BRAIN
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Association between ultrasonography foetal anomalies and autism spectrum disorder

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Multiple pieces of evidence support the prenatal predisposition of autism spectrum disorder (ASD). Nevertheless, robust data about abnormalities in foetuses later developing into children diagnosed with ASD are lacking. Prenatal ultrasound is an excellent tool to study abnormal foetal development as it is frequently used to monitor foetal growth and identify foetal anomalies throughout pregnancy.

We conducted a retrospective case-sibling-control study of children diagnosed with ASD (cases); their own typically developing, closest-in-age siblings (TDS); and typically developing children from the general population (TDP), matched by year of birth, sex and ethnicity to investigate the association between ultrasonography foetal anomalies and ASD. The case group was drawn from all children diagnosed with ASD enrolled at the National Autism Research Center of Israel. Foetal ultrasound data from the foetal anatomy survey were obtained from prenatal ultrasound clinics of Clalit Health Services in southern Israel.

The study comprised 659 children: 229 ASD, 201 TDS and 229 TDP. Ultrasonography foetal anomalies were found in 29.3% of ASD cases versus only 15.9% and 9.6% in the TDS and TDP groups [adjusted odds ratio (aOR) = 2.23, 95% confidence interval (CI) = 1.32–3.78, and aOR = 3.50, 95%CI = 2.07–5.91, respectively]. Multiple co-occurring ultrasonography foetal anomalies were significantly more prevalent among ASD cases. Ultrasonography foetal anomalies in the urinary system, heart, and head and brain were the most significantly associated with ASD diagnosis ($aOR_{Urinary} = 2.08$, 95%CI = 0.96–4.50 and $aOR_{Urinary} = 2.90$, 95%CI = 1.41–5.95; $aOR_{Heart} = 3.72$, 95%CI = 1.50–9.24 and $aOR_{Heart} = 8.67$, 95%CI = 2.62–28.63; and $aOR_{Head\&Brain} = 1.96$, 95%CI = 0.72–5.30 and $aOR_{Head\&Brain} = 4.67$, 95%CI = 1.34–16.24; versus TDS and TDP, respectively). ASD females had significantly more ultrasonography foetal anomalies than ASD males (43.1% versus 25.3%, $P = 0.013$) and a higher prevalence of multiple co-occurring ultrasonography foetal anomalies (15.7% versus 4.5%, $P = 0.011$). No sex differences were seen among TDS and TDP controls. ASD foetuses were characterized by a narrower head and a relatively wider ocular-distance versus TDP foetuses ($OR_{BPD} = 0.81$, 95% CI = 0.70–0.94, and $aOR_{Ocular\ distance} = 1.29$, 95%CI = 1.06–1.57). Ultrasonography foetal anomalies were associated with more severe ASD symptoms.

Our findings shed important light on the multiorgan foetal anomalies associated with ASD.

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Introduction

Autism spectrum disorder (ASD) is a multifactorial, life-long neurodevelopmental disorder characterized by impaired social communication and restrictive-repetitive behaviours.[1,2] However, many people with ASD manifest additional comorbidities and congenital anatomical abnormalities that further complicate their clinical picture.[3–16] Nonetheless, today, the diagnosis of ASD is based on behavioural symptoms,[2] which are typically manifested in the second year of life.[17] A growing body of evidence suggests that the initial signs of ASD emerge during early childhood[18–20] and possibly even before birth.[21–32] Indeed, recent postnatal studies have found indications of the prenatal onset of abnormal neurodevelopment in children with ASD,[22] and some prenatal studies have provided preliminary indications for abnormal brain development,[21,23–25,28–33] and higher rates of structural anomalies in the renal system of both ASD foetuses and children with specific genetic syndromes associated with ASD.[30,34–38] Taken together, these findings suggest that ASD may be associated with abnormal embryonic organogenesis of different body parts, which consequently leads to postnatal malformations in some children with ASD.[12,22,39,40] Accordingly, there is emerging interest in examining the prenatal organ development of foetuses later developing into children diagnosed with ASD.[21,23–25,28–32]

Prenatal ultrasound, a commonly used pregnancy monitoring tool, allows physicians to survey foetal growth and organ development and may hence reveal anomalies suggesting genetic and developmental problems that require further testing and follow-up. In prenatal monitoring protocols, one of the primary ultrasound screenings is the foetal anatomy survey, which is considered standard of care for examining foetal organ development and detecting foetal organ anomalies. The survey involves the screening of the different organ systems and the measurement of a number of markers.[41,42] The abnormalities that can be detected by the survey include structural anomalies and 'soft markers' that may indicate genetic abnormalities or other non-genetic embryonic insults such as intrauterine infections, but some may be considered, in isolation, as normal variants or transient. The discovery of either structural abnormalities or 'soft markers' during the foetal anatomy survey will usually prompt a thorough examination of the foetal anatomy and consideration of further diagnostic testing for chromosome abnormalities.[41–47]

Despite the emerging literature suggesting the prenatal onset of abnormal organogenesis and neurodevelopment in children with ASD and the possible genetic and environmental background of ASD, together with evidence of higher rates of congenital anomalies in ASD, very little has been done to investigate prenatal organ development in children with ASD, as reflected in the prenatal foetal anatomy survey. Specifically, all studies conducted to date only used basic biometric measures taken during the second and third trimesters, which do not allow thorough examination of foetal organ development. For this reason, we conducted the first study of ultrasound data from the foetal anatomy survey of foetuses developing into children later diagnosed with ASD in comparison with the ultrasound data for their unaffected siblings and for typically developing children from the general population.

