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Association between rare, genetic variants linked to autism and ultrasonography fetal anomalies in children with autism spectrum disorder

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Abstract

Background   Recent evidence suggests that certain fetal anomalies detected upon prenatal ultrasound screenings are associated with autism spectrum disorder (ASD). In this cross-sectional study, we aimed to identify genetic variants associated with fetal ultrasound anomalies (UFAs) in children with ASD.

Methods   The study included all children with ASD who are registered in the database of the Azrieli National Center of Autism and Neurodevelopment and for whom both prenatal ultrasound and whole exome sequencing (WES) data were available. We applied our in-house integrative bioinformatics pipeline, *AutScore*, to these WES data to prioritize rare, gene-disrupting variants (GDVs) probably contributing to ASD susceptibily. Univariate statistics and multivariable regression were used to assess the associations between UFAs and GDVs identified in these children.

Results   The study sample comprised 126 children, of whom 43 (34.1%) had at least one UFA detected in the prenatal ultrasound scan. A total of 87 candidate ASD genetic variants were detected in 60 children, with 24 (40%) children carrying multiple variants. Children with UFAs were more likely to have loss-of-function (LoF) mutations (aOR = 2.55, 95%CI: 1.13–5.80). This association was particularly noticeable when children with structural anomalies or children with UFAs in their head and brain scans were compared to children without UFAs (any mutation: aOR = 8.28, 95%CI: 2.29–30.01; LoF: aOR = 5.72, 95%CI: 2.08–15.71 and any mutation: aOR = 6.39, 95%CI: 1.34–30.47; LoF: aOR = 4.50, 95%CI: 1.32–15.35, respectively). GDVs associated with UFAs were enriched in genes highly expressed across all tissues (aOR = 2.76, 95%CI: 1.14–6.68). There was a weak, but significant, correlation between the number of mutations and the number of abnormalities detected in the same children ($r = 0.21$, $P = 0.016$).

Conclusions   The results provide valuable insights into the potential genetic basis of prenatal organogenesis abnormalities associated with ASD and shed light on the complex interplay between genetic factors and fetal development.

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Background

Autism spectrum disorder (ASD) is a multifactorial neurodevelopmental disorder with a remarkably heterogeneous clinical presentation and a wide range of associated clinical symptoms [1–3]. At present, ASD diagnosis is determined after birth [4], but a growing body of evidence suggests that in some cases initial signs of ASD can be detected *in utero* [5–12]. Indeed, several studies have reported that certain fetal abnormalities detected in standard prenatal ultrasound screening are associated with ASD [5, 7–11, 13–16].

Our group recently completed two studies that explored abnormalities in fetal development associated with ASD. In the first, using prenatal ultrasound biometric data from the second and third trimesters of gestation, we showed that children later diagnosed with ASD had abnormal fetal head growth compared with other fetuses [5]. In the second study, which focused on prenatal ultrasound data from the late anatomy survey conducted during mid-gestation, we showed that children later diagnosed with ASD had higher rates of fetal structural anomalies and fetal soft markers than their non-ASD counterparts [11, 17]. This ASD-specific excess of ultrasonography fetal anomalies (UFAs) was mainly seen in the head and brain, the heart, and the urinary system. Taken together, these findings suggest genetic or other gestational predisposition (i.e. maternal viral exposure) [18] to abnormal fetal development associated with ASD.

It is widely accepted that genetic factors play a significant role in ASD susceptibility [19–23]. In the past two decades, advances in next-generation sequencing (NGS) technologies have facilitated the identification of hundreds of rare genetic variants implicated in ASD susceptibility [24–28]. Importantly, some of the genetic variants associated with ASD have also been associated with various congenital diseases and malformations. For example, facial and head abnormalities usually occur in ASD-associated genetic syndromes that involve the *CHD8* [29, 30] and *ADNP* [31, 32] genes and genes in the *22q11.2* region [33]. Furthermore, comorbidity of ASD and congenital renal abnormalities may be found in people with Phelan-McDermid syndrome [34, 35], 17q12 microdeletion syndrome [13, 14, 36], or 16q24.2 deletions [16], while comorbidity of ASD and congenital heart defects is associated with 22q11.2 deletion syndrome [33] and with a set of genes involved in chromatin organization [37]. Taken together, these findings suggest shared molecular mechanisms underlying both ASD predisposition and abnormal embryonic organogenesis of various body parts.

