RESEARCH ARTICLE

A Prospective Study of Fetal Head Growth, Autistic Traits and Autism Spectrum Disorder

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Altered trajectories of brain growth are often reported in Autism Spectrum Disorder (ASD), particularly during the first year of life. However, less is known about prenatal head growth trajectories, and no study has examined the relation with postnatal autistic symptom severity. The current study prospectively examined the association between fetal head growth and the spectrum of autistic symptom severity in two large population-based cohorts, including a sample of individuals with clinically diagnosed ASD. This study included 3,820 children from two longitudinal prenatal cohorts in The Netherlands and Australia, comprising 60 individuals with a confirmed diagnosis of ASD. Latent growth curve models were used to examine the relationship between fetal head circumference measured at three different time points and autistic traits measured in postnatal life using either the Social Responsiveness Scale or the Autism-Spectrum Quotient. While lower initial prenatal HC was weakly associated with increasing autistic traits in the Dutch cohort, this relationship was not observed in the Australian cohort, nor when the two cohorts were analysed together. No differences in prenatal head growth were found between individuals with ASD and controls. This large population-based study identified no consistent association across two cohorts between prenatal head growth and postnatal autistic traits. Our mixed findings suggest that further research in this area is needed. *Autism Res* 2018, **11: 602–612**. © 2018 The Authors Autism Research published by International Society for Autism Research and Wiley Periodicals, Inc.

Lay Summary: It is not known whether different patterns of postnatal brain growth in Autism Spectrum Disorder (ASD) also occurs prenatally. We examined fetal head growth and autistic symptoms in two large groups from The Netherlands and Australia. Lower initial prenatal head circumference was associated with autistic traits in the Dutch, but not the Australian, group. No differences in head growth were found in individuals with ASD and controls when the data was combined. Our mixed findings suggest that more research in this area is needed.

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by difficulties in social communication and restrictive and repetitive behaviors. A large body of research has investigated a wide range of structural and functional brain abnormalities that may be associated with ASD [Minshew & Williams, 2007]. Studies of brain growth in children with ASD have reported early brain overgrowth during the first year of life, measured through changes in head circumference (HC) [Constantino et al., 2009; Courchesne et al., 2001; Green, Dissanayake, & Loesch, 2015; Hazlett et al., 2005; Piven, Arndt, Bailey, & Andreasen, 1996] and

changes in brain growth using imaging methods [Hazlett et al., 2017; Shen et al., 2017]. More recently, the existence and exact timing of early brain growth abnormalities have been challenged, due to several methodological limitations in the field, including the use of retrospective historic references and ascertainment bias [Raznahan et al., 2013]. Despite these methodological issues, there remains considerable interest in early brain growth in children diagnosed with ASD. In particular, there has been very little prospective data available to examine whether postnatal head overgrowth in the first year of life is the first manifestation of altered neurodevelopment, or whether differences in brain development are apparent earlier in life before overt behavioral symptoms are apparent.

It is possible that postnatal overgrowth may reflect a reaction or adaptation to earlier head growth patterns during prenatal life [Hobbs et al., 2007]. However, studies of HC at birth, as a proxy for brain volume [Cooke, Lucas, Yudkin, & Pryse-Davies, 1977], have been inconsistent. Although a few studies have reported an association between smaller HC at birth in individuals later diagnosed with ASD [Courchesne, Carper, & Akshoomoff, 2003; Courchesne et al., 2007; Mraz, Green, Dumont-Mathieu, Makin, & Fein, 2007], the majority of studies have shown no association between HC at birth for individuals with ASD when compared to a sample without ASD [Dissanayake, Bui, Huggins, & Loesch, 2006; Sacco et al., 2007]. There have been even fewer studies examining head growth trajectories in prenatal life in children subsequently diagnosed with ASD. Two earlier studies identified no group-level differences in second trimester prenatal HC between children later diagnosed with ASD and controls [Hobbs et al., 2007; Whitehouse, Hickey, Stanley, et al., 2011]. The only study of younger siblings of children with confirmed ASD, who are themselves at increased likelihood for later diagnosis of ASD [Ozonoff et al., 2011], revealed no differences in fetal HC compared to children without older siblings with ASD [Unwin et al., 2016]. However, it is these inconsistencies could be due to the use of data from retrospective ultrasounds or the lack of repeated measures of prenatal HC to determine differences in brain growth.

