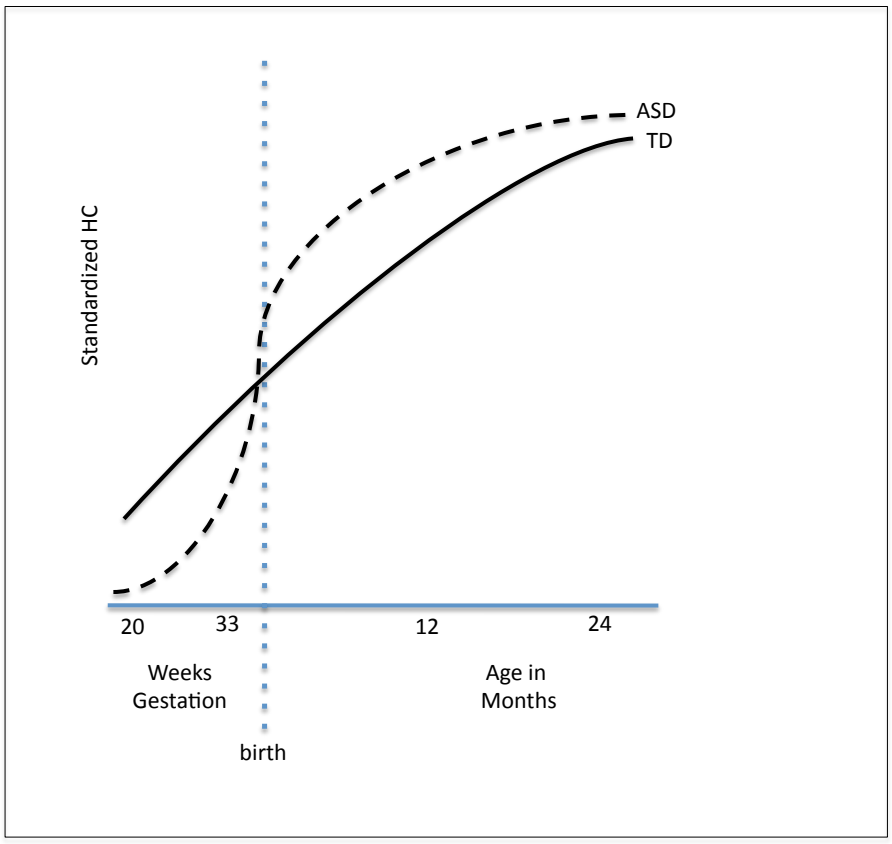

Figure 11. Proposed trajectory of standardized HC growth for ASD and TD controls from conception to 36 months of age. The proposed graph suggests brain underdevelopment at the second trimester, followed by a compensatory overgrowth through the first few years of life for participants who are later diagnosed with ASD. TD controls are depicted to follow a more normative trajectory of growth.

Limitations

One key limitation to the current study is small sample size, particularly for analyses of later gestational time points and rates of growth from second to third trimester. The retrospective nature of the current study design limited data to fetal ultrasound records that were collected as part of routine prenatal care. According to the senior vice president of public policy and government affairs for the March of Dimes, "Some [insurance] plans may cover just one ultrasound during pregnancy, while others will cover as many as the physician orders," (Hegwer & Frizzera, 2013). As mentioned earlier, the second trimester is generally the time at which mothers will receive their initial or only ultrasound examination. Casting a wider net to collect more medical records, or specifically targeting the recruitment of mothers who have had more than one ultrasound examination could be a way to improve sample size within a retrospective design.

However, other limitations still exist within the retrospective nature of the current study. Although fetal ultrasound examinations have strict guidelines and a high level of reliability when measuring fetal biometry, the lack of control over data collection increases chances for measurement error, differences in equipment, and overall variance. Fetal ultrasound is typically employed as a screening tool, thus the focus of data collection is not on precise measurements to assess for utility in predicting developmental outcomes. Prospective study designs would allow for more stringent data collection, including specific research protocols, reliability checks, and consistency of ultrasound technology and growth charts.

Continuing on the topic of growth charts, there is a potential limitation to the current study's use of the Hadlock et al. (1982,1984) scales of fetal growth. Although these scales are the most widely used normative reference data in fetal ultrasound, the growth charts are twenty years old and were normed on a relatively small sample of women (range = 338-533). The current study chose to use these reference data to remain consistent with their use in previous literature examining fetal growth in ASD; however, larger and more recent normative samples should be considered for future studies. Ioannou and colleagues (2012) conducted a systematic review of the methodology used in studies of fetal ultrasound aimed at creating growth charts. Their results indicated there is a positive correlation between the quality scores assigned to each study and year of publication. Thus, while the authors conclude quality has improved with time, they also document that considerable heterogeneity in study methodology is still observed in more recent normative growth chart creations. Future examinations should look to this review for guidance on choosing the most appropriate normative sample.

Future directions

One critical future direction for this line of research is to obtain a larger sample of participants. In particular, obtaining a larger sample of participants with both second and third trimester HC measurements is critical for further examining the relation between prenatal HC rate of growth and the development of ASD. The current study is the first to examine this relation and in spite of a small sample size, the current study presents preliminary evidence for the earliest detected brain difference in children with ASD. A

larger investigation into the phenomenon of *earliest* brain overgrowth will provide stronger evidence for the current finding.

Additional investigations should include comparisons of other fetal biometry, both in relation to, and independent of HC. Fetal brain development is a dynamic and highly interconnected process, so it is important to investigate fetal development in other brain regions. As mentioned previously, prenatal ultrasound examinations routinely record other head and body markers, including the biparietal diameter (i.e., head width), femur length, and abdominal circumference. Less routinely recorded, is the transcerebellar diameter (TCD). Allen and colleagues have proposed (manuscript in preparation), cerebellar development may also be delayed or reduced during fetal development. Examining the relation between fetal TCD and HC development may provide a better understanding for the mechanisms underlying the development of ASD.

Maternal age is another important factor that the current study design could address in future analyses. Although not considered causal, advanced maternal age has been identified as a potential risk factor for the development of ASD. Along with measurements of fetal biometry, maternal age is readily available on fetal ultrasound reports. Although the current study ensured that maternal age did not differ significantly between the ASD and TD groups, further analyses should look more closely at the proportion of mothers in each group who were considered of "advanced maternal age" (e.g., 35 or older). Advanced maternal age is associated with adverse outcomes in the perinatal period (Nelson, Telfer, & Anderson, 2012). Analyses should explore whether

the exclusion of this subset of mothers affects the relation between fetal HC growth and diagnostic and severity outcomes.