Despite the emerging literature suggesting a range of fetal anomalies associated with ASD, the specific mechanisms underlying this association are still vague. In this study, we investigated whether genetic variations associated with ASD are also associated with fetal anomalies in these children. In addition, we looked for the genetic variations associated with the specific fetal anomalies associated with ASD (i.e., those found in the heart, urinary system, and head&brain) [11].

Materials and methods

Study design

We conducted a cross-sectional study comprising all children diagnosed with ASD who are registered in the database of the Azrieli National Center of Autism and Neurodevelopment (ANCAN) [38], who are members of Clalit Health Services (CHS; the largest health services provider in Israel), and for whom both prenatal ultrasound and whole exome sequencing (WES) data are available (Fig. 1). Enrolment to the ANCAN database is conducted at the time ASD diagnosis is given to children in one of the associated clinics of ANCAN [38]. Then, participation in additional studies conducted at ANCAN is offered to the children's families. Approximately, one-third of these families consent to participate in the genetic study that involves WES analysis of the child and his/her parents.

ASD diagnosis

All ASD patients registered in the ANCAN database undergo the same multidisciplinary diagnosis process, which entails a comprehensive intake interview (sociodemographic and clinical factors), a behavioral evaluation with ADOS-2 [39], and a full neurocognitive assessment using either the Bayley Scales of Infant and Toddler Development - third edition (Bayley-III) [40] or the Wechsler Preschool and Primary Scale of Intelligence - version three (WPPSI-III) [41], all translated into Hebrew as described previously [38, 42]. In addition, the level of language development is assessed using the Preschool Language Scale - fifth edition (PLS-5) [43]. The final diagnosis of ASD is made by a pediatric psychiatrist or neurologist, according to DSM-5 criteria [44].

Fetal ultrasound and birth data

The study is based on prenatal ultrasound data from the fetal anatomy survey that is routinely conducted in Israel during gestational weeks 20–24. The fetal ultrasound data were obtained from all the prenatal ultrasound clinics of the CHS in southern Israel (the intake area for ANCAN).
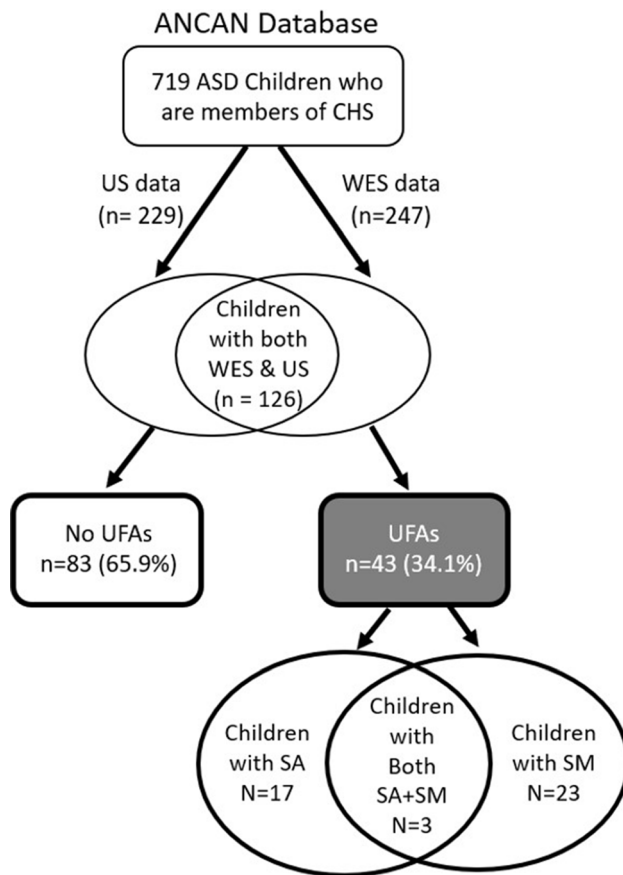
Fig. 1 Flowchart of children included in this study. ANCAN - Azrieli National Center for Autism and Neurodevelopment Research; ASD - autism spectrum disorder; CHS -Clalit Health Services; SA - structural anomalies; SM - soft markers; US - ultrasound; WES - whole exome sequencing