While most studies approach early brain or head growth differences using a binary approach of ASD versus typically developing children, an additional approach to studying the neurobiology of ASD involves the conceptualization of ASD as being on the extreme end of a severity continuum that extends into the general population [Constantino, 2011]. To our knowledge, there have been no studies that focus on prenatal head growth related to ASD on a trait-level. Identifying the earliest manifestations of neurobiological changes in trait-level ASD may help to identify neurodevelopmental mechanisms that underlie the disorder. The aim of this study was to measure the relationships between prenatal head circumference growth trajectories and autistic symptoms in two large general population samples. Secondly, we examined prenatal HC growth in a subset of 60 children within the broader cohorts who had a confirmed clinical diagnosis of ASD and compared this to HC growth amongst individuals who did not meet criteria for ASD.

This is the first large population-based study to assess trajectories of prenatal HC growth in ASD. We have combined data from two large population-based longitudinal pregnancy cohorts with repeated prenatal ultrasound assessments for measures of HC. This enabled us to reduce recruitment bias and bias associated with comparisons to standardized growth curve data [Raznahan et al., 2013]. We first hypothesized that alterations of fetal HC growth patterns would be related to later autistic traits in the general cohort. Second, we hypothesized that any atypical pattern of prenatal HC growth linked to autistic traits would be more pronounced in individuals who later met diagnostic criteria for ASD, consistent with a continuum in the neurobiology of ASD.

Methods
Participants

Generation R Cohort. The Generation R Study is a population-based cohort from the Netherlands of 8,879 mothers recruited prior to 18 weeks gestation between April 2002 and January 2006 [Jaddoe et al., 2012; Verburg et al., 2008]. The current study sample is a selected group of 2,950 Caucasian children for whom information on autistic traits and at least one measure of prenatal HC was available. The Medical Ethics Committee of the Erasmus MC approved the study and written informed consent was obtained from the mothers.

Raine Cohort. The Western Australian Pregnancy Cohort (Raine) Study is a longitudinal cohort of women recruited prior to 18 weeks gestation from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics, between May 1989 and November 1991 [Newnham, Evans, Michael, Stanley, & Landau, 1993]. To these women, 2,868 children were live born and available for postnatal follow-up. For 870 Caucasian children, information on autistic traits at age 20 was available, as well as at least one prenatal HC measurement. Participant recruitment from the study families was approved by the Human Ethics Committee at King Edward Memorial Hospital. Ethical approval for the 20-year follow-up was received from the Human Research Ethics Committee at the University of Western Australia. Participants provided written informed consent for data collection on autistic traits at approximately 20 years of age.

Prenatal Head Size and Growth

Generation R. Fetal ultrasound measurements were performed during the first (mean age 13.5 weeks), second (mean age 20.6 weeks), and third trimester (mean age 30.5 weeks) of pregnancy [Jaddoe et al., 2012; Verburg et al., 2008]. Crown-to-rump length was used for pregnancy dating until a gestational age of 12 weeks and 5 days, and biparietal diameter for pregnancy dating thereafter. HC was measured in a transverse section of the head by drawing an ellipse around the outline of the skull [Verburg et al., 2008]. Up to three HC measurements were available. Ultrasound examinations were performed using an Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA). Most ultrasounds were conducted in a research setting at a regional health facility, with the remaining examinations conducted in hospitals under guidance by Generation R staff. Frequent quality checks were conducted to ensure accuracy of fetal biometry. Fetal biometry in early pregnancy may be difficult; however, the intra- and inter-observer reliabilities of fetal biometry measurements during early pregnancy in a sample of 21 pregnancies at 9–14 weeks were excellent (all intra-class correlation coefficients greater than 0.99) [Verburg et al., 2008].