Materials and methods

Study population

All the participants in this study were born between 2004 and 2018 to mothers living in southern Israel—the Negev—which has ~700 000 inhabitants belonging to two main ethnic groups, Jews and Bedouins, that differ in their environmental exposures and genetic backgrounds. We included only foetuses from singleton pregnancies whose mothers were members of Clalit Health Services (CHS), Israel's largest health maintenance organization (HMO), serving ~75% of the Negev population. Members of CHS in this region receive most of their hospital-related health services (including ASD diagnosis) at the region's only tertiary hospital, the Soroka University Medical Center, and its associated outpatient clinics.

Study design

This retrospective case-sibling-control study comprised children diagnosed with ASD (cases); their own typically developing, closest-in-age siblings (TDS); and typically developing children from the (general) population (TDP), who were matched to cases by year of birth, sex (male/female) and ethnicity (Jewish/Bedouin). The case group was drawn from all children diagnosed with ASD in the Negev area, who are registered in the database of the National Autism Research Center of Israel (NARCI).[48,49] The diagnosis of ASD at the NARCI is a multidisciplinary process, which entails a comprehensive intake interview (socio-demographic and clinical factors), a behavioural evaluation with ADOS-2,[50] and a full neurocognitive assessment as described previously.[48,49] The final diagnosis of ASD is made by a paediatric psychiatrist or neurologist, according to DSM-5 criteria.[2]

Of the 704 singleton birth children with ASD in the NARCI database (database freeze, February 2020), there were 237 children (34%) for whom the relevant ultrasound scans were available in the database of the CHS prenatal ultrasound clinics. Among the 237 children, there were eight pairs of siblings with ASD (multiplex families). We randomly assigned one ASD sibling from each such multiplex family to the final study sample to reduce familial bias in our results. In addition, a sensitivity analysis using the second ASD sibling from these families was conducted. In total, the study cohort included 659 children: 229 with ASD, 201 TDS and 229 TDP (Fig. 1). An evaluation of socio-demographic and clinical differences between cases in the

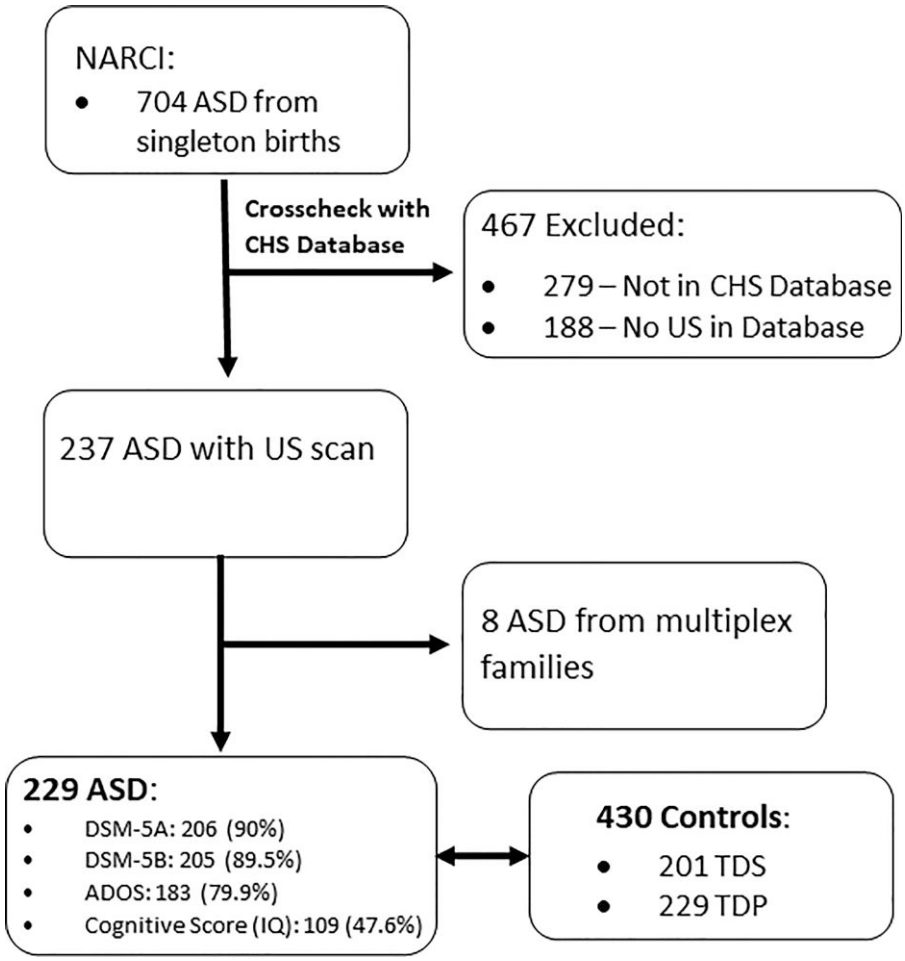

Figure 1 Flowchart of children included in this study.

study cohort and the other children with ASD in the NARCI database showed a lower proportion of Jews (versus Bedouin Israelis), a lower parental age, and a higher ADOS score for the ASD children in the study cohort (Supplementary Table 1). The lower proportion of Jews in the study cases can be explained due to the more frequent use of private insurance among Jewish parents to conduct a more comprehensive anatomy survey than that offered by the HMO.[51,52] The ethnic differences in the study cases can also explain the differences in parental age and ADOS scores, since Bedouins tend to have children at an earlier age than Jews,[49] and the diagnosis of ASD in the Bedouin population is usually made for those with more severe symptoms of the disorder.[49,53]