In these clinics, fetal anatomy surveys are performed by experienced physicians, who record fetal anomalies and biometric measures according to standard clinical guidelines [45, 46]. The ultrasound screening includes the examination of different anatomical landmarks according to the various body systems, including the head, brain, thorax, abdomen, spine, limbs, and umbilical cord. Any abnormalities in each examined organ are classified as structural anomalies or soft markers [45–47]. It should be noted that soft markers are not equivalent to structural anomalies; they may represent normal variations or transient conditions and are frequently not included in clinical reports or utilized in clinical decision-making. The list of abnormalities detected can be seen in Supplementary Table S1. Additional measurements of the fetal head and brain include head circumference, biparietal diameter, size of the cisterna magna, and lateral ventricle width [45, 48]. For the current study, the gestational age of each fetus was calculated from the last menstrual period (LMP) and confirmed by the crown-rump length (CRL) in the ultrasound scan obtained in the first

trimester. If the LMP date was not known, gestational age was calculated based on CRL.

Birth characteristics of the children included in the study were obtained from the database of the Division of Obstetrics and Gynecology, Soroka University Medical Center (SUMC). The birth characteristics of children born outside of SUMC were obtained from the "Ofek" database, which includes medical data from most hospitals in Israel.

Identification of candidate ASD genetic variants

WES was performed on DNA purified from saliva samples collected from the affected children and their parents with Genotek OG-500 and OG-575 saliva collection kits, as described previously [49]. An in-house integrative bioinformatics pipeline, *AutScore* [50], was then applied to the WES data to prioritize rare, gene-disrupting variants (GDVs) probably contributing to ASD susceptibly. *AutScore* anlyzes each candidate GDV through a variety of bioinformatics tools and databases and subsequently assigns a summary score to each GDV, based on its predicted clinical pathogenicity, its inheritence characteristics, and the relevance of the affected gene to ASD or other neurodevelopmental disorders. Genetic variants with *AutScore* ≥11 were included in this study as candidate ASD genetic variants.

Storage of the genetic data, data processing, and the detection of GDVs were conducted on a high-performing computer cluster in a Linux environment using Python version 3.5 and R Studio version 1.1.456.

Statistical analysis

We divided the study sample into children with (Cases) and without (Controls) any UFAs in their ultrasonography anatomical surveys as described in their medical records, and then further divided the UFA group into those with soft markers and those with structural anomalies (Fig. 1). Differences in sociodemographic and clinical characteristics between the study groups were tested using appropriate univariate statistics while adjusting for multiple testing using the Bonferroni correction. Spearman's correlation was used to assess the correlation between the number of mutations and the number of UFAs in the sample. Logistic regression was used to assess the association between the identified GDVs and the existence of any UFA as well as the existence of different types of UFAs while adjusting for the sex of the children.

We used data from the Genotype-Tissue Expression (GTEx) Multi Gene Query in the GTEx Portal [51] to assess the level of transcripts per million (TPM) of genes with identified variants in different tissues. Then, we applied the GTEx Multi Gene Query genetic clustering [51, 52] to identify four clusters of gene expression

patterns. Finally, we assessed the association of different types of UFAs with these clusters using logistic regression, as described above.

All statistical 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.

RESULTS

Sample characteristics
The study sample included 126 children with ASD (6 sibling pairs and 114 singletons) for whom both WES and ultrasound data were available (Fig. 1). The children in the study sample had a relatively lower age at diagnosis and a higher ADOS score compared to other children with ASD in the ANCAN database (Supplementary Table S2). Of the 126 children, 43 (34.1%) had at least one reported UFA in their anatomical ultrasonography surveys; of these 43 children, in turn, 17 (13.5%) had UFAs defined as structural anomalies, 23 (18.3%) had UFAs defined as soft markers, and 3 children (2.4%) had both structural anomalies and soft markers. In the remaining 83 children (65.9%), no UFAs were detected in the anatomical ultrasonography survey.

