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Association Between Abnormal Fetal Head Growth and Autism Spectrum Disorder

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Objective: Despite evidence for the prenatal onset of abnormal head growth in children with autism spectrum disorder (ASD), studies on fetal ultrasound data in ASD are limited and controversial.

Method: We conducted a longitudinal matched case-sibling–control study on prenatal ultrasound biometric measures of children with ASD, and 2 control groups: (1) their own typically developed sibling (TDS) and (2) typically developed population (TDP). The cohort comprised 528 children (72.7% male), 174 with ASD, 178 TDS, and 176 TDP.

Results: During the second trimester, ASD and TDS fetuses had significantly smaller biparietal diameter (BPD) than TDP fetuses (adjusted odds ratio for the z score of BPD [aOR_{zBPD}] = 0.685, 95% CI = 0.527–0.890, and aOR_{zBPD} = 0.587, 95% CI = 0.459–0.751, respectively). However, these differences became statistically indistinguishable in the third trimester. Interestingly, head biometric measures varied by sex, with male fetuses having larger heads than female fetuses within and across groups. A linear mixed-effect model assessing the effects of sex and group assignment on fetal longitudinal head growth indicated faster BPD growth in TDS versus both ASD and TDP in male fetuses ($\beta = 0.084$ and $\beta = 0.100$ respectively; $p <$ .001) but not in female fetuses, suggesting an ASD–sex interaction in head growth during gestation. Finally, fetal head growth showed conflicting correlations with ASD severity in male and female children across different gestation periods, thus further supporting the sex effect on the association between fetal head growth and ASD.

Conclusion: Our findings suggest that abnormal fetal head growth is a familial trait of ASD, which is modulated by sex and is associated with the severity of the disorder. Thus, it could serve as an early biomarker for ASD.

Key words: autism spectrum disorder, prenatal ultrasound, head growth, fetal development

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Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder with a complex etiology having both genetic and environmental determinants.[1] The wide range of pre- and perinatal risk factors,[2] together with the abnormalities in early brain development associated with ASD,[3-8] suggest that, at least in some cases, the predisposition to ASD begins during gestation.

Abnormal head growth has been proposed as an early biomarker for ASD. Specifically, various magnetic resonance imaging (MRI), postmortem, and head measurement studies have reported that infants who later developed ASD were born with a smaller or average head circumference (HC), followed by an excessive increase during the first years of life in head size, in extra-axial cerebrospinal fluid (CSF) volume, and in brain regions associated with the language, social, emotional, and communication functions affected by ASD.[5,6,9–18] These studies also reported correlations between clinical severity with postnatal head size, brain morphometry, and extra-axial CSF volume.[9,15,19,20]

Importantly, some of these head growth abnormalities have been associated with sex, an interesting finding because sex is a known risk factor of ASD, with the disorder being ~4 times more prevalent among male individuals, but symptoms tending to be more frequent and severe among female individuals.[21-23] Several postnatal studies have found sex-specific neuroanatomical profiles in children with ASD compared to sex-matched controls. Among these investigations, a large population-based study revealed sex-specific differences in HC at birth and throughout early childhood in children with ASD.[18] Similarly, MRI studies found sex-specific differences in total brain volume (in gray and white matter volume of the frontal and temporal lobes, cingulate cortex, amygdala, and cerebellum), alongside

different and abnormal growth trajectories throughout childhood.[14,16,20,24-26] These studies also showed sex-specific correlations between specific brain regions and the clinical severity of ASD.[20]

Previous postnatal studies suggested that the accelerated head growth among children with ASD during early childhood may be a reaction or an adaptation to abnormal prenatal head growth. This assertion is supported by an emerging ASD prenatal literature covering developmental abnormalities in certain fetal ultrasound (US) parameters,[27] and specifically in biometric measures.[28-32] Nevertheless, these studies have shown conflicting results. Specifically, 2 studies found indications for either wider heads with normal HC in children with ASD compared to typically developed population (TDP) controls,[28] or larger HC with normal shape compared to the population norm.[29] Two other studies found an association between smaller HC to either ASD phenotype[36] or autistic traits,[37] whereas the fifth study did not find any association between HC and ASD.[30] In addition, the reported studies were conducted in relatively small cohorts and did not account for potential confounders; for example, none took into consideration familial factors known to play a significant role in fetal growth[33] or to constitute a risk for ASD.[34] Similarly, several studies did not take into account the inherent male sex bias in ASD,[28,29] even though boys are known to have larger biometric measures.[35] Finally, these studies report findings from different gestational periods, which further complicates comparisons of their findings. Thus, conclusions regarding fetal head growth in children with ASD remain minimal, inconsistent, and controversial. The main goal of this study was therefore to assess fetal head growth in fetuses later diagnosed with ASD in comparison to their typically developed siblings as well as to a population control across different gestation periods. In this study, the largest and most comprehensive on the subject to date, we examined the association between fetal head growth and ASD. In addition, we asked whether differences in fetal head growth are modulated by the sex of the fetus and whether they are associated with the clinical severity of children with ASD.