Another critical avenue to explore is the potential for differences in fetal brain development based on familial risk for autism, and not just diagnostic outcome. As mentioned earlier, HC is highly correlated among family members, regardless of diagnosis. Additionally, the term broader autism phenotype applies to family members and siblings who also display social and communication deficits, though not necessarily severe enough to reach clinical thresholds. Future studies should investigate whether fetal brain development of AU sibs differs from that of low risk controls. In addition, questions should address whether aberrant fetal HC growth is associated with more severe social and communication deficits, as these are often still present in siblings who do not meet criteria for ASD.

Finally, a prospective study following at-risk siblings and low risk controls from conception may provide the methodological control and resources necessary to address all of the proposed future directions. Although current ultrasound practices are often limited to ultrasound scans at the second trimester, a prospective study could allow for more frequent, and more reliable measurements of a large sample of participants. While certain head and brain measurements are often checked for "normality," such as the TCD at subsequent ultrasound scans, a prospective design would ensure that all possible brain measurements are precisely measured and recorded at specified time points.

In addition to a thorough tracking of fetal growth, a large prospective study could reliably collect other significant family demographic information. Although studies of

fetal brain development have attempted to include some of this information, a prospective study would allow for the comprehensive inclusion of other important factors, such as family history of ASD, familial HC measurements, parental age, race, socio-economic status, and perinatal risk factors. Given the plausibility that prenatal development of ASD may actually cause some of the neonatal risk factors more common to children with a family history of ASD, a prospective study could provide a high level of monitoring and prenatal care to families at risk.

A prospective design could also include an exhaustive collection of developmental, diagnostic, and behavioral data to examine other clinical correlates. In addition to severity of social and communication deficits, exploring the relation between fetal brain development and other important clinical correlates is critical to understanding how early brain development may affect outcomes. Cognitive measures, language development scales, motor development scales, eye-tracking techniques, sensory information, and behavioral measures are just some of the additional tools that could be used to expand our understanding of the effects of early dysregulated brain development. A longitudinal design tracking these measures over time would be ideal for both researchers and parents in terms of early identification of developmental delays. Moreover, collecting information from families about early intervention services received will allow researchers to at least account for the impact that these services may have on measures of developmental and diagnostic outcomes.

Finally, a prospective study would allow for the continued measurement of HC from prenatal to postnatal development, providing insight to long-term trajectory of brain

development in ASD. While other studies have conducted meta-analyses to track longitudinal brain development over time in groups of children with ASD, a prospective study of HC development from prenatal to postnatal years would be the first of its kind. Ideally, this design would track HC growth in children with and without ASD throughout childhood, offering the longest and most comprehensive evaluation of HC development in ASD.

Appendix A: Literature Review

Autism Spectrum Disorder

Diagnosis of ASD and diagnostic changes.

Autism spectrum disorder (ASD) is a developmental disorder that is characterized by impairments in social communication skills, and by the presence of repetitive and stereotyped interests and behaviors (American Psychiatric Association [APA], 2013). The hallmark of ASD is a lack of social reciprocity and an impaired ability to develop typical social relationships. While language delay is common in ASD, abnormal communication features are even more characteristic, including echolalia, pragmatic deficits, pronominal reversal, and idiosyncratic use of language. The presence of stereotyped, repetitive patterns of behavior can manifest as obsessive routines or rituals, preoccupations, circumscribed interests, and particular motor stereotypies (Bailey, Phillips, & Rutter, 1996). In addition, the behavioral markers of ASD are often accompanied by abnormalities in cognitive functioning and sensory processing (Filipek et al., 2000).

The previous version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR, APA, 2000) reflected a view of autism as a group of pervasive developmental disorders (PDDs) with distinct subcategories. Within PDD, subtypes included Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS). A diagnosis of Autistic Disorder required delays in language development and abnormal social interaction, as well as the presence of at least one or more stereotypic or repetitive behavior, all prior to three years of age. A

diagnosis of Asperger's Disorder was given if abnormal communication and social reciprocity were present, but normal development of language, cognitive skills, and adaptive behavior were required. Finally, a diagnosis of PDD-NOS was given when an individual exhibited a marked impairment in reciprocal social interaction but did not meet full symptom criteria for Autistic Disorder or Asperger's Disorder.

Under the previous diagnostic criteria, practitioners demonstrated a low rate of reliability and validity when distinguishing between Autistic Disorder, Asperger's Disorder, and PDD-NOS (Klin, Lang, Cicchetti, & Volkmar, 2000; Ozonoff, South, & Miller, 2000; Lord, Risi, DiLavore, Shulman, Thurm, & Pickles, 2006). Diagnoses were often made based on severity, language level, and intelligence rather than on symptom criteria that distinguish between disorders. While the prevalence of ASD in the population was fairly consistent across studies, the prevalence of subtypes of ASD tended to vary widely from study to study, indicating diagnostic imprecision among clinicians (Lord & Bishop, 2010). Another study found that when the patient's language level and intelligence level were controlled for, there were wide discrepancies in clinician diagnosis (Rosenberg et al., 2009). Additionally, diagnostic stability over time for PDD-NOS is not high (Lord et al., 2006). When diagnosed prior to age three, PDD-NOS demonstrates stability rates of 35% (Rondeau et al., 2011).

Due to the limitations of DSM-IV diagnostic criteria, The DSM, Fifth Edition (DSM-5; APA, 2013) utilizes ASD in place of the previously distinct diagnoses. Additionally, as a result of combining the social and communication domains, the DSM-5 diagnostic criteria for ASD now fall under two symptom domains of (1) social-

communication and (2) restricted, repetitive interests. Within ASD, distinctions are based on severity of presentation, in relation to chronological age (Lord & Bishop, 2010), and require more symptom behaviors than the DSM-IV-TR. A recent study of 4,453 children with DSM-IV clinical PDD diagnoses found that most children with DSM-IV PDD diagnoses would remain eligible for an ASD diagnosis under the new DSM-5 criteria. Further, compared with the DSM-IV criteria for Asperger's disorder and PDD-NOS, the DSM-5 ASD criteria have greater specificity (Huerta, Bishop, Duncan, Hus & Lord, 2012). The first DSM-5 field trial found test-retest examinations of the new criteria to be reliable, and also showed that the large majority of children who met DSM-IV criteria for a disorder on the autism spectrum would retain a diagnosis of ASD (Reiger et al., 2012; Narrow et al., 2012; Clarke et al., 2012).

Prevalence and etiology.