Table 1 presents the sociodemographic and clinical characteristics of the study sample, including a comparison of these characteristics between children with and without UFAs. The study sample comprised 77.8% males and 73.8% children of Jewish ethnicity, without significant differences between cases and controls. There were also no significant differences between children with and without UFAs in terms pregnancy, birth, and ultrasound details, and clinical severity characteristics. Of note, children in the soft marker group had slightly higher, although not statistically significant, cognitive and language abilities compared to children with structural markers and children without UFAs (82.4±19.1 vs. 74.8±13.3 and 74.8±16.4, respectively, for IQ score, and 82.2±25.3 vs. 57.7±11.5 and 61.7±20.6, respectively, for PLS score).

Genetic findings
Overall, 87 GDVs with *AutScore*≥11 were identified in 60 children in the study sample (children with both ultrasound and WES data). Of these, 23 children (38.3%) carried more than one GDV. A list of these GDVs, their genetic characteristics, and their associated UFAs is given in Supplementary Table S3. Seventy-two of the detected GDVs were inherited (69 dominant, and 3 recessive), while 15 GDVs were de-novo variants. In terms of functional consequences, 43 variants were classified as loss-of-function (LoF) mutations (i.e., frameshift, stop gain/loss, and splice acceptor/donor variants), while the remaining 44 variants were missense mutations. The genetic characteristics of GDVs with *AutScore*≥11 did

not vary significantly between children with and without ultrasound data (Supplementary Table S4).

Association between UFAs and candidate ASD genetic variants
UFAs in children with ASD were associated with different characteristics of detected GDVs (Table 2). Specifically, children with UFAs were 2.5 times more likely to carry LoF mutations than children without UFAs (aOR=2.55, 95%CI: 1.13–5.80). This association was particularly noticeable when comparing children with structural anomalies or children with head and brain UFAs to children without UFAs (any mutation: aOR=8.28, 95%CI: 2.29–30.01; LoF: aOR=5.72, 95%CI: 2.08–15.71 and any mutation: aOR=6.39, 95%CI: 1.34–30.47; LoF: aOR=4.50, 95%CI: 1.32–15.35, respectively). Of note, given the significant overlap between structural anomalies and head and brain UFAs (Kappa=0.38, *P* value<0.001), the significant associations of genetic variants with these types of UFA are not mutually exclusive. In addition, there was a weak, but statistically significant, correlation between the number of detected GDVs and the number of detected UFAs in each child (*r*=0.21, *P*=0.016).

Association between UFAs and gene expression patterns
Figure 2 shows the tissue expression patterns of genes affected by the detected GDVs according to the GTEx Portal [51]. We selected the largest four clusters of co-expressed genes (≥10 genes per cluster) and examined their association with the detected UFAs in the study sample (Table 3). The genes of *cluster 4* that are highly expressed across all tissues were significantly more prevalent in children with any UFA (aOR=2.76, 95%CI: 1.14–6.68), and specifically in children with structural anomalies (aOR=4.07, 95%CI: 1.47–11.30) and UFAs in the head and brain (aOR=4.50, 95%CI: 1.31–15.42). Genes in *cluster 3*, which were characterized by moderate expression across all tissues, were more likely to be mutated in children with UFAs, specifically soft markers, in the urinary system, but these associations were not statistically significant (aOR=2.73 95%CI: 0.86–8.62; and aOR=3.31, 95%CI: 0.89–12.38, respectively).

Discussion
In this study, we examined the association between UFAs and GDVs in children with ASD. Approximately one-third of the study sample had UFAs in their fetal anatomy survey, a similar rate to the rate found in children with ASD in our previous study [11]. Furthermore, mutations in candidate ASD genes were mostly associated with structural anomalies in the head and brain, a finding that is consistent with the results of multiple genetic studies reporting a probable genetic cause of different head and