METHOD

Study Sample

The participants in this case/control study were singletons whose families were members of Clalit Health Services (CHS), Israel's largest health maintenance organization, serving about 75% of the approximately 700,000 residents of southern Israel, composed of Jews and Bedouins, 2 ethnic groups that differ in their genetic background and environmental exposures. Members of CHS in this region receive most of their hospital-related services (including ASD diagnosis) at the Soroka University Medical Center (SUMC), the region's only tertiary hospital. The diagnosis of ASD at SUMC is a multidisciplinary process, which entails a comprehensive intake interview (clinical and sociodemographic factors), a behavioral evaluation with the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), and a full neurocognitive assessment (eg, Bayley Scales of Infant and Toddler Development, Third Edition [Bayley-III] or Wechsler Preschool and Primary Scale of Intelligence, Third Edition [WPPSI-III]). Final diagnoses of ASD are made by a pediatric psychiatrist or neurologist, according to *DSM-5* criteria.[22] All sociodemographic and clinical data on these children are stored in the database of the National Autism Research Center of Israel (NARC).[36]

A flowchart showing the case/control assignment for this study is presented in Figure 1. We randomly selected from the NARC database 386 of the 719 children diagnosed with ASD (53.7%). Among these 386 cases, we identified those for whom there were prenatal ultrasound (US) scans in the CHS database. The 212 children (54.9%) for whom US scans were missing were excluded from the study, leaving us with a sample of 174 case individuals born between 2008 and 2017. There were no significant sociodemographic or clinical differences between this sample and other children with ASD in the NARC database, except that the proportion of Jews (versus Bedouin Israelis) was slightly lower for children included in the study, as was the parental age (see Table S1, available online).

Of the 174 cases included in the study, 85 had US scans from both the second and third trimesters, whereas 51 and 38 had scans from only the second or third trimester, respectively. For each trimester, we matched 2 types of control group for case individuals: (1) typically developing siblings (TDS): for each ASD case, we matched her/his own typically developed sibling who was closest in age; and (2) typically developed population (TDP): for each case, we identified a typically developed child matched by year of birth, sex, and ethnicity. In total, the study sample included 528 children: 174 with ASD, 178 TDS, and 176 TDP (Figure 1).

Fetal Ultrasound Data

All of the US scans had been performed by experienced sonographers or physicians that recorded fetal biometric measures using standardized US measurements of simple anatomic landmarks to the nearest millimeter. The following biometric measures were extracted from the CHS database for each trimester: HC and head width (BPD, biparietal diameter), that is, 2 biometric measures that

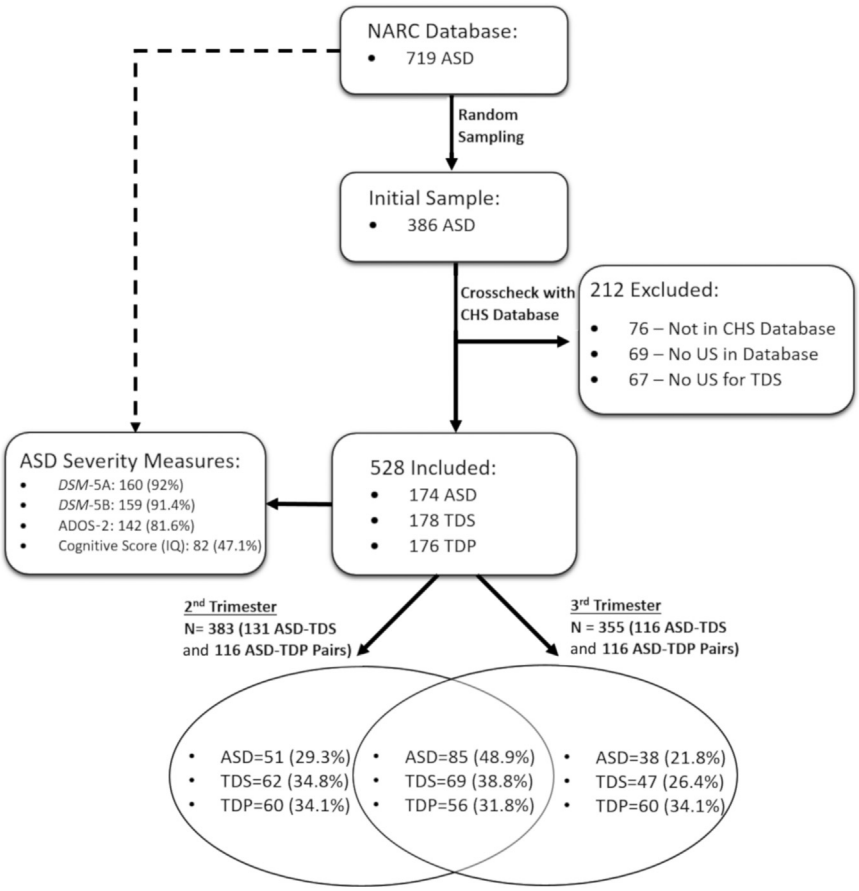
strongly correlate with brain size[37]; abdominal circumference (AC); and femur length (FL). HC was measured at the level of the BPD, taken at the widest point of the skull at the level of the thalamic peduncles and the cavum septum pellucidum; BPD was measured from outer skull wall to the inner wall directly opposite; AC was measured at the level of the junction of the vertebra, portal sinus, and fetal stomach; and FL was measured from the greater trochanter to the distal metaphysic.[28,38] It is known that for these US measurements, there is very high interrater reliability between experienced sonographers, particularly when transformation to standardized z scores are used.[28,31] The gestational age (GA) of each child was calculated from the last menstrual period (LMP) and confirmed by the crown−rump length (CRL) from the first trimester. If the date of LMP was unknown, GA was calculated based on CRL.