Steps towards improvements in diagnostic clarity are crucial as ASD is among the most prevalent and fastest growing developmental disabilities. Prior to the 1980s, estimates of autism included only one in every 2000 children (Fombonne, 2009; Rutter, 2005). In 1994, the DSM-IV estimated autism prevalence rates of 2-5 cases per 10,000 births (APA, 1994). Since then, prevalence rates have increased substantially, with as many as one in 110 children having ASD in 2009 [males: 1:70; females: 1:315](Autism and Developmental Disabilities Monitoring [ADDM], 2009) and one in every 88 children in 2012 [males: 1:54; females: 1:252](ADDM, 2012). Most recent estimates report that one in every 68 children [males: 1:42; females: 1:189] (ADDM, 2014).

Changes in diagnostic criteria, improvements in identification, and increased public awareness have all been suggested as contributing explanations for the dramatic increase in prevalence (Wing & Potter, 2002; Fombonne, 2005). In addtion, younger ages at diagnosis, differential migration, and inclusion of milder cases have all been posited as explanations for the observed increase. While some researchers have reported that changing practices in diagnosis have contributed to an increase in prevalence in certain geographical areas (King & Bearman, 2008), other research continues to suggest that these factors do not fully account for the growing incidence (Rutter, 2005; Shattuck, 2006; Hertz-Picciotto & Delwiche, 2009).

The increased prevalence has also raised major concerns about possible environmental causes. Indirect evidence for an environmental contribution to autism comes from studies demonstrating the sensitivity of the developing brain to toxins such as lead, ethyl alcohol and methyl mercury (Landrigan, 2010). Numberous studies and meta-analyses examining prenatal and neonatal risk factors have cited a broad number of risk factors associated with compromised infant and maternal health may increase susceptibility to ASD. Factors include umbilical-cord complications, fetal distress, prenatal viral exposure, birth injury or trauma, and neonatal anemia, maternal prenatal medication use (e.g., antidepressants), gestational diabetes, and advanced parental age. Still, there is not one clear factor contributing to an ASD diagnosis and studies have yet to find convincing evidence to support these hypotheses (Fallon, 2005; Kawashima et al., 2000; Libbey, Sweeten, McMahon, & Fujinami, 2005; Miller & Reynolds, 2009;

Lundström et al., 2010; Croen, Grether, Yoshida, Odouli, & Hendrick, 2011; Gardener, Spiegelman, & Buka, 2009; 2011).

In fact, researchers have questioned the causal nature of the relation between perinatal complications and the development of ASD. One such hypothesis, referred to as the "shared risk hypothesis," proposes that fetal complications may occur as a result of the development of ASD in the neonate. In other words, perinatal complications are a reflection of the aberrant development of a fetus with ASD. Evidence to support this hypothesis came from an association between suboptimal prenatal, perinatal, and neonatal composite scores and measures of autism severity and familiality. Additionally, probands with increased fetal complications had more family members characterized as broader autism phenotype (mild impairments in ASD domains) (Bolton, Murphy, Macdonald, Whitlock, Pickles, & Rutter, 1997). This question of causal directionality is critical and provides further possibility for a prenatal origin of ASD.

While an environmental cause of ASD remains unclear, other evidence points to a strong, yet complex genetic basis. In part, evidence comes from associations with specific genetic disorders. The genetic condition with the strongest association with ASD is Fragile X syndrome (FXS). FXS is the most common form of inherited intellectual disability and is the most common single-gene expression of autism (Brown et al., 1982; Bailey et al., 1993a; Piven et al., 1991; Belmonte & Bourgeron, 2006). Behavioral symptoms of FXS mirror many of those in ASD including, speech and language delay, tendency to avoid eye contact, hand clapping or biting, and motor delays (Bailey, Hatton,

Mesibov, Ament, & Skinner, 2000; Rogers, Wehner, & Hagerman, 2001; Kaufmann et al., 2004).

Other genetic evidence comes from heritability estimates calculated from twin concordance rates (Folstein & Rutter, 1997a,b; Ritvo, Freeman, Mason-Brothers, Mo, & Ritvo, 1985; Steffenburg et al., 1989; Bailey, Le Couteur, Gottesman, Bolton, & Siminoff, 1995; Gillberg & Coleman, 2000; for review, see Ronald & Hoekstra, 2011, 2014). The first small-scale twin study of autism challenged the idea that autism was caused by poor parenting, or other purely environmental causes, and lead to two very seminal findings (Folstein & Rutter, 1997a,b). First, significant concordance differences were found among monozygotic (36%) and dyzogotic (0%) twin pairs. Second, phenotypic variability within monozygotic pairs suggested a complicated genetic influence and the possibility of a diagnostic spectrum. Subsequent estimates have cited between 50% and 90% concordance among monozygotic twins (Bonora, Lamb, Barnby, Bailey, & Monaco, 2006; Boyle, Van Naarden Braun, & Yeargin-Allsopp, 2005; Hallmayer et al., 2011), making ASD one of the most heritable neuropsychiatric disorders (Bonora et al., 2006). The concordance rates are consistently reported as significantly lower in dizygotic twin pairs, ranging from 0% to 36% (Folstein & Rutter, 1997a,b; Bailey et al., 1995; Boyle et al., 2005; Ritvo et al., 1985; Hallmayer et al., 2011). When compared to recurrence rates among non-twin siblings, a recent study suggested that ASD recurrence rates among non-twin siblings is about 18.7% (Ozonoff et al., 2011) and lower than the reported 31% to 36% found among fraternal twins in the recent Hallmayer et al. (2011) study. The discrepancy among fraternal twin concordance

and sibling recurrence rates suggests that other shared environmental factors may be at play. As suggested by this recent study, and consistent with other neuropathology, the development of ASD is most likely a complex interaction between genetic susceptibility and environmental triggers. Further research into the underlying neurobiology of ASD may contribute to our understanding of this complex etiological interaction.

Neuropathology of ASD

Since the 1970s, research has demonstrated that ASD constitutes a biologically based neurodevelopmental disorder (Rutter, 1970; Rutter, 1979). Currently, a variety of data support the involvement of number of brain regions in the neuropathology of autism, including the limbic system, corpus callosum, basal ganglia, brainstem, and the cerebellum (Williams et al., 1980; Gaffney, Kuperman, Tsai, & Minchin, 1988; Hashimoto et al., 1995; Bailey, Phillips, & Rutter, 1996; Courchesne, 1997; Kemper & Bauman, 1998; Rodier et al., 1998; Baron-Cohen et al., 2000; Folstein & Rowen-Sheidley, 2001; Acosta & Pearl, 2004; Palmen et al., 2004; Allen, 2006; Weigel et al., 2010; Uppal et al., 2014). While specific studies have demonstrated varied and sometimes inconsistent results, it is clear that brain development is abnormal for individuals with ASD. Small sample sizes and the heterogeneity of ASD likely contribute to inconsistencies.