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

Variable		No UFAs (Controls) (n=83)	Any UFA (Cases) (n=43) SM (n=26)[*]	SA (n=20)[*]	P values[#]	
Sociodemographic background						
Ethnicity (Jewish)	No. (%)	60(72.3)	33(76.7)		1.00 [a]	
			20(76.9)	14(70.0)	1.00	1.00
Sex (male)	No. (%)	68(81.9)	30(69.8)		0.360 [a]	
			17(65.4)	15(75.0)	0.228	1.00
Pregnancy and birth details						
Mother's age (years)	Mean±SD	29.4±6.3	29.3±5.5		1.00[b]	
			28.4±5.7	30.5±4.7	1.00	1.00
Gestational age at birth (weeks)	Mean±SD	38.9±2.1	39.1±1.6		1.00[b]	
			39.5±1.4	38.5±1.7	0.933	1.00
C section	No. (%)	19(25.0)	13(32.5)		1.00 [a]	
			6(23.1)	8(47.1)	1.00	0.249[c]
Birth weight (g)	Mean±SD	3181±611	3165±563		1.00[b]	
			3073±541	3169±603	1.00	1.00
1-min low APGAR score (<7)	No. (%)	7(20.6)	3(14.3)		1.00[a]	
			2(15.5)	1(9.1)	1.00	1.00
Ultrasound details						
Gestation age assessed by last menstrual period	No. (%)	50(70.4)	25(73.5)		1.00[a]	
			15(68.2)	13(86.7)	1.00	1.00[c]
Gestational age at ultrasound (weeks)	Mean±SD	23.7±3.3	23.5±2.9		1.00[b]	
			22.9±1.9	24.3±3.6	0.291	1.00
Breech presentation on ultrasound scan	No. (%)	22(28.2)	10(25.6)		1.00[a]	
			8(30.8)	3(18.8)	1.00	1.00[c]
Normal amniotic fluid	No. (%)	80(96.4)	41(95.3)		1.000 [c]	
			24(92.3)	19(95.0)	1.00	1.00
Clinical severity						
Diagnosis age (years)	Mean±SD	2.7±1.1	3.1±1.3		0.363 [b]	
			3.1±1.4	3.0±1.0	0.459	1.00
Cognitive score (IQ)	Mean±SD	74.8±16.4	80.3±16.6		0.659 [b]	
			82.4±19.1	74.8±13.3	0.477	1.00
PLS score	Mean±SD	61.7±20.6	74.5±24.1		0. 237 [b]	
			82.2±25.3	57.7±11.5	0.141	1.00
ADOS Comparison Score	Median (IQR)	8(6–9)	8.5(6.75-10)		0.783 [d]	
			8.5(6.25–9.75)	8.5(6.75-10)	0.627	
DSM5-A (Social Domain), no. (%)	RS	6(8.3)	4(12.5)		1.00[a]	
			3(15.8)	2(12.5)		
	RSS	25(34.7)	8(25.0)			
			3(15.8)	6(37.5)	0.693	1.00
	RVSS	41(56.9)	20(62.5)			
			13(68.4)	8(50.0)		
DSM5-B (Restrictive-Repetitive Domain), No. (%)	RS	8(11.1)	4(12.5)		1.00[a]	
			3(15.8)	1(6.3)		
	RSS	36(50.0)	16(50.0)			
			9(47.4)	9(56.3)	1.00	1.00
	RVSS	28(38.9)	12(37.5)			
			7(36.8)	6(37.5)		

ADOS=Autism Diagnostic Observation Schedule; RS=requiring support; RSS=requiring substantial support; RVSS=requiring very substantial support; SM=soft marker; SA=structural anomaly; UFAs=ultrasonographic fetal anomalies

[*]Three children had both structural anomalies and soft markers

[#]*P* values for the comparison between the control group (No UFA) and the three case groups (Any UFA, SA, and SM) based on the following tests: [a] Chi-square; [b] Two-sided t-test; [c] Fisher; [d] Mann-Whitney. All *P* values are Bonferroni corrected for multiple comparisons (*N*=3)