Statistical Analysis

We converted all biometric fetal measures to gestation-matched standardized z scores using the Hadlock approach,[38-41] the most widely used standardization approach in this field. In addition, we created 5 new variables by subtracting body parameter (AC and FL) z scores from head parameter (HC and BPD) z scores and by subtracting the HC z scores from the BPD z scores to assess fetal head growth relative to the other biometric measures.[28,30] If there was more than 1 US measure for a fetus in a particular trimester, we used the mean of its z scores as a representative measure. Notably, biometric measures with z scores greater than ±6 or that deviated by greater than 3 SD from the average for that trimester were considered outliers and removed from further analyses (29 outliers: 12 ASD, 9 TDP, 8 TDS).

Differences in sociodemographic characteristics between case individuals and the 2 control groups were assessed by using the appropriate univariate statistics. Differences in biometric measures between cases and each of the 2 matched control groups (TDS and TDP) were assessed using paired t tests and multivariable conditional regression adjusting for sex and maternal age. Differences in

FIGURE 1 Flowchart of Children Included in This Study



Note: *ADOS-2 = Autism Diagnostic Observation Schedule, Second Edition; ASD = autism spectrum disorder; CHS = Clalit Health Services; NARC = National Autism Research Center of Israel; TDP = typically developed population; TDS = typically developing siblings.*

biometric measures between the 2 matched control groups were assessed via unpaired t tests and multivariable unconditional logistic regression adjusting for sex, maternal age, and ethnicity. For a fetus with longitudinal data, a linear mixed-effect model was used to assess the association between fetal growth rate (measured as the difference in biometric measures throughout pregnancy) and ASD, taking into account repeated biometric measures, ethnicity, and gestational age.[31] These analyses were conducted separately for male and female fetuses to examine the effect of fetal sex on fetal growth rate. Finally, correlation coefficients were calculated in cases between biometric measures and ASD severity according to ADOS-2, *DSM-5,* and cognitive measures. The p values of analyses with multiple testing were adjusted using the Bonferroni correction. All analyses were conducted using SPSS Statistics V. 25 and R software. A 2-sided test significance level of .05 was used throughout the entire study.

Ethics

This study was approved by the "Helsinki Committee" of SUMC (SOR 295-18).

RESULTS

Sociodemographic and Clinical Characteristics

Clinical and sociodemographic characteristics of the study sample are shown in Table 1. As expected, the sex ratio for the TDS group was significantly different from that for the ASD group, close to 1:1 versus 4:1 male/female, respectively ($p < .001$). In addition, for the TDP group, US scans were performed earlier in the second trimester compared to those for cases (21.4 ± 2.8 versus 22.3 ± 1.8 weeks, respectively; $p = .006$). No other statistically significant differences were seen between cases and controls.

Biometric Measures

Differences in fetal US biometric measures between cases and controls are shown in Table 2 and Figures S1 and S2, available online. In the second trimester, both ASD and TDS fetuses had significantly narrower heads (smaller BPD) than TDP fetuses (adjusted odds ratio for the z score of BPD [aOR_{zBPD}] = 0.685, 95% CI = 0.527−0.890 and aOR_{zBPD} = 0.587, 95% CI = 0.459−0.751, respectively), giving the head a dolichocephalic shape. Interestingly, the BPD of the TDS controls was the smallest of all groups, leading to a smaller HC, which was also significantly smaller than the HC for the TDP group (adjusted odds ratio for the z score of HC [aOR_{zHC}] = 0.598, 95%CI=0.426-0.838). The heads of ASD and TDS fetuses were also proportionally smaller versus other body parts compared to TDP fetuses (Table 2), indicating that these differences are specific to the head and are not due to differences in fetal growth or fetal age. By the third trimester, these differences in biometric measures between groups had attenuated and had become statistically indistinguishable, although the BPD of the ASD fetuses had become the smallest of all groups (Table 2).

To verify that our results were not biased by the different samples in the 2 trimesters (Figure 1), we repeated these analyses in a subsample containing only subjects with US biometric data from both trimesters. The repeat analysis confirmed the above findings, with some differences in head size between ASD and TDP becoming even clearer in this subsample (see Table S2, available online).

Head biometric measures were consistently smaller for female than for male fetuses within and across groups (Figure 2; also see Table S3 and Figures S1 and S2, available online), indicating a significant sex effect in fetal growth measures. Therefore, we re-evaluated the group differences in biometric measures separately for female and male fetuses (see Table S4, available online). In the second trimester, the BPD in both ASD and TDS male fetuses was significantly smaller compared to that for TDP, but no such differences were seen in HC measures. As a result, ASD and TDS male fetuses had narrower and more elongated heads compared to TDP male fetuses (z of BPD-HC [zBPD-zHC] = −0.271 ± 1.04; p = .027 and zBPD-zHC = −0.539 ± 1.08; $p < .001$ for ASD-TDP and TDS-TDP, respectively). In the third trimester, the differences in head size and shape were attenuated and became statistically nonsignificant. In female fetuses, HC and BPD were proportionally smaller in ASD and TDS compared to TDP in both pregnancy trimesters (see Table S4, available online), thus suggesting that such head shape abnormality (ie, narrower and elongated) during mid gestation is an exclusive characteristic of male fetuses in ASD families.