Raine. All pregnant women enrolled in the Raine Study underwent fetal ultrasound measurement at or close to 18 weeks gestation. As part of a previously described trial examining the use of multiple ultrasound scans during pregnancy [Newnham et al., 1993], women were randomly allocated to an intensive ultrasound protocol (including ultrasounds at approximately 18 weeks, and then at 24, 28, 34, and 38 weeks), or a less intensive protocol, with one ultrasound at 18 weeks [Stoch et al., 2012]. Gestational age was calculated from the date of the last menstrual period and confirmed via ultrasound biometrics at 18 weeks. Ultrasound examinations were performed with one of two General Electric 3600 machines (Milwaukee, USA) and conducted by a qualified sonographer at King Edward Memorial Hospital in Perth. Fetal head circumference was taken as the maximal biparietal head circumference. Intra- and inter-observer reliabilities of fetal biometry were not available for this study.

Autistic Traits

Generation R. At 6 years of age, mothers completed the Social Responsiveness Scale (SRS), which is a questionnaire of autistic traits for children between 4 and 18 years of age [Constantino, Przybeck, Friesen, & Todd, 2000; Constantino & Gruber, 2005]. It represents the parent's observation of the child's social behavior during the previous six months. Each item is scored from 0 ("never true") to 3 ("almost always true"). Higher total scores indicate more autistic traits. The data collected within the Generation R Study included an abbreviated version of the SRS with a total of 18 items [Román et al., 2013] to reduce participant burden. The correlation between total scores derived by the SRS short-form and the complete SRS in the Missouri Twin Study [Constantino & Todd, 2003] was 0.93 in monozygotic male twins ($n = 98$) and 0.94 in dizygotic male twins ($n = 134$).

Raine. At the 20-year follow-up, Raine Study participants were asked to complete the Autism Spectrum Quotient (AQ). The AQ is a self-report questionnaire that provides a quantitative measure of autistic traits in the general population [Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001]. Subjects were provided with 50 statements and asked to indicate on a 4-point scale how well each statement applies to them (strongly agree, agree, disagree, strongly disagree), and scores are coded such that higher total scores indicate a greater level of autistic-like traits [Baron-Cohen et al., 2001]. The total AQ is known to have good test–retest reliability ($r = 0.7$), and validation studies have found that scores in the general population follow a normal distribution [Baron-Cohen et al., 2001; Ruzich et al., 2015; Whitehouse, Hickey, & Ronald, 2011].

For both the SRS and the AQ, item scores were summed to obtain total scores, where a maximum of 25% missing items were allowed and scores were weighted for the number of items completed, square-root transformed to approach normality, and z-scored to facilitate comparison between cohorts.

Clinical ASD Diagnoses

Generation R. In the Generation R Study, medical records were examined for children who scored screen-positive for ASD in one or more of several stages of a multifaceted screening procedure. If a potential diagnosis of ASD could be confirmed through the medical records, the child was considered a clinically confirmed case of ASD. In the Netherlands, the general practitioners hold the central medical records, including information on treatment by medical specialists. A diagnosis of ASD is generally based on clinical consensus by a specialized multidisciplinary team. The diagnostic workup typically involves an extensive developmental case history obtained from parents, as well as school information and repeated observations of the child.

To confirm general practitioners' records, we selected those children for which one of three sources of information signaled possible ASD. All children were formally screened with the SRS. The authors of the scale

recommend cutoffs for screening in population-based settings, consistent with short-form SRS weighted scores of 1.078 for boys and 1.000 for girls [Constantino & Todd, 2003]. In addition, to rule out false negatives, children who scored in the top 15% on the total score of the Child Behavior Checklist (1.5–5) underwent a more specific screening using the Social Communication Questionnaire (SCQ), a 40-item parent-reported screening instrument for ASD [Berument, Rutter, Lord, Pickles, & Bailey, 1999]. Scores of 15 or above on the SCQ were considered screen-positive [Berument et al., 1999]. Further, psychiatric diagnoses and treatment were routinely assessed at contact moments between ages 6 and 9 (center visits and questionnaires). Within the Generation R study, 86 children who were screen-positive, but for whom a diagnosis could not be confirmed were excluded from the control group. In the final sample, there were 53 children with medical-record confirmed ASD and at least one ultrasound. Children with SRS data and at least one ultrasound ($n = 2,887$) were considered controls.