Total cerebral volume.

While further research will help to elucidate the underlying brain differences found in ASD, increased cerebral volume and head size have remained one of the most consistent findings. Structural MRI studies have repeatedly reported a trend for increased

total brain volume in individuals with ASD (Filipek et al., 1992; Aylward et al., 1999, 2002; Haznedar et al., 2000; Piven et al., 1995; Courchesne et al., 1999; Rojas et al., 2002; Sparks et al., 2002; Hardan et al., 2003; Tsatsnanis et al., 2003; Herbert et al., 2003; Kates et al., 2004; for review, see Vaccarino, Grigorenko, Smith & Stevens, 2009; Schumann et al., 2010; for review, see Chen, Jiao, & Herskovits, 2011; Shen et al., 2014).

The first MRI investigation of brain size in toddlers (aged 2-4) with ASD found total cerebral volume (TCV) was 10% greater than normal with 90% of children with volumes greater than average (Courchesne et al., 2001). Numerous subsequent investigations also found this same pattern of overgrowth in children 18 months to 4 years (Carper et al., 2002; Sparks et al., 2002; Sparks et al., 2004; Hazlett et al., 2005; Bloss & Courchesne, 2007; Amaral, Schumann, & Nordahl, 2008; Schumann et al., 2010; Amaral et al., 2011; Hazlett et al., 2011); however, it is less clear whether this abnormal enlargement continues into adolescense and adulthood (Courchesne et al., 2001; Sparks et al., 2002; Amaral, Schumann, & Nordahl, 2008; Aylward et al., 2002). A recent meta-analysis of 586 longitudinal and cross-sectional MRI scans demonstrated early brain overgrowth during infancy and the toddler years in autistic boys and girls, followed by an accelerated rate of decline in size from adolescence to late middle age in (Courchesne et al., 2011). Brain overgrowth has been attributed to increases in both gray and white matter and seems to be concentrated in the frontal, temporal, and cingulate regions of the cerebral cortex (Carper et al., 2002; Courchesne & Pierce, 2005; Schumann et al., 2010). Additionally, a recent investigation of neural connectivity in 2 year olds found significantly decreased local and global efficiency over temporal, parietal and

occipital lobes in high-risk infants later classified as ASD (Lewis et al., 2014). Taken together, this body of research supports the hypothesis that the cortical regions of the brain, which experience a prolonged timeline of neuronal development, may be more adversely affected by growth dysregulation.

In addition, recent prospective studies have investigated structural differences in even younger children. The Infant Brain Imaging Study (IBIS), a longitudinal imaging study of infants at high risk for autism, conducted the first systematic investigation into brain development prior to age 2. This cross-sectional analysis compared brain volumes at 6 months of age in high-risk infants and infants without family members with autism. The authors did not observe significant group differences for head circumference, brain volume, or abnormalities in radiologic findings from the group of infants at high risk for autism. However, the authors are unable to conclude that these abnormalities are not present in infants who later go on to receive a diagnosis of autism. Moreover, a different prospective MRI investigation found the presence of excessive cerebrospinal fluid in the subarachnoid space above the frontal lobes in infants as early as 6 months who later developed ASD (Shen et al., 2014). Further investigations into the earliest fundamental biological underpinnings of autism are critical.

Head circumference.

Given these documented increases in cerebral volume, it is not surprising that increased head circumference (HC) is another well documented biological phenomena in ASD. HC and brain volume increase at similar rates in early childhood (Lainhart et al., 2005) and the correlation between total cerebral volume and HC in young children is

strong (0.88) (Hazlett et al., 2005). While brain growth seems to plateau around the age of 13, HC continues to grow until around the age of 18, resulting in a smaller, but still significant correlation between HC and TCV (0.67) (Piven et al., 1996).

When compared to normative data, enlarged HC occurs at an unusually high frequency among children with ASD and their family members (Dvidovitch et al., 1996; Woodhouse et al., 1996; Lainhart et al., 1997; Stevenson et al., 1997; Fombonne et al., 1999; Fidler et al., 2000). One of the most replicated findings in ASD is the increased prevalence of macrocephaly, defined as head circumference (HC) greater than the 97th percentile for age and sex. While less than 3% of the normal population is expected to have macrocephaly, about 20% of children diagnosed with ASD meet criteria (Lainhart et al., 1997; Fombonne et al., 1999; Miles et al., 2000; Tager-Flusberg & Joseph, 2003). Another study reported that 59% of infants diagnosed with ASD showed a HC increase of 2 standard deviations or more during the first year of life as compared to 6% of normal infants (Courchesne et al., 2003).

Other more recent studies have investigated increased HC in ASD when compared to normative controls. After adjusting for height, weight, and age, head circumference in a sample of males with ASD was significantly increased from 6 to 9 months after birth, reaching a peak at 6 months after birth. No difference was found in the female ASD group (Fukumoto et al., 2011). Consistent with this finding, another study indicated that at 3-5 months and 6-12 months HC was significantly greater in an ASD group compared to typically developing controls, again controlling for height and weight (Muratori et al., 2012).

Current Controversy.

Although, increased HC has historically been considered a consistent physical feature of ASD (Stevenson et al., 1997) and Kanner's original description of the disorder noted a predominance of "large heads" in the children he described (Kanner, 1943), it is important to consider the recent literature that has called to question the impact of different head circumference growth charts on the prevalence of macrocephaly in ASD. In an effort to detect whether several of the reference norms used to define early brain overgrowth in ASD may be biased toward detecting HC overgrowth, Raznahan and colleages (2013) systematically reviewed all published HC studies in children with ASD and found that comparisons with locally recruited control subjects were significantly less likely to identify early brain overgrowth in ASD than norm-based studies. Another recent study tested the hypothesis that the presence of macrocephaly might vary depending on the specific growth chart used. Using the Nellahus, CDC, and recent Rollins et al. (2010) revision head circumference charts, the authors plotted the HC of 253 children with neurodevelopmental disorders (59 with ASD) between 12 to 36 months. The CDC and Rollins et al. head circumference charts identified more cases of macrocephaly and fewer cases of microcephaly than did the older Nellhaus chart but did not significantly differ in their identification of macrocephaly in children with autism (Morhardt, Barrow, Jaworksi, & Accardo, 2013). Another investigation assessed whether HC could be used to screen for ASD in young males with developmental delay. While no differences were found between the group of children with autism and developmental delay compared with the group with developmental delay only, the children with autism were found to have

significantly smaller head circumferences at birth and significantly larger head circumference at 18.5 months of age when the sample was compared with a range of selected Centers for Disease Control normative medians (Gray, Taffe, Sweeney, Forster, & Tonge, 2011). Future research should continue to assess the potential bias of normative HC data.