Longitudinal Growth

For approximately one-half of the ASD fetuses and one-third of the controls, there were biometric measures for both the second and third trimesters (Figure 1). These data allowed us to examine differential rates of organ growth during pregnancy (Figure 2; and see Table S5, available online). Head width (zBPD) showed the most considerable growth compared to other body parts in both cases and controls and in both male and female fetuses. TDS male fetuses exhibited significantly faster BPD growth than both ASD and TDP male fetuses (β = 0.084 and β = 0.100, respectively; $p < .001$). As a result, the gap between TDS and TDP groups in the second trimester closed in the third trimester. In contrast, ASD male fetuses did not exhibit fast BPD growth, and thus their head width remained smaller in the third trimester versus that in both control groups.

TABLE 1 Clinical and Sociodemographic Characteristics for Children Included in This Study

Variable	ASD (n = 174)	TDS (n = 178)	TDP (n = 176)
Ethnicity, Jewish			
n (%)	127 (73)	131 (73.6)	139 (79)
p^a		1	.380
Sex, male			
n (%)	140 (80.5)	98 (55.1)	146 (83)
p^a		**<.001**	1
Pregnancy number			
Median (IQR)	2 (1−3)	2 (2−4)	2 (1−3)
p^b		.208	.172
Previous abortions			
Median (IQR)	0 (0−1)	0 (0−1)	0 (0−1)
p^b		1	.312
Cesarean delivery			
n (%)	31 (18.5)	23 (13.4)	26 (17.9)
p^a		.400	1
Gestational age at birth, wk			
Mean (SD)	38.8 (2.5)	39.0 (2)	39.3 (1.4)
p^c		1	.164
Birth weight, g			
Mean (SD)	3,174 (579)	3,186 (552)	3,324 (523)
p^c		1	.052
1-min low Apgar score, <7			
n (%)	4 (5)	5 (5)	6 (6)
p^d		1	1
Mother's age, y			
Mean (SD)	28.6 (5.8)	28.6 (5.2)	29.2 (4.8)
p^c		1	.510
Examined by physician			
n (%)	136 (85)	140 (78.7)	90 (79.6)
p^a		.264	.496
Breech presentation on US			
n (%)	51 (30.9)	39 (22.4)	40 (24.1)
p^a		.334	.308
Gestational age assessed by last menstrual period			
n (%)	115 (74.7)	129 (75.9)	85 (79.4)
p^a		1	.742
Gestational age at second trimester US, wk			
Mean (SD)	22.3 (1.8)	22.5 (1.5)	21.4 (2.8)
p^c		.632	**.006**
Gestational age at third trimester US, wk			
Mean (SD)	32.5 (2.6)	32.6 (2.4)	33.2 (2.9)
p^c		1	.132

Note: Boldface type indicates p < .05. *All* p *values are Bonferroni corrected for multiple comparison (n = 2). ASD = autism spectrum disorder; IQR = interquartile range; TDP = typically developed population; TDS = typically developed sibling; US = ultrasound.*

[a] χ^2 *Test.*

[b] Mann−Whitney *U* test.

[c] Two-sided *t* test.

[d] Fisher exact test.