Foetal ultrasound data

Foetal ultrasound data from the foetal anatomy survey, which is conducted during gestational Weeks 20–24 in Israel, were obtained from all the prenatal ultrasound clinics of CHS in southern Israel. In these clinics, foetal anatomy surveys are performed by experienced physicians, who record foetal anomalies and biometric measures according to standard clinical guidelines.[41,42] The anatomy survey includes examination of different anatomical landmarks according to the various body systems, including the head, brain, thorax, abdomen, spine, limbs and umbilical cord. Abnormalities in each examined organ are classified as either structural anomalies or 'soft markers.[41–44] In addition, the following biometric measures are recorded: head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), femur length (FL), cisterna magna size, cerebellar diameter, lateral ventricle width, and ocular distance.[41,54] The physician also assesses the foetal wellbeing according to a biophysical profile, which includes examination of the amniotic fluid index (AFI), breathing, movement, and tone, giving a score of 0–8.[55] For the current study, the gestational age (GA) of each foetus was calculated from the last menstrual period (LMP) and confirmed by the crown-rump length (CRL) from the ultrasound scan in the first trimester. If the date of LMP was unknown, GA was calculated based on CRL.

Statistical analysis

We converted the basic biometric foetal measures (HC, BPD, AC, FL) to gestation-matched standardized Z-scores using the Hadlock approach, the most widely used standardization approach in this field.[56–58] In addition, the proportions of the ocular distance and of the cerebellum width out of the BPD were calculated (e.g. ocular distance × 100/BPD) in light of the strong relationships between these measures and head width. Differences in socio-demographic and clinical characteristics and in the proportion of anomalies between cases and each of the two matched control groups (TDS and TDP) were assessed using appropriate univariate statistics. Multivariable conditional regression or logistic regression models were used to assess the independent association of each

Table 1 Clinical and sociodemographic characteristics for children included in this study

Variable		ASD ($n = 229$)	TDS ($n = 201$)	TDP ($n = 229$)
Sociodemographic background				
Ethnicity (Jewish)	No. (%)	163 (71.2)	141 (70.1)	163 (71.2)
	P-value[a]		1	1
Sex (male)	No. (%)	178 (77.7)	114 (56.7)	178 (77.7)
	P-value[a]		**<0.001**	1
Pregnancy details				
Mother's age (years)	Mean ± SD	28.7 ± 5.5	28.5 ± 5.0	27.9 ± 4.6
	P-value[b]		1	0164
Pregnancy number	Median (IQR)	2 (1–3)	2 (2–4)	2 (1–3)
	P-value[c]		0.242	0.234
Previous abortions	Median (IQR)	0 (0–1)	0 (0–1)	0 (0–0)
	P-value[c]		1	0.070
Gestational age at birth (weeks)	Mean ± SD	38.8 ± 2.5	39.1 ± 1.9	39.2 ± 1.6
	P-value[b]		0.496	0.188
C-section	No. (%)	40 (17.8)	27 (14.4)	34 (14.9)
	P-value[a]		0.696	0.818
Birth weight (g)	Mean ± SD	3148 ± 606	3163 ± 593	3259 ± 531
	P-value[b]		1	0.128
1-min low APGAR score (<7)	No. (%)	5 (4.7)	5 (5.6)	9 (6.8)
	P-value[d]		1	0.966
Ultrasound details				
Gestation age assessed by last menstrual period	No. (%)	157 (76.2)	130 (75.6)	166 (82.2)
	P-value[a]		1	0.276
Gestational age at US (weeks)	Mean ± SD	22.9 ± 1.9	22.7 ± 1.8	22.9 ± 1.5
	P-value[b]		0.412	1
Placenta position, no. (%)	Fundus	13 (5.7)	13 (6.8)	14 (6.3)
	Front wall	124 (54.6)	102 (53.7)	109 (48.7)
	Back wall	96 (39.6)	74 (38.9)	100 (44.6)
	Placenta previa	0 (0)	1 (0.5)	1 (0.4)
	P-value[a]		1	0.948
Placental grading	Median (IQR)	1 (0–2)	1 (0–2)	1 (0–2)
	P-value[c]		1	0.578
Breech presentation at US	No. (%)	74 (32.9)	58 (29.6)	59 (26.1)
	P-value[a]		0.934	0.228
Normal amniotic fluid	No. (%)	222 (97.4)	195 (98.5)	219 (97.8)
	P-value[d]		1	1
Weight at US (g)	Mean ± SD	578 ± 197	555 ± 176	577 ± 145
	P-value[b]		0.406	1

Boldface type indicates *P-value* < 0.05. All P-values are Bonferroni corrected for multiple comparison ($n = 2$).
ASD = autism spectrum disorder; SD = standard deviation; TDS = typically developing siblings; TDP = typically developing population; US = ultrasound.
[a]Chi-square.
[b]Two-sided *t*-test.
[c]Mann–Whitney U-test.
[d]Fisher's Exact Test.

ultrasound foetal measure/biomarker with ASD risk after adjusting for potential confounders. Details concerning the specific statistical tests conducted for each variable can be seen at the footnote of each table. Finally, the association between clinical severity and foetal abnormalities was assessed using appropriate univariate statistics. 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 two-sided test significance level of 0.05 was used throughout the entire study.

Ethics statement

The study was approved by the SUMC Ethics Committee per the Declaration of Helsinki SOR 295-18. Importantly, to protect patient confidentiality, all ultrasound data were 'de-identified' manner (i.e. without the mother's ID or name, or any other identifiable information about the mother or the child).

Data availability

Raw data were generated at the National Autism Research Center of Israel. Derived data supporting the findings of this study are available from the corresponding author upon reasonable request.