Clinical Correlates.

A body of ASD research has been conducted to explore the relationship between head size in ASD and other demographic and clinical variables. A multi-site investigation found that while the distribution of HC in ASD is normal in shape, the mean, variance and rate of macrocephaly is increased. Effects of gender, age, SES, and IQ were not present. ASD diagnosis, height, and parental head circumference were all predictive of HC in participants with ASD. In addition, increased HC was associated with more severe scores on the social domain of the Autism Diagnostic Interview-Revised (ADI-R) and with delayed onset of language (Lainhart et al., 2006). Courchesne and colleagues (2003) also found that increased occipitofrontal circumference (OFC) was associated with later onset of first word, more severe stereotyped and repetitive behaviors, and higher likelihood of autism diagnosis, compared to diagnosis of PDD-NOS. In contrast, other studies have found that OFC was associated with less severe core features of ASD (Lainhart et al., 1997) and accelerated head growth was linked to higher levels of adaptive functioning (Dementieva et al., 2005). Finally, some reports indicate that regressive symptomatology is associated with brain overgrowth (Amaral, 2011), while others refute such associations (Muratori et al., 2012). In an attempt to make sense of the

conflicting findings, a group of researchers recently examined data from 7225 individuals from the Simons Simplex Collection cohort. Results indicated gender, height, weight, genetic ancestry, and ASD status were significant predictors of HC. In addition, larger HC was associated with ASD symptom severity and regression (Chaste et al., 2013). Future research should continue to explore the relation between aberrant HC growth and symptom severity.

Early Brain Overgrowth in ASD

In spite of the controversy surrounding the prevalence of macrocephaly in ASD due to potentially biased normative data, the phenomena of early brain overgrowth is supported by a wealth of direct evidence through structural MRI studies. In addition, studies employing HC as a proxy for head size further support early brain overgrowth in ASD, particularly when compared to local typically developing controls. Multiple studies cite early pathological overgrowth in the first postnatal years (Hazlett et al., 2005, Dementieva et al., 2005, Dissanayke et al., 2006, Dawson et al., 2007, Mraz et al., 2007, Webb et al., 2007, Elder et al., 2008; Fukumoto et al., 2008, 2011; Muratori et al., 2012), yet the biological underpinnings and exact timeline of early brain overgrowth remain unclear.

Timing of overgrowth.

A great deal of research has focused on determining when this period of brain overgrowth, and thus increased measures of HC, first occurs. Gillberg and de Souza (2002) reported that for children who were macrocephalic at 16 months, 14 out of 24 children with ASD had been macrocephalic at birth. Previously, Courchesne et al. (2003)

also found smaller than normal HC at birth with rapidly increasing rates at 1-2 months, meeting the 84th percentile by 6-14 months. A meta-analysis of 187 children suggested that the period of pathological growth was restricted to the first year of life (Redcay & Courchesne, 2005), a finding that was later supported by a sample of 28 infants and children with ASD (Dawson et al., 2007). However, another sample of 28 children with ASD found accelerated growth to manifest in the second and third years of life (Dissanayake et al., 2006). When comparing the HC of children with ASD to typically developing children and normative data, initial findings were similar to Courchesne and colleagues (2003), with smaller HC at birth, followed by significantly larger than normal HC at 10-14 months. However, after controlling for height and weight, no significant differences were found in HC among groups (Mraz et al., 2007). Another study did find significant differences in HC during 7 to 10 months, even after controlling for height (Webb et al., 2007). A more recent comparison of groups of children reported difference in HC during a wider postnatal time period encompassing 3 through 12 months (Mratori et al., 2012). Still, the most recent investigation of over 400 children with ASD reports that rates of macrocephaly at birth were significantly higher than children from the control group (Grandgeorge, Lemonnier, & Jallot, 2013).

Given the difference in prevalence rates among males and females with ASD, some investigations have explored the variable of gender. One study compared 85 children with ASD to normative data found no difference in HC at birth, but reported accelerated HC growth in males from 1-6 months of age (Fukumoto et al., 2008). Other studies have found gender differences as well, citing accelerated head growth in a

disproportionate amount of males with ASD (Dementieva et al., 2005). In contrast, another study found that mean birth HC of 8 girls with autism was greater than the comparison mean, while a sample of 37 boys did not differ from the mean. Two out of the 9 children in this study who later identified as macrocephalic were macrocephalic at birth (Lainhart et al., 2006). On the other hand, a recent study reported that children with ASD had smaller HC at birth compared to Centers for Disease Control (CDC) normative data (Gray et al., 2011).

Overall, the body of research surrounding the timeline of early brain overgrowth in ASD seems to indicate that enlarged HC occurs within the first year of life. Whether or not group differences are detectable at birth remains less consistent. While a majority of studies report no significant difference, some indicate decreased HC in ASD, and still others have cited increased HC. Again, discrepancies in findings may be related to small samples sizes and heterogeneity within ASD. Still, Hobbs and colleagues (2007) suggested that among children later diagnosed with ASD, the pooled prevalence of macrocephaly at birth from previous studies was more than double the population rate (6.7% vs. 3%). Further research into early HC growth in ASD is crucial as it provides insight into the underlying neurobiological mechanisms driving the development of ASD.

Mechanisms of overgrowth.

While HC provides an index of underlying brain changes, other techniques such as MRI and postmortem brain tissue analysis provide additional hypotheses into the neurodevelopmental origins of ASD. Neuroanatomical evidence suggests various aberrant processes may be at play in the development of ASD, including increased

neurogenesis (due to altered generation or death of neurons), increased gliogenesis, altered mylenation, inappropriate synaptic pruning, altered cortical connectivity, and altered neuronal signaling (for review, see Vaccarino & Muller Smith, 2009). The hypothesis for a dysregulated increase in the number of neurons is supported by research citing increased total brain volume, high incidence of macrocephaly, and altered trajectory of brain growth in ASD. More specifically, the overgrowth pathology may be a result of overproduction of nerve cells and reduced synaptic pruning during the course of development of the central nervous system.

This theory of overgrowth was originally based on evidence from a series of studies in the early 2000s (Courchesne et al., 2001; Carper et al., 2002; Sparks et al., 2002). A more recent postmortem examination by Courchesne and colleages provides strong preliminary evidence to confirm this hypothesis. An analysis of prefrontal brain tissue in male children aged 2 to 16 years revealed that children with ASD had 67% more neurons in the prefrontal cortex (PFC) when compared to control samples. More specifically, there were 79% more neurons in the dorsolateral PFC and 29% in the mesial PFC. Brain weight was also greater than normative mean weight by 17.6% in the ASD group and 0.2% in the control group. These results provide preliminary confirmation a neural basis for the early overgrowth seen in children with ASD (Courchesne et al., 2011).