TABLE 2 Differences in Fetal Biometric Measures

Variable	Group comparison	Mean difference ± SD	p^a	Adjusted odds ratio (aOR)[b]	95% CI	p^b
Second trimester: 131 ASD-TDS pairs, 116 ASD-TDP pairs						
zBPD	ASD (Ref) TDS	0.420 ± 1.63	**.012**	1.261	0.998–1.594	.156
	ASD (Ref) TDP	−0.402 ± 1.53	**.018**	0.685	0.527–0.890	**.015**
	TDS (Ref) TDP	−0.870 ± 1.74	**<.001**	0.587	0.459–0.751	**<.001**
zHC	ASD (Ref) TDS	0.181 ± 1.24	.300	1.087	0.795–1.487	1
	ASD (Ref) TDP	−0.216 ± 1.11	.12	0.693	0.486–0.987	.126
	TDS (Ref) TDP	0.441 ± 1.26	**<.001**	0.598	0.426–0.838	**.009**
zAC	ASD (Ref) TDS	0.094 ± 1.09	.984	0.991	0.701–1.400	1
	ASD (Ref) TDP	−0.180 ± 1.27	.402	0.809	0.595–1.100	.531
	TDS (Ref) TDP	−0.285 ± 1.25	**.036**	0.792	0.571–1.097	.480
zFL	ASD (Ref) TDS	−0.071 ± 1.07	1	0.838	0.596–1.177	.921
	ASD (Ref) TDP	−0.099 ± 1.04	.933	0.846	0.591–1.212	1
	TDS (Ref) TDP	−0.026 ± 1.02	1	1.061	0.733–1.536	1
zBPD–zHC	ASD (Ref) TDS	0.257 ± 1.13	**.033**	1.577	1.081–2.302	.054
	ASD (Ref) TDP	−0.181 ± 0.96	.141	0.621	0.407–0.948	.081
	TDS (Ref) TDP	−0.442 ± 1.12	**<.001**	0.521	0.364–0.746	**<.001**
zHC–zAC	ASD (Ref) TDS	0.101 ± 1.06	.837	1.159	0.811–1.657	1
	ASD (Ref) TDP	−0.025 ± 1.16	1	0.939	0.679–1.298	1
	TDS (Ref) TDP	−0.153 ± 1.10	.369	0.715	0.498–1.027	.210
zBPD–zAC	ASD (Ref) TDS	0.324 ± 1.41	**.027**	1.369	1.041–1.800	.072
	ASD (Ref) TDP	−0.238 ± 1.49	.276	0.767	0.588–1.000	.150
	TDS (Ref) TDP	−0.592 ± 1.46	**<.001**	0.572	0.431–0.758	**<.001**
zHC–zFL	ASD (Ref) TDS	0.278 ± 1.03	**.009**	1.489	1.009–2.197	.135
	ASD (Ref) TDP	−0.109 ± 1.10	.882	0.813	0.575–1.149	.720
	TDS (Ref) TDP	−0.411 ± 1.07	**<.001**	0.502	0.341–0.738	**<.001**
zBPD–zFL	ASD (Ref) TDS	0.520 ± 1.50	**<.001**	1.482	1.136–1.934	**.012**
	ASD (Ref) TDP	−0.295 ± 1.50	.111	0.733	0.562–0.955	.063
	TDS (Ref) TDP	−0.858 ± 1.50	**<.001**	0.489	0.368–0.650	**<.001**
Third trimester: 116 ASD–TDS pairs, 116 ASD–TDP pairs						
zBPD	ASD (Ref) TDS	0.061 ± 2.05	1	0.920	0.743–1.140	1
	ASD (Ref) TDP	−0.174 ± 2.13	1	0.925	0.773–1.106	1
	TDS (Ref) TDP	−0.351 ± 2.21	.270	0.985	0.819–1.184	1
zHC	ASD (Ref) TDS	0.040 ± 1.34	1	0.924	0.670–1.274	1
	ASD (Ref) TDP	−0.028 ± 1.34	1	0.968	0.729–1.287	1
	TDS (Ref) TDP	−0.163 ± 1.51	.738	0.977	0.749–1.275	1
zAC	ASD (Ref) TDS	−0.084 ± 1.38	1	0.868	0.637–1.182	1
	ASD (Ref) TDP	−0.048 ± 1.73	1	0.966	0.777–1.202	1
	TDS (Ref) TDP	0.019 ± 1.73	1	1.050	0.835–1.321	1
zFL	ASD (Ref) TDS	−0.176 ± 1.06	.237	0.745	0.502–1.106	.432
	ASD (Ref) TDP	0.082 ± 1.09	1	1.150	0.813–1.627	1
	TDS (Ref) TDP	0.218 ± 1.16	.135	1.407	0.994–1.992	.162
zBPD–zHC	ASD (Ref) TDS	0.039 ± 1.37	1	0.908	0.651–1.266	1
	ASD (Ref) TDP	−0.103 ± 1.48	1	0.910	0.702–1.178	1
	TDS (Ref) TDP	−0.167 ± 1.54	.735	1.022	0.781–1.337	1
zHC–zAC	ASD (Ref) TDS	0.181 ± 1.11	.273	1.244	0.845–1.830	0.804
	ASD (Ref) TDP	0.044 ± 1.47	1	1.043	0.805–1.351	1
	TDS (Ref) TDP	−0.164 ± 1.51	.726	0.923	0.708–1.204	1
zBPD–zAC	ASD (Ref) TDS	0.094 ± 1.70	1	0.922	0.711–1.196	1
	ASD (Ref) TDP	−0.097 ± 1.89	1	0.947	0.774–1.158	1
	TDS (Ref) TDP	−0.307 ± 1.95	.276	0.984	0.797–1.215	1
zHC–zFL	ASD (Ref) TDS	0.241 ± 1.07	.063	1.235	0.829–1.841	.897

(continued)

TABLE 2 Continued

Variable	Group comparison	Mean difference ± SD	p^a	Adjusted odds ratio (aOR)[b]	95% CI	p^b
	ASD (Ref) TDP	−0.148 ± 1.10	.498	0.782	0.552−1.109	.504
	TDS (Ref) TDP	−0.401 ± 1.44	**.009**	0.765	0.574−1.018	.198
zBPD−zFL	ASD (Ref) TDS	0.201 ± 1.88	.792	0.986	0.781−1.245	1
	ASD (Ref) TDP	−0.284 ± 1.85	.333	0.845	0.684−1.043	.348
	TDS (Ref) TDP	−0.544 ± 2.09	**.018**	0.894	0.734−1.088	.786

Note: Boldface type indicates p < .05. All p values are Bonferroni corrected for multiple comparison (n = 3). AC = abdominal circumference; ASD = autism spectrum disorder; BPD = biparietal diameter; FL = femur length; HC = head circumference; TDP = typically developed population; TDS = typically developed sibling.

[a]Paired *t* test: ASD-TDS, ASD-TDP; 2–sided *t* test: TDS-TDP.

[b]*ASD–TDS: adjusted to maternal age and sex; ASD-TDP: adjusted to maternal age; TDS-TDP: adjusted to maternal age, gender, and ethnicity.*

Association With ASD Phenotypes

Finally, we examined the association between fetal biometric measures with the severity of ASD symptoms (Figure 3). Three major trends were seen in this analyses: (1) smaller biometric measures (ie, HC, BPD, AC, and FL) were associated with worse ASD symptoms in childhood, especially for male fetuses in the second trimester (Figure 3A) and female fetuses in the third trimester (Figure 3D); (2) larger head-to-body ratios were positively correlated with more severe ASD symptoms in childhood, especially in the second trimester in both male and female fetuses (Figures 3A and 3B, respectively); and (3) for female fetuses, different trends were seen between the 2 trimesters. In the second trimester, most biometric measures were positively correlated with ASD severity (Figure 3C), whereas in the third trimester, they were inversely correlated with symptom severity (Figure 3D). Of note, none of these correlations remained statistically significant after Bonferroni correction for multiple testing.