Results

Socio-demographic and clinical characteristics

Clinical and socio-demographic characteristics of the study sample are shown in Table 1. The anatomy survey was performed at the gestational age of 22.85 ± 1.7 weeks, with no significant differences between the groups. Similarly, there were no significant differences between the groups in all other clinical characteristics, except for the inherent male bias in the ASD group compared to their unaffected siblings (77.7% versus 56.7%, respectively; $P < 0.001$).

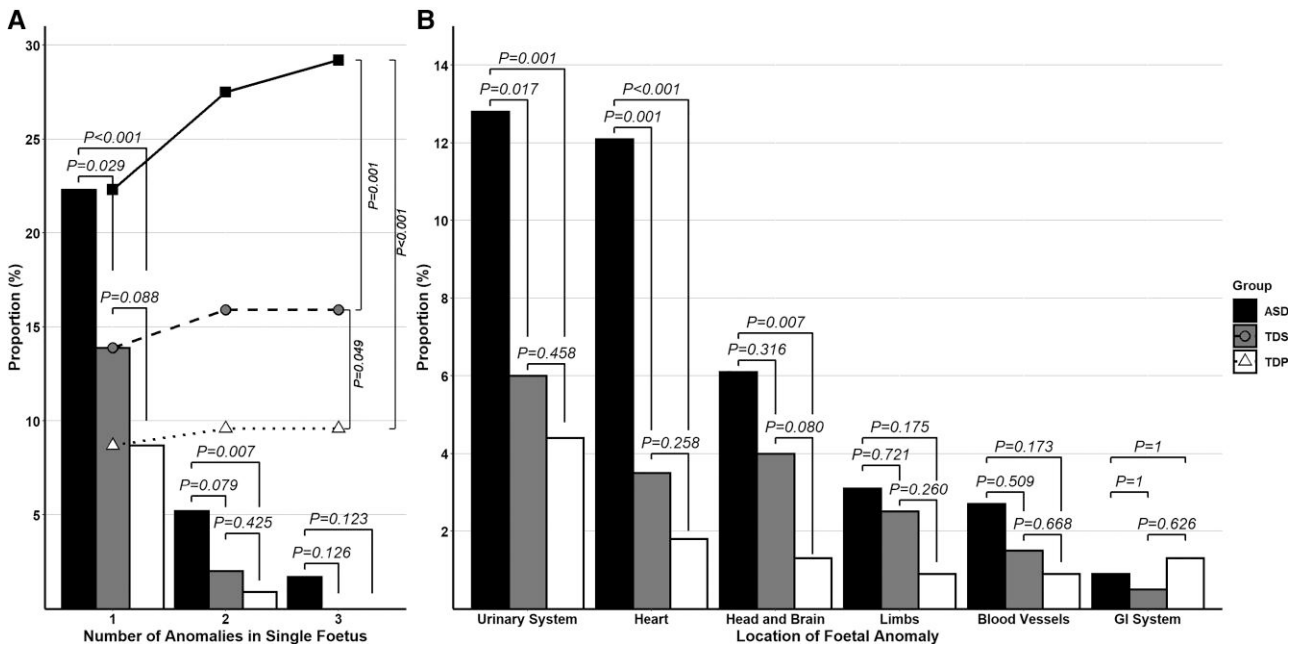

Figure 2 Proportions of anomalies in different foetal organ systems. (**A**) Proportion of UFAs in each group. The bars represent the proportion of foetuses with one, two and three co-occurring UFAs. The lines with symbols represent the cumulative proportion of anomalies in each group: ASD (solid black line and black squares), TDS (dashed black line and grey circles), and TDP (dotted black line and white triangles). (**B**) Proportion of UFAs in the different organs. Black bars = ASD; grey bars = TDS; white bars = TDP.

Ultrasonography foetal anomalies

Case-control differences in ultrasonography foetal anomalies (UFAs) are depicted in Fig. 2, Table 2 and Supplementary Table 2. Overall, UFAs were found in 67 (29.3%) of the ASD cases compared to 32 (15.9%) and 22 (9.6%) in the TDS and TDP groups [adjusted odds ratio (aOR) = 2.23, 95% confidence interval (CI) = 1.32–3.78, and aOR = 3.50, 95%CI = 2.07–5.91, respectively]. In addition, more ASD cases had multiple anatomic anomalies than controls, with 7% of cases having multiple anatomic anomalies compared to only 2% of TDS and 0.9% of TDP controls ($P = 0.014$ and $P = 0.001$, respectively) (Fig. 2A). Most UFAs in the ASD cases were seen in the urinary system and the heart (12.8% and 12.1%, respectively), followed by the head and brain (always taken together in this study; 5.7%), limbs (3.1%), blood vessels (2.7%), and gastrointestinal system (0.9%) (Fig. 2B). Of note, cardiac UFAs were significantly associated with higher odds of an ASD diagnosis ($aOR_{Heart} = 3.72$, 95%CI = 1.50–9.24, and $aOR_{Heart} = 8.67$, 95%CI = 2.62–28.63 compared to TDS and TDP, respectively). UFAs in the urinary system were also associated, although to a lesser extent, with elevated odds of an ASD diagnosis ($aOR_{Urinary} = 2.08$, 95%CI = 0.96–4.50 and $aOR_{Urinary} = 2.90$, 95%CI = 1.41–5.95 compared to TDS and TDP, respectively), with dilation of the renal pelvis (pyelectasis or hydronephrosis) being the most frequently detected anomaly. Finally, UFAs in the head and brain were significantly higher in the ASD cases compared to the TDP group but not to the TDS group ($aOR_{Head\&Brain} = 4.67$, 95%CI = 1.34–16.24, and $aOR_{Head\&Brain} = 1.96$, 95%CI = 0.72–5.30, respectively), with anomalies in CSF circulation seen among 4% of ASD cases compared to 1.3% of TDP ($P = 0.078$). ASD cases also had higher rates of UFAs in most other organs examined in the study, although these differences did not reach statistical significance (Table 2 and Fig. 2B).