Raine. At the 5-, 8-, 10-, 13-, and 16-year follow-ups of the Raine Study, parents were asked whether their child had ever received a diagnosis of ASD by a health professional.

Diagnosis of ASD in Western Australia mandates consensus by a multidisciplinary team comprising a pediatrician, psychologist and speech-language pathologist under DSM guidelines [APA, 2000]. Parent report indicated that 16 children in the Raine cohort had received a diagnosis of ASD. At least one ultrasound was available for 10 Caucasian children. All other Caucasian children with AQ data and at least one ultrasound ($n = 865$) were considered controls.

Covariates

Covariates were carefully chosen based on factors known to influence prenatal head growth and ASD. In both studies, information on maternal age at the time of recruitment in early pregnancy, education, prenatal smoking and alcohol use was obtained by self-report questionnaires. Analyses involving HC at birth were additionally adjusted for mode of delivery.

In the Generation R Study, maternal educational level was categorized in three levels: primary, secondary, and higher education [Netherlands, 2003]. Prenatal smoking and alcohol use were categorized into 'No', 'Until pregnancy was known' and 'Continued during pregnancy', based on the information of repeated questionnaires [Roza et al., 2007].

In the Raine Study, maternal educational level was categorized in six levels: none, trade certificate, professional registration, college diploma, university degree or other.

Information on maternal alcohol use during pregnancy was categorized in zero, once a week or less and several times a week. Maternal smoking was categorized as zero, 1–10 cigarettes or more than 11 cigarettes per day.

In both studies, mode of delivery was categorized as spontaneous vaginal delivery, instrumental vaginal delivery, planned Cesarean section or emergency Cesarean section.

Data processing. HC measurements were transformed to gestational-age adjusted SD scores. In both studies, we used study-specific reference curves for this purpose [Verburg et al., 2008; White et al., 2016].

Statistical Analysis

Prenatal head circumference growth. We assessed the relation between repeatedly measured fetal HC and autistic traits using latent growth curve modeling. Latent growth curve models consider change over time through underlying latent growth parameters (e.g., intercept and linear slope), and capture individual variations around these growth parameters as random effects. Within this modeling framework, trajectories of growth can be related to an outcome of interest. In these analyses, all participants with a valid measurement of autistic traits and at least one HC measurement were included. Participants in the Generation R study had a maximum of three HC measurements, whereas the Raine study had up to 13 measurements. We used up to three measurements for the Raine Study for consistency and to avoid confounding by indication introduced by extra measurements. Where we had additional measurements, we chose measurements that were closest to the Generation R trimester-mean in terms of gestational age across early, mid, and late gestation (*Generation R*: mean [SD] gestational age for ultrasound measurements in weeks: early=13.31 [1.67], mid=20.63 [1.09], late=30.49 [1.01]; *Raine*: early=19.89 [2.16], mid=25.18 [3.34], late=30.07 [2.52]). From the repeated measures of SD scores of fetal HC, two components of growth were estimated (an intercept, which indicates initial level and a linear slope, which denotes the growth). The time intervals between repeated ultrasound measurements were fixed at group level for each of the cohorts. Latent growth curve analyses were performed with Mplus version 7.31 for the latent growth curve analyses [Muthén & Muthén, 2012]. To account for the skewness of the included variables, a maximum likelihood estimator with robust standard errors was used. We used full information maximum likelihood estimation in Mplus, which accommodates missing values in the analyses. We determined model fit using the comparative fit index and root mean square error of approximation, using recommended cutoffs for interpretation of goodness of fit [Bentler & Bonett, 1980].