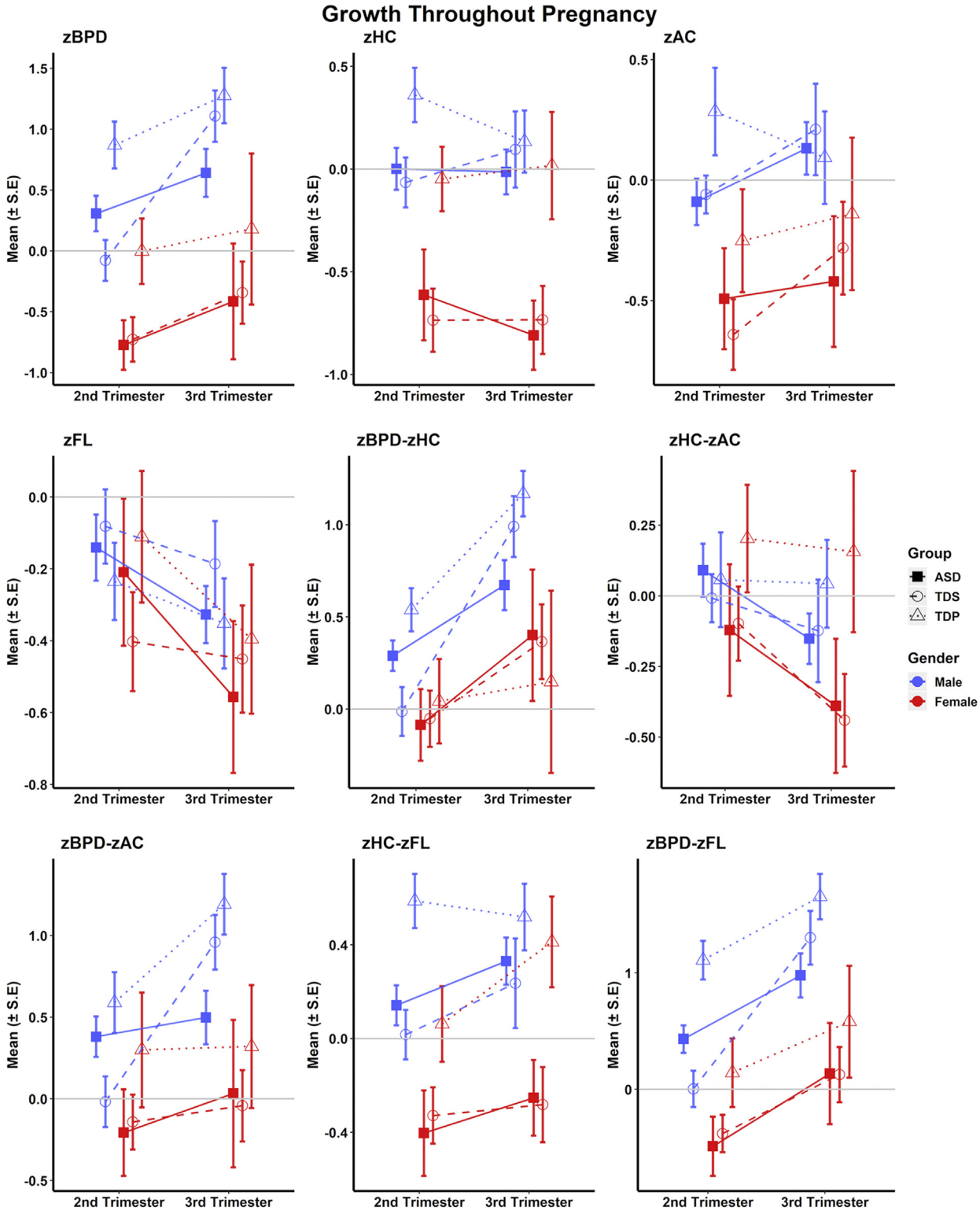
DISCUSSION

This is the largest and most comprehensive study to date to examine the association between fetal growth parameters and ASD. Three main findings (discussed in detail below) emerged from this study. First, fetuses later diagnosed with ASD and their TDS have narrower heads during mid-gestation compared to TDP, thus suggesting that such fetal head growth abnormality is a familial trait of ASD. Second, ASD-related head growth abnormalities are modulated by the sex of the fetus, with male and female fetuses showing different head shapes during gestation. Third, fetal head anomalies appear to be associated with the severity of ASD.

The abnormal fetal head growth associated with ASD that we report in this study suggests that brain abnormalities associated with ASD begin during gestation. Further support for prenatal brain anomalies in ASD can be found in a recent review, which summarizes findings from multiple studies of ASD about abnormal prenatal development of the brain including cell proliferation, neurogenesis, migration, laminar organization, and neurite outgrowth during the first and second trimester alongside neurite outgrowth, synaptogenesis, and synapse functioning in the third trimester and early postnatal life.[8] Additional support may be drawn from postmortem studies that found clues of aberrant neuronal migration during early prenatal brain development in the prefrontal and temporal cortical tissue, alongside evidence of curtailment of maturation of the forebrain limbic system and abnormalities in the cerebellar circuits, all of which are brain regions held to be affected ASD.[3,4,6,7] In addition, 2 postnatal MRI studies in preterm infants indicated structural brain asymmetry and reduced brain volume in preterm fetuses later diagnosed with ASD, again suggesting that abnormal head growth begins early in prenatal life, although ASD in the context of extreme prematurity might have a different pathogenic pathway compared to ASD in the general population.[42,43] Moreover, a recent MRI study found a 15% increase in extra-axial CSF volume among children with ASD during early childhood.[9] This increase is categorized as benign external hydrocephalus, characterized by increased CSF mainly above the frontal lobes, and is suggested to accrue due to delayed maturation of arachnoid granulations, which leads to an imbalance between the production and absorption of CSF.[44] Our finding of a narrow and elongated head among male feuses subsequently diagnosed with ASD is consistent with this finding, and may suggest that the increase in CSF above the frontal lobes may begin during prenatal life due to delayed maturation of arachnoid granulations, leading to the abnormal and elongated head shape.