Common biometric measures evaluated during the anatomy survey are presented in Table 3. While ASD cases had significantly smaller (HC) and narrower (BPD) heads compared to TDP controls ($aOR_{HC} = 0.76$, 95%CI = 0.62–0.93; $aOR_{BPD} = 0.81$, 95%CI = 0.70–0.94), their ocular distance, relative to their BPD, was significantly larger than that in TDP controls ($aOR_{Ocular\ Distance} = 1.29$, 95%CI = 1.06–1.57). ASD foetuses also had a lower biophysical profile than TDP foetuses (aOR = 0.58, 95%CI = 0.38-0.89), suggesting abnormal foetal neurodevelopment in the ASD cases. Finally, there were no significant differences in the sizes of the cisterna magna, cerebellum, or lateral ventricles between ASD foetuses and the two control groups.

Sex differences

Sex differences in UFA rates are depicted in Supplementary Table 3. UFAs were significantly more common among ASD females than males (43.1% versus 25.3%, $P = 0.013$; for females and males, respectively). The most significant sex differences were seen in UFAs in heart (23.5% versus 8.7%, $P = 0.007$), head and brain (11.8% versus 4.5%, $P = 0.056$), and gastrointestinal system (3.9% versus 0%, $P = 0.051$). In addition, ASD females had a higher prevalence of multiple co-occurring UFAs compared to ASD males (15.7% versus 4.5%, $P = 0.011$). TDS and TDP controls had no significant differences between females and males.

Association with ASD severity

Finally, we examined the association between UFAs and biometric measures with the severity of ASD symptoms (Table 4). The most significant associations were those between foetal cardiac anomalies and younger age at diagnosis ($P = 0.031$), and between UFAs of the head and brain and DSM5-A criteria ($P = 0.017$). Specifically, children for whom there were observable cardiac anomalies during gestation were diagnosed with ASD 6 months earlier than other children with ASD (33.6 ± 11.7 versus 39.4 ± 16.5 months, $P = 0.031$).

Table 2 Anomalies in different foetal organ systems

Variable		Group[a]	No. (%)	Adjusted odds ratio (aOR)	95% CI	P-value
Any foetal organ abnormality		ASD	67 (29.3)	REF		
		TDS	32 (15.9)	2.23	1.32–3.78	**0.006**[b]
		TDP	22 (9.6)	3.50	2.07–5.91	**<0.001**[b]
Total	Urinary system	ASD	29 (12.8)	REF		
		TDS	12 (6.0)	2.08	0.96–4.50	0.126[b]
		TDP	10 (4.4)	2.90	1.41–5.95	**0.008**[b]
Dilation of renal pelvis		ASD	26 (11.5)	REF		
		TDS	12 (6.0)	1.82	0.82–4.02	0.280[b]
		TDP	10 (4.4)	2.60	1.25–5.39	**0.020**[b]
Bladder		ASD	1 (0.4)	REF		
		TDS	0 (0)	NA		1[c]
		TDP	0 (0)	NA		0.994[c]
Other malformations		ASD	2 (0.9)	REF		
		TDS	0 (0)	NA		1[c]
		TDP	0 (0)	NA		0.494[c]
Total	Heart	ASD	27 (12.1)	REF		
		TDS	7 (3.5)	3.72	1.50–9.24	**0.010**[b]
		TDP	4 (1.8)	8.67	2.62–28.63	**<0.001**[b]
EIF		ASD	16 (7.2)	REF		
		TDS	6 (3)	3.35	1.21–9.28	**0.040**[b]
		TDP	1 (0.4)	16.00	2.12–120.647	**0.014**[b]
VSD		ASD	11 (4.9)	REF		
		TDS	1 (0.5)	5.80	0.67–50.18	0.220[b]
		TDP	3 (1.3)	5.00	1.10–22.82	0.076[b]
Total	Head and Brain	ASD	14 (6.1)	REF		
		TDS	8 (4.0)	1.96	0.72–5.30	0.374[b]
		TDP	3 (1.3)	4.67	1.34–16.24	**0.030**[b]
Ventricles		ASD	4 (1.8)	REF		
		TDS	2 (1)	1.50	0.27–8.52	1[b]
		TDP	2 (0.9)	2.00	0.37–10.92	0.846[b]
Mega cisterna magna		ASD	2 (0.9)	REF		
		TDS	1 (0.6)	1.37	0.12–15.48	1[c]
		TDP	0 (0)	NA		0.996[c]
Choroid plexus cyst		ASD	5 (2.3)	REF		
		TDS	2 (1.2)	2.01	0.39–10.48	0.942[c]
		TDP	1 (0.5)	1.71	0.84–3.50	0.284[b]
Cerebellum		ASD	1 (1.1)	REF		
		TDS	0 (0)	NA		1[c]
		TDP	0 (0)	NA		1[c]
Skull		ASD	2 (0.9)	REF		
		TDS	0 (0)	NA		1[c]
		TDP	0 (0)	NA		0.490[c]
Microcephaly		ASD	2 (0.9)	REF		
		TDS	2 (1)	1.41	0.12–16.33	1[b]
		TDP	0 (0)	NA		0.496[c]
Total	Limbs[d]	ASD	7 (3.1)	REF		
		TDS	5 (2.5)	1.65	0.46–5.92	0.880[b]
		TDP	2 (0.9)	3.50	0.73–16.85	0.236[b]
Total	Blood vessels[d]	ASD	6 (2.7)	REF		
		TDS	3 (1.5)	2.04	0.44–9.38	0.722[b]
		TDP	2 (0.9)	3.00	0.61–14.86	0.356[b]
Total	Gastrointestinal system[d]	ASD	2 (0.9)	REF		
		TDS	1 (0.5)	1.79	0.16–19.93	1[c]
		TDP	3 (1.3)	0.67	0.11–3.99	1[b]