Prenatal Development in ASD

It has been hypothesized that early brain overgrowth in the first year, followed by a period of arrested development, may significantly disrupt the development and

organization of the infant brain, which is shaped through interaction with the environment in the first years of life (Courchesne & Pierce, 2005). It is equally important to consider the earlier points of the developmental timeline and hypothesize about what may be causing early brain overgrowth. In order to address this important question, we must look at the evidence for a prenatal origin in the development of ASD.

Perinatal risk factors.

As mentioned earlier, a number of perinatal risk factors have been associated with increased risk of developing ASD. In general, advanced parental age, multiple pregnancies, and low birth weight have all been cited as factors that increase a child's risk of developing ASD (Zhang et al., 2010; Gardener, Spiegelman, & Buka, 2011; Croen et al., 2011). Additionally, there are a number of maternal factors that have been documented as risk factors. These include, but are not limited to, maternal infection during pregnancy, (Shi et al., 2009; Hsiao, McBride, Cho, Mazmanian, & Patterson, 2012), exposure to medications during pregnancy (Gardner et al., 2011), exposure to high levels of air pollution during pregnancy (Volk, Lurmann, Penforld, Hertz-Picciotto, & McConnell, 2013), maternal history of rheumatoid arthritis and celiac disease (Atladóttir et al., 2009), maternal obesity (Krakowiak et al., 2012), and maternal diagnosis of asthma or allergies during the second trimester (Croen, Grether, Yoshida, Odouli, & Van de Water, 2005). Additionally, maternal consumption of folic acid around the time of conception has been associated with decreased risk of the development of ASD (Surén et al., 2013). Taken together, this body of research not only outlines many risk factors that may increase a child's risk of developing ASD, but it also strongly suggests that the

prenatal environment is vulnerable to factors that may influence the development of ASD.

Neurobiological evidence.

Still, the most direct evidence of a prenatal origin for the development of ASD may come from neurobiological studies. As previously mentioned, smaller cerebella and brain stems have been documented in children with ASD compared with control subjects. Additionally, Hashimoto and colleagues (1995) found that the pattern of growth of these structures after birth was consistent with normal, suggesting an early insult and hypoplasia rather than a postnatal growth disturbance. Another critical finding includes an increased number of cortical minicolumns in ASD (Casanova, Buxhoeveden, Switala, & Roy, 2002). The number of minicolumns that organize brain development is determined by the number of founder cells generated early in gestation, which typically occurs before 40 days (Rakic, 1995). Thus, Casanova and colleagues have hypothesized increased minicolumns suggest a fetal onset of brain abnormality in autism (Casanova, Buxhoeveden, & Gomez, 2003; Casanova et al., 2006).

Cerebellar evidence.

Other evidence comes from aberrant cerebellar development, one of the most consistent sites of brain abnormality in ASD. In almost all cases of postmortem investigations of cerebellar development in ASD a reduction in Purkinje cells have been cited (Bailey et al., 1998; Fehlow, Bernstein, Tennstedt, & Walther, 1993; Bauman & Kemper, 2005; Kemper & Bauman, 1998; Lee et al., 2002; Ritvo et al., 1986; Vargas et al., 2005; Wegiel, 2004; Williams, Hauser, Purpura, DeLong, & Swisher, 1980). Initial

descriptions of Purkinje cell (PC) reduction cited a lack of gliosis (Kemper & Bauman, 1998; Ritvo et al., 1986; Williams et al., 1980), empty basket cells (Bailey et al., 1988), or retrograde neuron loss in the inferior olive (Kemper & Bauman, 1998). Gliosis is a reactive cellular process that occurs after insult to the central nervous system, and is a normative process that occurs during the pruning of neuronal connections. Basket cells are neurons whose fibers form a basket-like nest in which a Purkinje cell rests. A decrease in Purkinje cell numbers without the expected gliosis or empty basket cells suggests that these abnormalities were acquired early in development. More specifically, the preservation of olivary neurons in the presence of markedly reduced numbers of Purkinje cells in ASD suggests that the cause of these processes may occur at or before approximately 30 weeks of gestation (Bauman & Kemper, 1994; Kemper, 2010). Similarly, others researchers have suggested a prenatal onset at or before 32 weeks gestation (Bailey et al., 1998).

Further support for a prenatal onset of cerebellar pathology comes from a recent direct examination of in vivo prenatal cerebellar development. Allen and colleagues (manuscript in preparation) conducted a retrospective investigation of prenatal ultrasound records, examining second trimester fetal measurements of the transverse cerebellar diameter (TCD). When compared to a normative sample, the TCD was significantly smaller for children who later developed ASD. This study is consistent with previous evidence for cerebellar hypoplasia in ASD and represents the first direct evidence for anatomical abnormalities the prenatal brain of children later diagnosed with ASD. Given the cerebellum's anatomical connections to prefrontal and parietal cortices (Allen et al.,

2005), early aberrant cerebellar development may likely coincide with dysregulated prenatal development in these regions as well.

Brain overgrowth evidence.

Finally, evidence for prenatal onset of early brain overgrowth comes from the finding of over 65% excess cortical neurons in the prefrontal cortices of children with ASD (Courchesne et al., 2011). In addition to providing a mechanism for the underlying cause of HC overgrowth in ASD, an excess of cortical neurons indicates a timeline of prenatal onset because all cortical neurons are generated prenatally (Samuelsen et al., 2003; Bhardwaj et al., 2006; Larsen et al., 2006; Gohlke, Griffith, & Faustman, 2007; Rabinowicz, de Courten-Myers, Petetot, Xi, & de los Reyes, 1996). Fetal brain development is a powerful process. The vast majority of neurons are created between weeks 10 and 20 of gestation, resulting in a normative overabundance of neurons by as much as 100% (Gohlke, Griffith, & Faustman, 2007). However, during the third trimester of pregnancy and early life of an infant, about half of those neurons are removed in a process referred to as pruning or apoptosis (programmed cell death) (Kanold, 2009). As proposed by Courchesne and colleagues (2011), dysregulation of these key early developmental processes would likely create a pathological overabundance of cortical neurons, and could lead to overall larger head volume.

Prenatal Head Circumference in ASD

Previous studies.