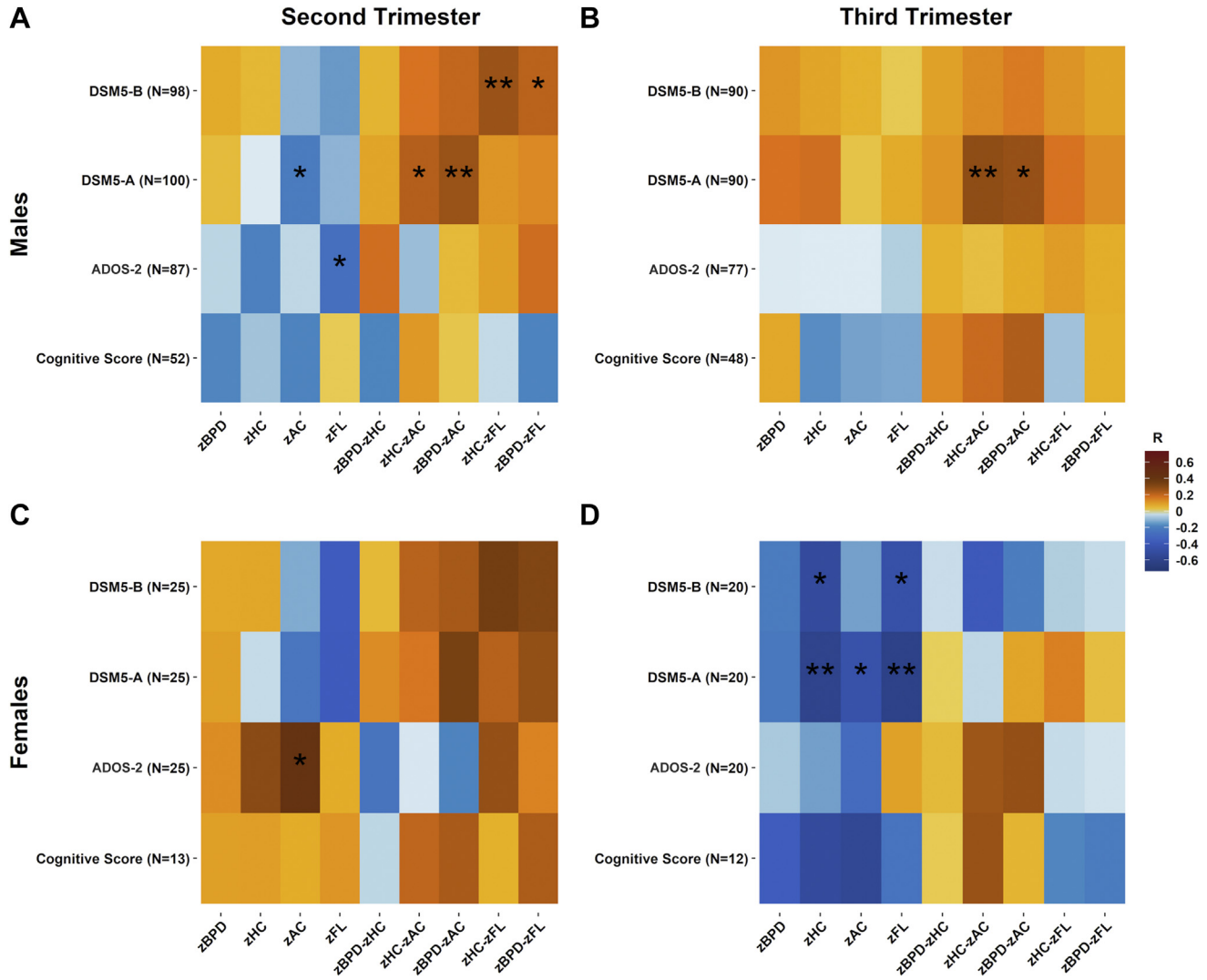
Our findings suggest that abnormalities in the fetal head during gestation that are later associated with ASD is a familial trait. Support for this premise can be drawn both

FIGURE 2 Changes in Biometric Measures Between Second and Third Trimesters



Note: *The mean and standard error of different biometric measures are shown for male (blue) and female (red) fetuses in the 3 study groups: individuals with autism spectrum disorder (ASD; squares), typically developed sibling (TDS; open circles), and typically developed population (TDP; open diamonds). A steeper line between the 2 trimesters indicates a faster growth rate between the 2 trimesters. Please note color figures are available online.*

FIGURE 3 Correlation Between Autism Spectrum Disorder (ASD) Severity and Fetal Biometric Measures in the Second and Third Trimesters of Pregnancy in Male and Female Fetuses