[a]ASD = 229, TDS = 201, TDP = 229.
[b]Conditional logistic regression, adjusted to foetal sex.
[c]Fisher's Exact Test.
[d]Details about anomalies in specific parts of the limbs, blood vessels, and gastrointestinal system are provided in Supplementary Table 2. All *P*-values are Bonferroni corrected for multiple comparison ($n = 2$). Boldface type indicates *P*-value < 0.05. EIF = echogenic intracardiac focus; NA =; REF = reference; VSD = ventricular septal defect.

Table 3 Risk of ASD associated with foetal measures

Variable	Group	Mean ± SD	Odds Ratio (OR)	95% CI	P-Value	Adjusted odds ratio (aOR)	95% CI	P-value
zHC	ASD = 227	−0.11 ± 1.0	REF					
	TDS = 199	−0.20 ± 1.1	1.08	0.90–1.30	0.782	0.98	0.71–1.19	1[a]
	TDP = 227	0.12 ± 0.8	0.77	0.63–0.94	**0.020**	0.76	0.62–0.93	**0.018**[a]
zBPD	ASD = 228	0.08 ± 1.4	REF					
	TDS = 200	−0.07 ± 1.4	1.08	0.94–1.24	0.536	1.01	0.88–1.16	1[a]
	TDP = 228	0.42 ± 1.2	0.82	0.71–0.95	**0.014**	0.81	0.70–0.94	**0.010**[a]
zAC	ASD = 229	−0.31 ± 0.9	REF					
	TDS = 199	−0.28 ± 1.0	0.97	0.80–1.18	1	0.89	0.73–1.09	0.540[a]
	TDP = 228	−0.13 ± 0.9	0.81	0.66–0.99	0.076	0.81	0.66–0.99	0.072[a]
zFL	ASD = 229	−0.20 ± 0.9	REF					
	TDS = 200	−0.13 ± 0.8	0.91	0.73–1.14	0.838	0.89	0.70–1.12	0.606[a]
	TDP = 227	−0.15 ± 0.8	0.93	0.74–1.16	1	0.93	0.74–1.16	1[a]
Ocular distance, %	ASD = 35	66.31 ± 3.1	REF			REF		
	TDS = 23	65.65 ± 2.6	1.07	0.90–1.28	0.858	1.11	0.89–1.37	0.712[b]
	TDP = 28	63.61 ± 3.4	1.31	1.09–1.57	**0.008**	1.29	1.06–1.57	**0.020**[b]
Cerebellum, %	ASD = 91	45.53 ± 2.6	REF			REF		
	TDS = 69	45.20 ± 2.3	1.06	0.93–1.20	0.818	1.04	0.91–1.19	1[b]
	TDP = 81	45.01 ± 2.6	1.08	0.96–1.22	0.380	1.08	0.96–1.22	0.282[b]
Cisterna magna, mm	ASD = 88	4.91 ± 1.3	REF			REF		
	TDS = 60	4.64 ± 1.5	1.15	0.90–1.50	0.518	1.10	0.86–1.41	0.926[b]
	TDP = 81	5.00 ± 1.4	0.95	0.76–1.19	1	0.95	0.76–1.19	1[b]
Lateral ventricles, mm	ASD = 93	5.74 ± 1.3	REF			REF		
	TDS = 68	5.33 ± 1.3	1.27	0.99–1.63	0.116	1.26	0.98–1.62	0.142[b]
	TDP = 78	5.63 ± 1.4	1.06	0.85–1.33	1	1.07	0.85–1.34	1[b]
Amniotic fluid index, cm	ASD = 19	16.63 ± 4.5	REF					
	TDS = 26	17.73 ± 3.5	0.93	0.79–1.09	0.718	0.86	0.71–1.03	0.208[b]
	TDP = 19	18.37 ± 4.0	0.90	0.76–1.06	0.444	0.92	0.77–1.10	0.716[b]
Biophysical profile, score (1–8)	ASD = 53	7.09 ± 1.0	REF					
	TDS = 31	6.90 ± 1.0	1.21	0.78–1.89	0.678	1.11	0.68–1.83	1[b]
	TDP = 67	7.46 ± 0.9	0.67	0.46–0.98	0.074	0.58	0.38–0.89	**0.026**[b]

All *P*-values are Bonferroni corrected for multiple comparison ($n = 2$). Boldface type indicates *P*-value < 0.05
AC = abdominal circumference; BPD = biparietal diameter; FL = femur length; HC = head circumference; SD = standard deviation.
[a]Conditional logistic regression, adjusted to foetal sex.
[b]Logistic regression, adjusted to foetal sex and gestational age.

Furthermore, children for whom there were observable head and brain UFAs were diagnosed as requiring more support than other children with ASD according to DSM5-A criteria.

Sensitivity analysis

The study sample included one randomly selected ASD case from each of the eight multiplex families in the study. We repeated all the reported analyses in this study using a sample that included the other ASD case in each family. The results of these analyses are reported in Supplementary Tables 4–6 and show the same differences in UFA rates between the study groups.