Table 1. Characteristics of Children and Their Mothers Included in the Current Study

Generation R (n = 2950)		Raine (n = 870)	
Child characteristics			
Sex (% male)	50.4	Sex (% male)	51.6
N prenatal ultrasounds available (%)		N prenatal ultrasounds available (%)	
one	3.0	one	42.4
two	29.5	two	4.5
three	67.5	three	53.1
Autistic traits		Autistic traits	
Age at measurement, M years (SD)	6.13 (0.45)	Age at measurement, M years (SD)	19.70 (0.71)
Weighted total score, M (SD)	0.20 (0.23)	Weighted total score, M (SD)	0.43 (0.09)
Maternal characteristics			
Age at birth of child, M years (SD)	31.7 (4.3)	Age at birth of child, M years (SD)	28.9 (5.7)
Education level (%)		Education level (%)	
High	69	None	42.9
Medium	24.8	Trade certificate	7.4
Low	6.2	Professional registration	12.8
		College diploma/degree	18.6
		University degree	13.0
		other	5.4
Alcohol consumption in pregnancy (%)		Alcohol consumption in pregnancy (%)	
Never	29.6	Zero	55.9
Until pregnancy was known	15.7	Once a week or less	38.9
Continued occasionally	41.9	Several times a week or more	5.2
Continued frequently	12.8		
Smoking during pregnancy (%)		Smoking during pregnancy (%)	
Never	79.2	Zero	79.6
Until pregnancy was known	7.6	1–10 cigarettes	10.3
Continued	13.2	11+ cigarettes	10.2

In addition to predicting autistic traits, we also examined whether the growth components predicted a diagnosis of ASD. For this analysis, intercept and slope were related to the dichotomous outcome ASD (yes/no).

Head circumference. We tested whether HC at any of three time points in pregnancy (early, mid, or late pregnancy) was associated with later autistic traits. For each time point in pregnancy, we assessed the relation between HC and the continuous measure of autistic traits, using linear regression with autistic traits as the dependent variable. Models were adjusted for sex, maternal educational level, maternal age, maternal alcohol use, and maternal smoking during pregnancy. Analyses involving HC at birth were additionally adjusted for mode of delivery.

In addition to predicting autistic traits, we also examined whether the growth components predicted a diagnosis of ASD. For this analysis, intercept and slope were related to the dichotomous outcome ASD (yes/no) in a logistical regression model. In both the trait-based analyses and the analyses with a dichotomous outcome of ASD, we used group or level of autistic traits as the dependent variable to preserve the chronology of the measurements and to test whether we could predict a postnatal diagnosis of ASD or a level of autistic traits from prenatal growth measurements.

Similarly, we tested if there were differences in HC in individuals later diagnosed with ASD compared to those with no diagnosis. This was tested using logistic regression models for each time point in pregnancy, with ASD (yes/no) as the dichotomous outcome. Covariates in each of these analyses were as described above. Missing values of covariates were imputed using multiple imputation, with 10 imputed datasets. The analyses and imputations were performed using IBM SPSS statistics version 21 [2012].

Pooled estimates. Analyses were conducted separately for each cohort. Subsequently, a pooled estimate was obtained, taking into account sample size and direction of effect, using Metal [Willer, Li, & Abecasis, 2010].

Results
Demographics

Table 1 presents characteristics of the study samples from both cohorts used for the main analyses. Of 53 children diagnosed with ASD in the Generation R study, 47 were male and 6 were female. For 45 of these children, an SRS score was known and the mean weighted score of this group was 0.96 (SD = 0.68). Of 10 children diagnosed with ASD in the Raine study,

Table 2. Statistical Models Examining the Relationship Between Autistic Traits in Two Population-Based Samples and (A) Prenatal Head Circumference, and (B) Cross-Sectional Measure of Prenatal Head Circumference

	Generation R (SRS) $n = 2,950$	Raine (AQ) $n = 870$	Pooled z-statistic	P
A. Head growth[a]				
Intercept (SE); P	−0.060 (0.030); .05	0.055 (0.041); .18	−1.08	.28
Slope (SE); P	−0.042 (0.028); .13	0.003 (0.094); .98	−1.39	.16
B. Head size[b]				
Early pregnancy	$n = 2,084$	$n = 870$		
B (SE)	−0.027 (0.019)	0.052 (0.042)		
P	.15	.21	−0.53	.60
Mid pregnancy	$n = 2,843$	$n = 501$		
B (SE)	−0.026 (0.017)	0.019 (0.046)		
P	.14	.67	−1.20	.23
Late pregnancy	$n = 2,882$	$n = 462$		
B (SE)	−0.041 (0.018)	0.001 (0.042)		
P	.02	.98	−2.15	.03