Note: *Spearman correlation coefficients (R) are color coded, with asterisks indicating statistically significant correlations. For all tests, a positive R means that a larger biometric measure is correlated with a worse outcome on a specific clinical test, as follows* DSM-5-B: Restrictive-Repetitive Domain *has a range of 3 values: (1) Requiring Support, (2) Requiring Substantial Support, and (3) Requiring Very Substantial Support. A higher score means a worse outcome.* DSM-5-A: Social Domain *has a range of 3 values: (1) Requiring Support, (2) Requiring Substantial Support, (3) Requiring Very Substantial Support. A higher score means a worse outcome. Autism Diagnostic Observation Schedule, Second Edition (ADOS-2): A higher score means a worse outcome. Cognitive Score: Cognitive abilities of child. In the original test, a higher score means a better outcome. To avoid confusion, we multiplied correlation outcome by −1 so that in this test also, a positive correlation means a worse outcome. Please note color figures are available online.*

*p < .05; **p ≤ .01.

from a prospective study showing smaller fetal heads in families with ASD compared to fetuses from families without ASD[31] and from a study that found an association between smaller HC and various autistic traits,[32] which are more prevalent in ASD families.[45] Additional support comes from genetic studies suggesting that HC is highly heritable[46] and that genetic variations associated with HC are also associated with risk of ASD.[47] Another indication of a familial link between abnormal fetal head growth and risk of ASD lies in some maternal endocrine functions during pregnancy that have been associated with both of these traits.[48,49] Interestingly, fetuses of our TDS group demonstrated accelerated head growth during late gestation to compensate for their relatively smaller heads, a finding consistent with previous study demonstrating slightly faster growth rate among TDS compared to TDP.[31] Similar

accelerated head growth in early infancy has been reported in children with ASD,[5,11-15] providing another familial trait of head growth abnormality associated with ASD. Furthermore, adjustment of the brain developmental rate has also been shown as a type of whole-brain adaptation in response to early environmental adversity to maximize its fit with the external environment.[50]

Our findings also suggest that ASD is associated with different prenatal head growth abnormalities in male and female fetuses. Similar sex-specific head size abnormalities were seen in a large population-based study at birth and throughout early childhood in children with ASD.[18] In addition, differences in total brain volume, and in gray and white matter volumes in different brain regions, were seen throughout childhood in boys and girls with ASD.[14,16,18,20,24–26] Furthermore, our study suggests that the observed sex-specific prenatal head growth abnormalities in fetuses later diagnosed with ASD are correlated with symptom severity in both sexes. This finding is consistent with previous postnatal studies that reported correlations between postnatal head size, brain morphometry, and extra-axial CSF volume on the one hand, to behavioral development, IQ,[19] social deficits,[15] sleep disturbances, lower nonverbal ability, poorer motor ability,[9] and ADOS scores,[20] on the other. We therefore suggest that postnatal sex-specific differences in ASD severity are associated with differences in fetal brain development between male and female fetuses later diagnosed with ASD.

Our study has several advantages over previous investigations of prenatal US data of children with ASD. The use of 2 distinct control groups, TDS and TDP, enabled us to adjust our findings to multiple familial and prenatal confounders that are known to have a considerable effect on both ASD risk and fetal growth (eg, sex,[21-23,35] shared genetics among siblings[1,33,34]), making our findings more compelling. In this context, previous findings suggesting larger HC[29] or BPD[28] in ASD fetuses may be attributed to bias resulting from the large and unaddressed proportion of male cases (~90% in both), for which biometric measures are usually larger.[35] Another important advantage of our study is the availability of longitudinal biometric measures from both second and third trimesters for a significant portion of our sample, thus allowing us to account for sampling bias between the 2 pregnancy periods and to investigate prenatal growth trajectories of these biometric measures in ASD and the 2 control groups. Using these data, we show that significant head growth abnormalities that are seen in mid-gestation (second trimester) for the ASD and TDS groups are attenuated toward the end of the pregnancy. Although this attenuation could be due to accelerated growth in some fetuses, as is seen for the TDS male fetuses, it could also be attributed to the typical higher variability of gestation-based biometric measures during the third trimester.[38] Finally, this study is the first to examine correlations between prenatal US measures and ASD clinical metrics, allowing us to understand the associations between fetal growth and ASD severity.

Our study has several limitations. First, the sample size of the study, despite being the largest of its kind to date, is still too small to address associations within subgroups. This is particularly relevant to the longitudinal analyses (Figure 2) and to the correlations between the biometric measures and ASD symptoms (Figure 3), where data was available only for subsets of our sample. The same limitation applies to the sex-specific analyses, as female fetuses comprised only ~20% of the cases due to the inherent male bias of ASD.[22] In addition, parents of children in the study sample were slightly younger than those of the other children with ASD in the NARC cohort. However, this small difference in parental age is unlikely to affect the study findings. Finally, we had only limited data about potential birth and pregnancy confounding factors for subjects in our study, such as weight at birth. Nonetheless, the comparison of ASD cases to both TDS and TDP control groups probably minimized the effect of such confounders.

In conclusion, our findings suggest that abnormal fetal head growth is a familial trait of ASD, which is modulated by sex and is associated with the severity of the disorder. Thus, it could serve as an early biomarker for ASD. Integration of our findings with fetal brain research will shed important light on neuroanatomical embryonic milestones in ASD development.

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