Table 2 Association between UFAs and genetic mutations

UFA type	Mutation type		N(%)	Adjusted odds ratio (aOR) [a]	95% CI
Any UFA (N=43)	Any mutation	No	16(24.2)	Ref.	
		Yes	27(45.0)	**2.57**	**1.19–5.52**
	Loss of function	No	26(28.3)	Ref.	
		Yes	17(50.0)	**2.55**	**1.13–5.80**
	De novo	No	38(33.6)	Ref.	
		Yes	5(38.5)	1.09	0.34–3.66
Structural anomalies (N=20)	Any mutation	No	3(4.5)	Ref.	
		Yes	17(28.3)	**8.28**	**2.29–30.01**
	Loss of function	No	8(8.7)	Ref.	
		Yes	12(35.3)	**5.72**	**2.08–15.71**
	De novo	No	17(15.0)	Ref.	
		Yes	3(23.1)	1.65	0.41–6.74
Soft marker (N=26)	Any mutation	No	14(21.2)	Ref.	
		Yes	12(20.0)	0.90	0.37–2.18
	Loss of function	No	19(20.7)	Ref.	
		Yes	7(20.6)	0.97	0.36–2.63
	De novo	No	23(20.4)	Ref.	
		Yes	3(23.1)	0.95	0.23–3.94
Head and brain (N=12)	Any mutation	No	2(3.0)	Ref.	
		Yes	10(16.7)	**6.39**	**1.34–30.47**
	Loss of function	No	5(5.4)	Ref.	
		Yes	7(20.6)	**4.50**	**1.32–15.35**
	De novo	No	9(8.0)	Ref.	
		Yes	3(23.1)	3.46	0.79–15.23
Urinary system (N=14)	Any mutation	No	4(6.1)	Ref.	
		Yes	10(16.7)	3.09	0.91–10.50
	Loss of function	No	10(10.9)	Ref.	
		Yes	4(11.8)	1.08	0.31–3.73
	De novo	No	13(11.5)	Ref.	
		Yes	1(7.7)	0.54	0.06–4.64
Heart (N=18)	Any mutation	No	10(15.2)	Ref.	
		Yes	8(13.3)	0.85	0.31–2.32
	Loss of function	No	12(13.0)	Ref.	
		Yes	6(17.6)	1.42	0.48–4.16
	De novo	No	17(15.0)	Ref.	
		Yes	1(7.7)	0.40	0.05–3.39

Ref. = Reference group

[a] Logistic regression, adjusted for child's sex

Boldface type indicates statistically significant aOR at $a < 0.05$

brain congenital anomalies in ASD children. For example, three children with fetal macrocephaly (a structural anomaly) in our study sample had genetic mutations in genes previously linked to this phenotype. One of these genes is *CEP290*, a gene linked to Joubert Syndrome, which has been associated with multiple fetal anomalies, including macrocephaly, increased biparietal diameter, and ventriculomegaly, on fetal MRI and ultrasound [53]. In addition, two children in our sample, one with a mutation in *PTPN11*, and the other with mutations in *TSC2* and SRCAP, also exhibited macrocephaly (a structural anomaly), as reported previously [54–57]. Furthermore, one child in our sample with a de-novo mutation