[a] Values are standardized regression coefficients from latent growth curve analyses on gestational age adjusted SD scores for head circumference (constructed using growth reference curves). Models were adjusted for age, sex, maternal educational level, maternal age, maternal alcohol and maternal smoking. Fit indices for the Generation R model were RMSEA 0.024 and CFI 0.983. Fit indices for the Raine Study model were RMSEA: 0.019 and CFI 0.992.

[b] Values are regression coefficients from linear regression analysis of autistic traits on gestational age adjusted SD scores for head size. Models were adjusted for maternal educational level, maternal age, maternal alcohol and maternal smoking.

8 were male and 2 were female. For 5 of them, an AQ score was known and the mean weighted score of the group was 0.33 (SD = 0.07).

Prenatal Head Circumference Growth and Autistic Traits

We examined whether fetal HC growth trajectories were related to the severity of later autistic traits (Table 2A). This was addressed in a separate model for each of the cohorts, in which autistic trait scores were regressed on the latent growth parameters of gestational-age-adjusted SD scores of HC. Models were adjusted for sex, maternal educational level, maternal age, maternal alcohol use, and maternal smoking during pregnancy. These models had good fit indices: comparative fit index = 0.983 and root mean square error of approximation = 0.024 for the Generation R Study and comparative fit index = 0.992 and root mean square error of approximation = 0.019 for the Raine Study.

For the Generation R Study, there was a trend-level negative association between the intercept HC growth during pregnancy and autistic traits: children with growth trajectories characterized by a lower intercept had more autistic traits at age 6 (β intercept = −0.060, P = .05). For the slope, there was no association (β slope = −0.042, P = .13). For the Raine Study, there was no association between these growth parameters and later autistic traits. When the results for both cohorts were pooled, we found no association of the intercept or the slope of growth (i.e., growth rate) with autistic traits (intercept: pooled $z = -1.08$, P = .28; slope pooled $z = -1.39$, P = .16).

For the Generation R Study, lower HC in late pregnancy was significantly related to higher autistic traits (Table 2B). In early and mid-pregnancy, this relationship did not reach statistical significance, although effect estimates were consistently negative. Scatterplots of the unadjusted association between autistic traits and HC in each cohort, by stage of pregnancy, can be found in the supplementary material. Pooled results indicate that children with smaller HC in late pregnancy had higher levels of autistic traits later in life (pooled $z = -2.15$, P = .03).

Prenatal Head Circumference Growth in Children with ASD

Evaluating prenatal HC growth trajectories of children who were later diagnosed with ASD, we found no association between prenatal HC growth and a diagnosis of ASD (Table 3A) (Intercept: pooled $z = -0.25$, P = .81, Slope: pooled $z = -0.33$, P = .74). Similarly, there was no association between HC at any of three time points in pregnancy and an ASD diagnosis (Table 3B) (all P-values > .05).

Birth Head Circumference and Autistic Traits

We found no association between birth head circumference and autistic traits (Generation R Study: $B = -0.017$, P = .41; Raine Study: $B = -0.006$, P = .80).

Birth Head Circumference in Individuals with ASD

There was no association between birth head circumference and later diagnosis of ASD in both the Generation

Table 3. Statistical Models Examining the Relationship Between Clinically Diagnosed ASD in Two Population-Based Samples and (A) Prenatal Head Circumference, and (b) Cross-Sectional Measure of Prenatal Head Circumference