Discussion

This study is the first to comprehensively examine prenatal organ development in ASD children via an examination of the foetal anatomy survey. We show that foetuses developing into children later diagnosed with ASD had significantly higher rates of UFAs compared to both their typically developing siblings and to matched typically developing children from the general population. These finding highlight the association of certain UFAs with ASD susceptibility of the developing foetus. These UFAs, which can be detected in standard prenatal anatomy ultrasound surveys conducted during mid-gestation, could form the basis of new prenatal screening approaches for ASD. The results of such prenatal screening will reveal foetuses at risk to develop ASD and may facilitate their earlier diagnosis, a factor that has already been shown to optimize the long-term outcomes of ASD treatment.[59–61]

Most of the identified UFAs were observed in the urinary system, heart, and head and brain, suggesting a shared aetiology for the abnormal development of these organs in ASD. Dilation of the renal pelvis (pyelectasis or hydronephrosis) was the most prevalent UFA among ASD cases in our study (11.5%), significantly higher than observed in the TDS and TDP controls (6% and 4.4%, respectively) and higher than the reported prevalence of 2–5% of this anomaly in the general population.[62] This finding is consistent with a previous study demonstrating higher rates of pyelectasis in a subset of ASD foetuses.[30] Pyelectasis is considered a 'soft marker' associated with an underlying foetal genetic risk.[43,45] Furthermore, a number of genetic syndromes associated with ASD are characterized by various renal anomalies. For example, children with the 16q24.2 deletion or the17q12 microdeletion usually manifest both ASD and various congenital abnormalities of the kidney and urinary tract, including dilation of the renal pelvis,[37,38,63–65] some of these congenital abnormalities could indeed be identified in prenatal ultrasound scans.[34–36,38] Another example is Phelan-McDermid syndrome, caused by 22q13 deletion or by

Table 4 Association between clinical severity and foetal abnormalities

Abnormality type	Clinical test		Abnormality		P-value
			Yes	No	
Any foetal organ abnormality	Cognitive score, mean (SD)		73.18 ± 15.8	76.97 ± 14.2	0.218[a]
	Diagnosis age, months, mean (SD)		38.69 ± 17.1	38.65 ± 15.6	0.986[a]
	ADOS, median (IQR)		8 (6–10)	8 (6–9)	0.142[b]
	DSM5-A, no. (%)	RS	7 (11.7)	9 (6.2)	0.173[c]
		RSS	18 (30)	61 (41.8)	
		RVSS	35 (58.3)	76 (52.1)	
	DSM5-B, no. (%)	RS	6 (10)	14 (9.7)	0.671[c]
		RSS	30 (50)	82 (56.6)	
		RVSS	24 (40)	49 (33.8)	
Heart	Cognitive score, mean (SD)		73.80 ± 15	75.95 ± 14.2	0.623[b]
	Diagnosis age, months, mean (SD)		33.61 ± 11.7	39.39 ± 16.5	**0.031**[a]
	ADOS, median (IQR)		8.5 (6–10)	8 (6–9)	0.427[b]
	DSM5-A, no. (%)	RS	2 (8.3)	14 (7.9)	0.840[c]
		RSS	8 (33.3)	70 (39.5)	
		RVSS	14 (58.3)	93 (52.5)	
	DSM5-B, no. (%)	RS	1 (4.2)	19 (10.8)	0.393[c]
		RSS	16 (66.7)	94 (53.4)	
		RVSS	7 (29.2)	63 (35.8)	
Urinary system	Cognitive score, mean (SD)		74.92 ± 16.8	75.96 ± 14.6	0.694[b]
	Diagnosis age, months, mean (SD)		35.72 ± 14.7	39.17 ± 16.2	0.280[a]
	ADOS, median (IQR)		9 (6–10)	8 (6–9)	0.201[b]
	DSM5-A, no. (%)	RS	2 (7.7)	14 (7.9)	0.890[c]
		RSS	9 (34.6)	70 (39.3)	
		RVSS	15 (57.7)	94 (52.8)	
	DSM5-B, no. (%)	RS	4 (15.4)	16 (9)	0.341[c]
		RSS	11 (42.3)	100 (56.5)	
		RVSS	11 (42.3)	61 (34.5)	
Head and brain	Cognitive score, mean (SD)		69.83 ± 17.3	76.18 ± 14.6	0.344[b]
	Diagnosis age, months, mean (SD)		41.78 ± 15.7	38.47 ± 16.0	0.373[b]
	ADOS, median (IQR)		8 (6–10)	8 (6–9)	0.643[b]
	DSM5-A, no. (%)	RS	3 (23.1)	13 (6.7)	**0.017**[c]
		RSS	1 (7.7)	78 (40.4)	
		RVSS	9 (69.2)	102 (52.8)	
	DSM5-B, no. (%)	RS	1 (7.7)	19 (9.9)	0.127[c]
		RSS	4 (30.8)	108 (56.3)	
		RVSS	8 (61.5)	65 (33.9)	

Each category includes all the abnormalities specified in Table 1. RS = requiring support; RSS = requiring substantial support; RVSS = requiring very substantial support; SD = standard deviation. Boldface type indicates *P*-value < 0.05.

[a]Two-sided *t*-test.

[b]Mann–Whitney U-test.