Given the recent evidence that dysregulation of prenatal neurogenesis may be at the root of the abnormal brain overgrowth observed in many children with ASD,

researchers have begun exploring prenatal HC in children later diagnosed with ASD. As mentioned previously, measurements of HC at birth have been mixed, but the pooled prevalence of macrocephaly reported in these studies suggests macrocephaly is twice the population average in children with ASD at birth (Hobbs et al., 2007). Through the use of prenatal ultrasound, two separate research groups have explored mean differences in HC at midgestation in children later diagnosed with ASD and control children (Hobbs et al., 2007; Whitehouse et al., 2011).

The first study of fetal head and body size was a retrospective investigation of children later diagnosed with ASD. Second trimester fetal ultrasounds were collected for 45 children with ASD and compared to 222 control subjects through case-control comparisons obtained from medical records. Diagnostic disconfirmation was not available. Subjects were matched on gestational age, calculated from the standard composite of fetal biometry (Hadlock et al., 1984), and year of ultrasound. Sixty percent of the ASD sample underwent diagnostic confirmation using the gold standard assessments. HC, biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) (see Appendix B) measurements were extracted from ultrasound records and standardized. Results indicated the ASD group did not differ significantly from control subjects on any of the individual standardized fetal measures. Trends toward increased discrepancy between standardized BPD and HC measures suggested a tendency for brain width to grow at an increased rate relative to the brain as a whole. Unique limitations to this study included a relatively small sample size for anthropometric studies and lack of confirmation that control children were indeed typically developing.

A more recent prospective study of fetal brain development collected HC and BPD measurements from over 2,000 children around 18 weeks gestation and shortly after birth. During follow-up visits, parents were asked whether their child had ever received a diagnosis of ASD by a health professional. Out of the original sample, 14 children reported ASD diagnosis and had ultrasound data available. Each child with ASD was matched with four control children according to a number of factors. Other developmental or learning disabilities were ruled out for control subjects. In this case, fetal head measurements were standardized independent of head biometry (e.g., HC and BPD), using only FL reference data (Hadlock et al., 1982, 1984). Birth measurements were standardized using gender-stratified reference data. Independent samples *t*-tests found no difference between cases and controls at second trimester or at birth. Examination of 'difference scores', which quantified head circumference relative to body size, revealed that a small number of children with ASD had a disproportionately large head size at the second trimester (n = 5) and birth (n = 2) assessments, although statistically significant group differences were not reached. Unique limitations to this study included lacking diagnostic confirmation and clinician consistency through the use of gold standard observational tools (i.e. Autism Diagnostic Observation Schedule-Generic; Lord et al., 2000; Lord, Rutter, DiLavore, & Risi, 2008) and a small ASD sample.

Future directions.

Although both studies found no significant mean differences in prenatal head measurements among children with and without ASD, each study offered methodological

limitations and encouraged further investigation into prenatal HC in ASD. One area that remains to be explored is HC in later gestation. Second trimester ultrasound remains the standard of care for fetal anatomical evaluation (Salomon et al., 2010), and thus typically provides the largest sample of ultrasounds in retrospective designs. Given that the normative process of neurogenesis is quite different from second trimester to third trimester, it is critical to look at HC size during the third trimester, as well as the rate of growth from second to third trimester. If an excess of cortical neurons is indeed the underlying mechanism driving the overgrowth seen in many children with ASD, examining the point at which pruning of these neurons is expected to occur may provide the earliest evidence for this growth dysregulation.

Another important addition to this line of investigation may be to expand the ways in which the relationship between HC and ASD is explored. First, both previous studies examining prenatal HC in ASD have compared mean HC between ASD and control groups of children. Although rates of macrocephaly are higher in children with ASD, large HC is not present in every child with ASD. Given the heterogeneity of ASD, and that the etiology remains unknown, comparing group means between ASD and control samples may not be the most appropriate way to initially address the questions surrounding the relationship between prenatal brain development and the diagnosis of ASD. Rather, exploring whether the probability of developing ASD is associated with larger prenatal HC through may be of interest, and may provide greater clinical utility. Second, the recent controversy surrounding the use of national normative growth charts for HC provides an impetus to explore new ways to assess HC size in ASD. Comparisons

to local community controls and analysis of raw measurements may provide sound ways to address these concerns.

Finally, although postnatal HC has been correlated to symptom severity and other diagnostic variables, this connection has yet to be explored using prenatal HC. Although skill acquisition and symptom severity is undoubtedly affected by early developmental factors such as age of diagnosis, early intervention, and early developmental experiences, it is reasonable to predict that symptom severity may be positively correlated to earliest detected thus most influentional differences in brain development. Accordingly, understanding the mechanisms governing such early neuropathology is essential to making gains in the areas of etiology, early detection, and early intervention.

Summary

ASD is a heterogeneous neurodevelopmental disorder that is among the most prevalent and fastest growing developmental disabilities. The etiology of ASD is poorly understood, but research suggests an interaction between environmental and genetic causes. A number of brain regions are involved in the neuropathology of ASD, including a body of evidence that individuals with ASD have an overall abnormally large brain size. These studies have demonstrated a phenomenon of early brain overgrowth in ASD, with the most significant overgrowth of brain size occurring within the first few years of life.

In conjunction with early brain overgrowth, one of the most consistent findings remains abnormal head circumference (HC). It is estimated around 20% of children with ASD meet criteria for macrocephaly, and enlarged HC occurs at an unusually high

frequency among children with ASD as well as their family members. While a body of research has consistently demonstrated this neurobiological phenomena in ASD, the exact timeline, related risk factors, and relation to earlier brain development is not well understood.

A growing body of research points to a prenatal origin in the development of ASD. A number of perinatal influences have been identified as risk factors, suggesting that the prenatal environment is vulnerable to factors that may influence the development of ASD. Other more direct evidence comes from neurobiological observations of aberrant development known to take place during prenatal neurogenesis. Representing the first direct evidence for abnormal prenatal brain development in ASD, a recent study found fetal measurements of the cerebellum were significantly smaller for children who later developed ASD when compared to a normative sample. Finally, another recent critical finding confirmed an excess of cortical neurons in children with ASD. Not only does this provide evidence for the underlying cause of brain overgrowth —due to dysregulation of neurogenesis—but also strongly indicates a prenatal origin.

Few previous studies have examined mean prenatal HC in children diagnosed with ASD. Through the use of prenatal ultrasound, both retrospective and prospective investigations have compared the mean HC of children with ASD to typically developing controls. Although both studies found no significant mean differences in prenatal head measurements among children with and without ASD, the investigations were limited to comparing HC measurements at the second trimester, neglecting later points in gestation when critical regulatory and developmental processes may be going awry. In addtion,

these analyses may be influenced by biased normative data, as has been recently suggested by various research groups. Finally, the connection between prenatal HC and later developmental outcomes has yet to be explored.