	Generation R	Raine	Pooled z-statistic	P
A. Head growth[a]				
n ASD/n controls	53/2,879	10/865		
Intercept OR (95% CI)	0.92 (0.54; 1.43)	1.11 (0.36; 3.35)		
P	.75	.86	−0.25	.81
Slope OR (95% CI)	0.95 (0.54; 1.43)	0.42 (0.05; 3.12)		
P	.76	.39	−0.33	.74
B. Head size[b]				
Early pregnancy				
n ASD/n controls	39/2,029	10/865		
OR (95% CI)	0.86 (0.64; 1.17)	1.42 (0.65; 3.08)		
P	.34	.38	−0.45	.65
Mid pregnancy				
n ASD/n controls	51/2,768	8/496		
OR (95% CI)	1.05; (0.80;1.39)	1.00 (0.47; 2.14)		
P	.73	.99	0.33	.75
Late pregnancy				
n ASD/n controls	51/2,807	7/458		
OR (95% CI)	0.93 (0.70; 1.23)	0.85 (0.41; 1.78)		
P	.59	.67	−0.65	.51

[a] Values are standardized Odds ratios from latent growth curve analyses of ASD on gestational age adjusted SD scores for head circumference. Regression coefficients and Odd's ratios are standardized for slope and intercept. Models were adjusted for sex, maternal educational level, maternal age, maternal alcohol and maternal smoking.
[b] Values are Odds ratios from logistic regression analysis of ASD on gestational age adjusted SD scores for head size. Models were adjusted for sex, maternal educational level, maternal age, maternal alcohol and maternal smoking.

R Study (OR = 1.01, P = .98) and the Raine Study (OR = 1.03, P = .88).

Discussion

In a study based on two large population-based pregnancy cohorts, we found no relation between prenatal head growth and later autistic traits or a diagnosis of ASD. While we found a weak association between smaller fetal HC and greater levels of autistic traits postnatally in the Dutch cohort, these findings were not replicated in the Australian cohort nor in a subset of individuals with a clinical diagnosis of ASD. In addition, we found no association between HC at birth and later autistic traits or ASD in either cohort.

While there was no relationship between prenatal head growth and postnatal autistic traits that was consistent across cohorts, the trend-level association between lower intercept of prenatal HC growth in individuals with high levels of autistic traits in the Generation R cohort warrants discussion. We found a trend-level association between the intercept of growth and later autistic traits in the Generation R study. Since all human beings begin life as a single cell, this finding implicates decreased early growth rate, prior to the first ultrasound measurement, which remains constant over subsequent ultrasound measurements. This result is largely consistent with the results in the cross-sectional analyses, where we found negative effect estimators for the relation between HC and autistic traits at each of the three separate time points in the Generation R study, although the relation did not always reach statistical significance.

Several biological mechanisms could potentially underlie an association between prenatal HC growth and autistic traits. A recent study of postmortem neural tissue of children with ASD identified local disorganization across different cortical layers suggestive of aberrant prenatal neuronal migration [Stoner et al., 2014]. Environmental factors, such as maternal thyroid dysfunction or vitamin D deficiency may lead to autism-like symptoms through restriction of prenatal brain growth [Korevaar et al., 2016; Román et al., 2013; Vinkhuyzen et al., 2016; Whitehouse et al., 2013]. Alternatively, autistic traits and prenatal HC growth may share a similar genetic background. Interestingly, evidence from histopathological studies demonstrates high expression of ASD candidate genes in the fetal cortex, although the role of these genes in prenatal brain growth is unknown [Birnbaum, Jaffe, Hyde, Kleinman, & Weinberger, 2014; Willsey et al., 2013]. Future studies should clarify whether this trend-level association suggesting a relation between smaller fetal HC and autistic traits is indicative of a neurobiological pathway of ASD, or may reflect features not necessarily specific to ASD.