[c]Chi-square.

disruptive mutations in *SHANK3*, one of the most common monogenic causes of ASD, with renal abnormalities being found in 25–38% of children with this syndrome.[11,66]

As mentioned above, higher odds of an ASD diagnosis were significantly associated with cardiac UFAs, including echogenic intracardiac focus, which is considered a 'soft marker' associated with various genetic anomalies,[43] and ventricular septal defect, which is a structural malformation that may progress to congenital heart disease (CHD) after birth.[4,40] Indeed, there is emerging evidence supporting a possible association between CHD and ASD, with several population-based studies reporting a higher risk of ASD in children with CHD.[4–6,9,13,16,67–69] Furthermore, recent findings from exome sequencing studies demonstrate a striking overlap between genes associated with ASD and CHD.[4,70] A genetic link between ASD and CHD can also be seen in several genetic syndromes associated with ASD; for example, ~3–25% of children with Phelan-McDermid syndrome also manifest various cardiac abnormalities,[11,66] and comorbidity of ASD and cardiac defects is also seen in children with the 22q11 deletion.[71,72] Heart and brain development occur simultaneously during foetal development. Due to the depth and complexity of these shared morphogenetic programmes, disruption of organogenesis in one organ may impact the development of the other. For example, CHD is well known to be associated with abnormal cerebral development—smaller brain volumes, white matter maldevelopment and punctate lesions that are not detectable by ultrasound. These children suffer intrauterine maldevelopment due to their abnormal circulations, but then often have postnatal cerebral insults because of hypoxia or ischemia, before, during or after surgery. Also, children undergoing heart surgery have an increased risk of ASD.[4,13,67–69,73,74]

Relatively high UFA rates were also seen for the head and brain. These UFAs consisted mainly of anomalies in the CSF circulation, including choroid plexus cysts, enlarged lateral ventricles, and mega cisterna magna, suggesting abnormal development of CSF circulation in ASD compared to TDP. Indeed, increased pre- and postnatal ventricle volumes have been proposed as early structural

markers of altered development of the cerebral cortex and increased risk for neuropsychiatric disorders, including ASD.[25,28,75] In addition, ventriculomegaly, enlarged cisterna magna, hydrocephalus, and increased extra-axial CSF were associated with ASD in multiple MRI and population-based studies.[7,25,28,75–81] Finally, children with 22q13 deletion syndrome associated with ASD are characterized by abnormalities in the CSF circulation, including ventricle dilation, enlarged cisterna magna, and arachnoid cysts.[11,66,82]

Abnormalities in the CSF circulation in the extra-axial space may lead to an accumulation of CSF above the frontal lobes,[78–81] resulting in an abnormal and elongated (dolichocephalic) head shape, as revealed in this analysis and our previous study,[21] or to other head growth abnormalities in ASD foetuses as reported in other prenatal biometric studies.[21,23,24,29–31] These abnormalities may also be related to relatively wider set eyes observed in ASD foetuses versus the other foetuses in the study cohort and which is in line with evidence from postnatal head image analysis demonstrating wide-set eyes in a subgroup of children with ASD.[83] Both dolichocephaly and wide-set eyes have been linked to several genetic anomalies associated with ASD, including copy-number variants in the 16p11.2[84] and 22q13[11,85,86] chromosomal loci and mutations in the *CHD8* gene.[87]

Our findings also suggest a positive association between foetal structural anomalies and ASD severity. Indeed, congenital anomalies have been shown to be more prevalent among individuals with autism and intellectual disability,[12] and ASD children with CHD or wider-set eyes have worse cognitive, language, and attention disabilities than other children with ASD.[13,68,83] ASD children with CHD usually also suffer from developmental delay and tend to be diagnosed earlier than most other children with ASD.[68] It is hard to know whether these children were diagnosed earlier because they were followed more closely due to their other medical conditions, or if they had more severe ASD leading to earlier diagnosis. In addition, the amount of extra-axial CSF volume detected as early as 6 months is predictive of more severe ASD symptoms.[80,81] Finally, children with genetic syndromes that include both ASD and congenital malformations usually manifest additional cognitive and clinical impairments that lead to a more severe ASD outcome.[37,71,72]

We show that ASD females have more UFAs and multiple co-occurring UFAs compared to ASD males. These findings are in line with the higher prevalence of comorbidities, including congenital anomalies, among ASD females,[6,8,88] and with our previous report about sex differences in prenatal head growth in children with ASD.[21] These findings are also in line with the reported higher prevalence of genetic abnormalities in ASD females compared to ASD males,[88–92] and with the known more severe manifestation of ASD in females.[8,88,93] Altogether, these evidence are consistent with theories about diverged aetiologies of ASD in males and females.[88,94,95]

This study is the first to systematically examine organogenesis in foetuses later developing into children with ASD by exploiting retrospectively the foetal ultrasound anatomy survey. The use of two 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 foetal growth (e.g. sex[2,21] and shared genetics among siblings[1,96,97]), making our findings more compelling.

Study limitations

The results of this study should be considered in the context of the following limitations. Less than half of the children with ASD at the NARCI database had prenatal ultrasound data and therefore included in the study sample. This may result in a selection bias of children in the study sample that were different from the other children in the database in parental age, ethnicity and ADOS score. In addition, we used a case-sibling-control design to minimize the number of confounders affecting the result of the study. Indeed, no significant differences were found between cases and controls in a range of socio-demographic and clinical characteristics. Yet, the associations between UFAs and ASD found in our study could still be confounded by other unmeasured variables. Additional limitations includes the use of ultrasound scans from pregnancy centres in the community and not in a dedicated research lab, which may add some noise to the raw data. Nevertheless, all ultrasound anatomy scans in the study were conducted by experienced physicians according to strict guidelines, which reduce heterogeneity. Finally, despite the large size of the study cohort, consisting of over 650 children, it still lacked sufficient statistical power to enable us to draw conclusions about rare UFAs (e.g. UFAs in the cerebellum, cisterna magna, great arteries, and gastrointestinal system) or about variables with a significant fraction of missing data such as biometric measures and clinical severity.

Conclusions

The association of UFAs with ASD, especially in the urinary system, heart, head and brain, sheds important light on the abnormal multiorgan embryonic development of this complex disorder. Given the novelty of these findings, they need to be conirmed in further studies before considering UFAs for clinical use as ultrasonography markers for ASD.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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