The current study sought to expand upon current literature by examining both second and third trimester prenatal HC measurements in children with ASD, as well as the rate of growth from one prenatal timepoint to the next. Additionally, the current study investigated prenatal HC as a potential risk factor for developing ASD, rather than comparing group means. Finally, the current study explored the relation between prenatal HC growth and later symptom severity. Examining HC later in gestation contributes to a more complete understanding of how and when brain growth dysregulation occurs in the development of ASD. Given the hypothesis that the underlying biological cause of brain overgrowth in children with ASD is an excess of neurons, the current study did not expect differences in neuronal growth to be apparent during the second trimester. It was however hypothesized that differences in fetal HC will be detected when dysregulation of neuronal pruning begins in the third trimester, and thus the rate of HC growth from second to third trimester is expected to differ and predict the development of ASD. Finally, a more dysregulated rate of growth (e.g. faster) is expected to predict greater symptom severity at the time of diagnosis.

Appendix B: Fetal Biometry Ultrasound Measurement

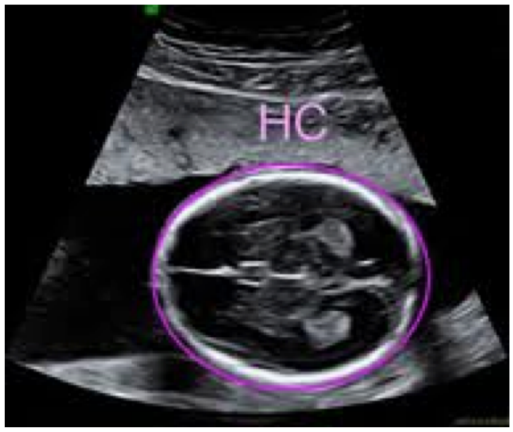

Figure 1. Fetal head circumference

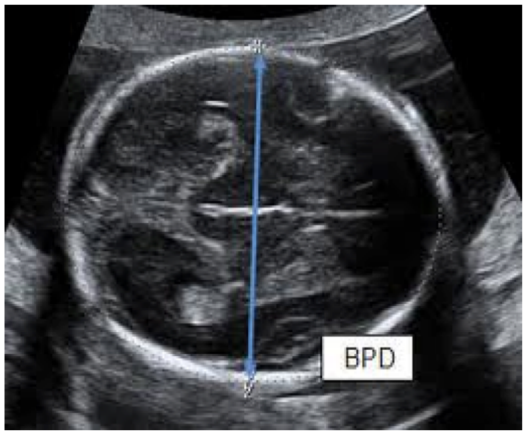

Figure 2. Fetal biparietal diameter

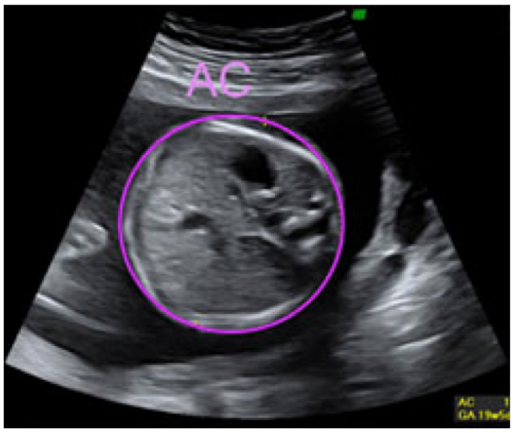

Figure 3. Fetal abdominal circumference

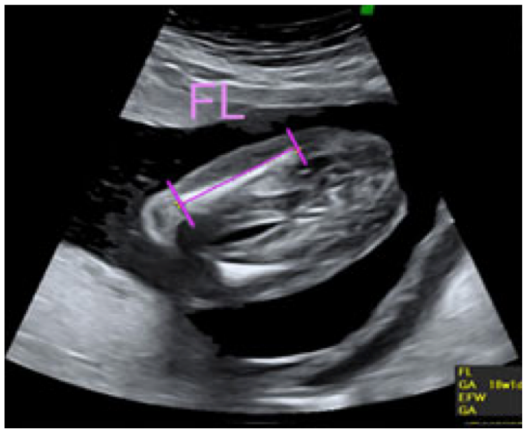

Figure 4. Fetal femur length

Source: From www.fetal.com/Screening/s03StandardExamination.html. Referenced from The American College of Ostebtrics and Gynecology, American Institute of Medicine, and American College of Radiology.

Appendix C: Femur Length Reference Values

TABLE 9. REFERENCE VALUES FOR FEMUR LENGTH

Menstrual age (wk)	Femur length (cm)				
	Percentiles				
	3rd	10th	50th	90th	97th
14.0	1.2	1.3	1.4	1.5	1.6
15.0	1.5	1.6	1.7	1.9	1.9
16.0	1.7	1.8	2.0	2.2	2.3
17.0	2.1	2.2	2.4	2.6	2.7
18.0	2.3	2.5	2.7	2.9	3.1
19.0	2.6	2.7	3.0	3.3	3.4
20.0	2.8	3.0	3.3	3.6	3.8
21.0	3.0	3.2	3.5	3.8	4.0
22.0	3.3	3.5	3.8	4.1	4.3
23.0	3.5	3.7	4.1	4.5	4.7
24.0	3.8	4.0	4.4	4.8	5.0
25.0	4.0	4.2	4.6	5.0	5.2
26.0	4.2	4.5	4.9	5.3	5.6
27.0	4.4	4.6	5.1	5.6	5.8
28.0	4.6	4.9	5.4	5.9	6.2
29.0	4.8	5.1	5.6	6.1	6.4
30.0	5.0	5.3	5.8	6.3	6.6
31.0	5.2	5.5	6.0	6.5	6.8
32.0	5.3	5.6	6.2	6.8	7.1
33.0	5.5	5.8	6.4	7.0	7.3
34.0	5.7	6.0	6.6	7.2	7.5
35.0	5.9	6.2	6.8	7.4	7.8
36.0	6.0	6.4	7.0	7.6	8.0
37.0	6.2	6.6	7.2	7.9	8.2
38.0	6.4	6.7	7.4	8.1	8.4
39.0	6.5	6.8	7.5	8.2	8.6
40.0	6.6	7.0	7.7	8.4	8.8

Adapted from Hadlock FP, Deter RL, Harrist RB, Park SK. Estimating fetal age: computer-assisted analysis of multiple fetal growth parameters. *Radiology* 1984;152:497–501.

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