Despite this suggestive finding of lower initial level of HC growth prenatally and increased autistic traits

postnatally in the Generation R cohort, we did not detect differences in individuals with ASD in either cohort. There are several potential explanations for this. While this was the largest study to date on prenatal HC growth in clinically confirmed ASD, it is likely still underpowered to identify subtle effects [Raznahan et al., 2013]. Furthermore, the etiological pathways contributing to ASD are known to be heterogeneous, and only a subset of which may involve altered brain growth [Betancur, 2011; Campbell, Chang, & Chawarska, 2014]. Different variants may operate through distinct pathways, so that the relation between brain growth and ASD symptoms may depend on the specific genetic background of an individual. For example, in a study of postnatal head growth, a positive association of autistic symptoms with HC was found only in children with ASD classified as simplex and not in children with ASD from multiplex families [Davis, Keeney, Sikela, & Hepburn, 2013]. It is possible that the ASD group includes a heterogeneous mixture of effects in both directions. Postnatally, both microcephaly and macrocephaly have been reported in so-called 'syndromic' ASD [Fombonne, Rogé, Claverie, Courty, & Frémolle, 1999]. Alternatively, it is possible that ASD is not characterized by gross differences in prenatal HC growth. This is consistent with several smaller studies that did not identify differences between ASD and typically developing groups in prenatal HC growth and at birth [Courchesne et al., 2007; Hobbs et al., 2007; Whitehouse, Hickey, Stanley, et al., 2011]. While dysregulation of brain development in ASD is undisputed, any prenatal head growth differences present in ASD may be more regional and thus not translate into global HC differences prenatally or at birth. While ultrasound measures provide reliable measures of HC, which is an excellent proxy for fetal brain size [Cooke et al., 1977], more advanced methods, such as 3D ultrasounds, may provide more information about prenatal growth of specific structures in the brain. Our ultrasound measures consisted of an oval placed on one axial slice through the head, which is less sensitive than the current 3D ultrasound approaches. Interestingly, we did find differences in the Generation R Study with the third ultrasound measure, which in light of the considerable head growth during the third trimester, is the most accurate measurement.

The current study has several strengths, including the involvement of two large, pregnancy-cohort studies that enabled the largest investigation to date of prenatal HC growth trajectories and autistic symptoms in postnatal life. In both cohorts, systematic and prospective data collection began during fetal life, in which standardized research-based approaches to ultrasound measurements were collected at multiple time points during pregnancy [Verburg et al., 2008]. We were able to study HC growth trajectories prospectively, beginning in fetal life and in the context of appropriate controls, thus avoiding the bias introduced by using published normative data [Raznahan et al., 2013]. Further, we combined results obtained in these two independent cohort studies using a meta-analytic approach, which builds a form of replication into the study design. Finally, the large sample size with repeated measurements allowed us to model growth curves with greater precision and to adjust for potential confounding variables.

We also acknowledge several limitations of the study design. In both studies, the first ultrasound measurement was used for dating of the pregnancy in addition to the last menstrual period, which potentially masks very early growth differences related to ASD. Another potential limitation concerns the different techniques for measuring autistic traits across the two cohorts. While the two checklists (AQ and SRS) assessing autistic traits were designed to measure similar constructs, the items differ between questionnaires, and in the current study age of administration and the informant differed between the Generation R (parent report of child in middle childhood) and Raine (self-report during early adulthood) studies. While these differences between cohorts potentially complicates direct comparison, we note that the AQ and SRS have good convergent validity [Armstrong & Iarocci, 2013] and that autistic traits have been found to be relatively stable across development [Robinson et al., 2011; Whitehouse et al., 2011]. In addition, there were some differences in the procedures to identify ASD cases between the two cohorts and it is possible that some cases were missed.

In conclusion, the current study found no clear evidence of an association between prenatal HC growth trajectories and autistic symptom severity (nor clinical ASD) in two independent, prospective, longitudinal pregnancy-cohort studies. Despite these null findings, the recent neuroimaging evidence of hyperexpansion of the cortical surface area commencing at 6-month-old in infants later diagnosed with ASD [Hazlett et al., 2017] suggests that this remains a potentially important area of investigation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1a. Association between SRS and head circumference in early pregnancy in the Generation R study.

Figure S1b. Association between SRS and head circumference in mid pregnancy in the Generation R study.

Figure S1c. Association between SRS and head circumference in late pregnancy in the Generation R study.

Figure S2a. Association between AQ and head circumference in early pregnancy in the Raine study.

Figure S2b. Association between AQ and head circumference in mid pregnancy in the Raine study.

Figure S2c. Association between AQ and head circumference in late pregnancy in the Raine study.