in *MTOR*, which is a key member in the mTOR pathway that regulates cell growth and proliferation [58, 59], also exhibited fetal microcephaly (a structural anomaly). It has been reported for animal models that alterations in the mTOR pathway lead to microcephaly during prenatal life as a result of reduced cell number and cell size [58]. Moreover, a recent study on ASD children found an increased risk of microcephaly in mTOR variant carriers as compared to carriers of mutations in non-mTOR genes [59]. Finally, three children with ventriculomegaly (a structural anomaly) in our sample had genetic mutations in *DPYD*, *NAA15*, and *SEZL6*. Consistent with these findings, ventriculomegaly has previously been
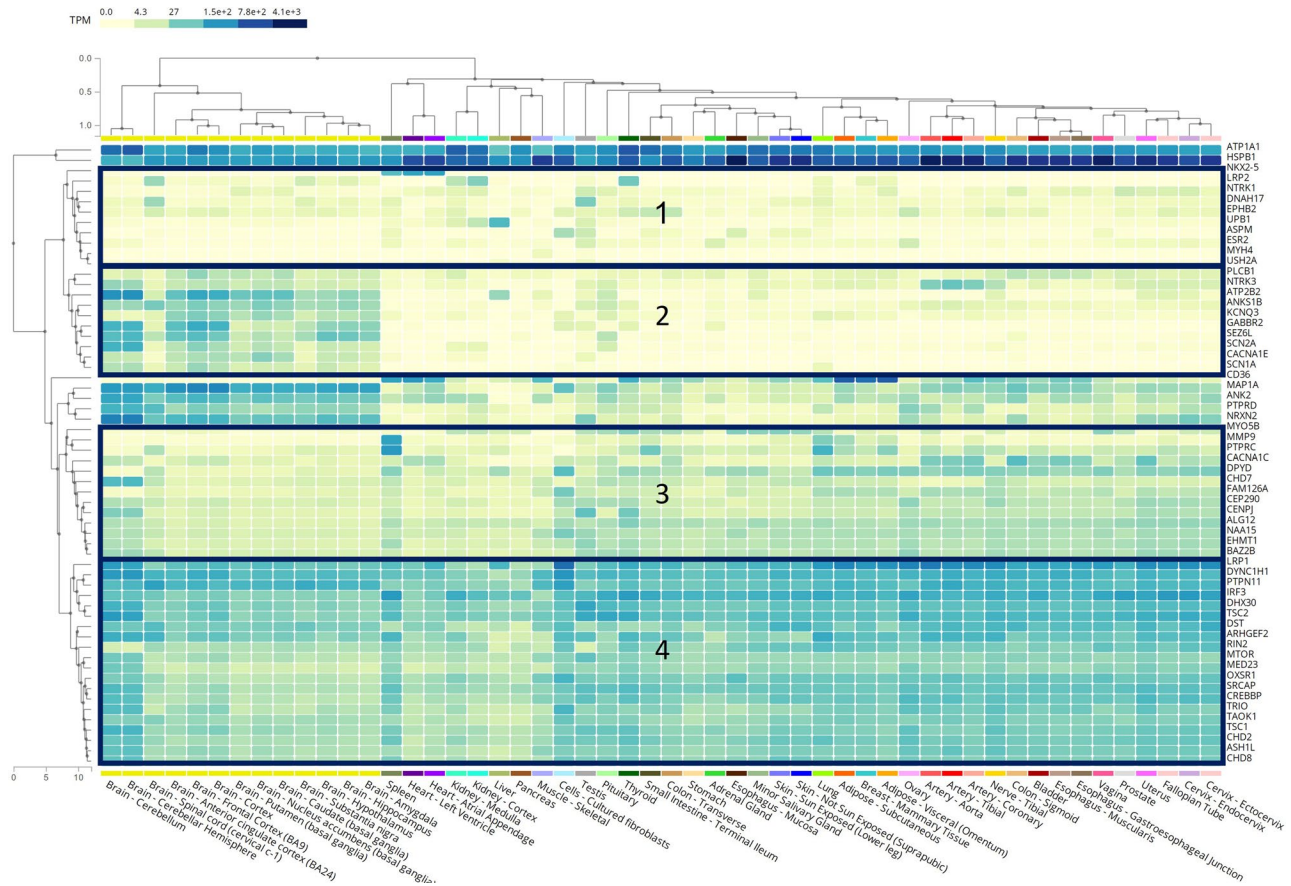
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Fig. 2 Heatmap of gene expression in different organs. The numbers represent the 4 gene clusters

reported in a child with a *DPYD* mutation [60] as well as in children with a 4q31.1 deletion and 16p11.2 duplications, which include the *NAA15* and *SEZL6* genes, respectively [61, 62].

Our sample included children from several multiplex ASD families. For two siblings with ASD with the same missense/dominant genetic variant in *ANK2*, there were UFAs in their urinary systems. The scan of the older child, who also had a LoF mutation in *MMP9*, exhibited pyelectasis (a soft marker), while that of the younger one showed a polycystic kidney (a structural anomaly). *ANK2* mutations are associated with an increased number of excitatory synapses with augmented axonal branching, which supports the presence of altered connectivity and penetrant behavioral impairments in humans carrying the ASD-related *ANK2* mutation [63]. In addition, *ANK2* is located on the 4q deletion, previously linked to congenital anomalies affecting different body systems, including developmental delay, cardiac involvement and polycystic kidney or isolated kidney [64]. Importantly, *MMP9*, which was disrupted in one of these siblings, is known to be involved in prenatal kidney organogenesis and thus may contribute to the upper urinary tract dilatation [65, 66]. In another multiplex family, the fetal ultrasound scan

for a child with missense/dominant mutations in both *CHD7* and *CHD8* genes showed a single kidney (a structural anomaly). For this child, two older siblings with fetal ultrasonography hydronephrosis and dilated bowels died shortly after birth. *CHD8* and its paralog *CHD7* have been previously linked to the CHARGE syndrome in which renal anomalies have been described [12, 67]. In addition, in studies on both animals and humans, mutations in *CHD8* were characterized by macrocephaly due to expansion of the forebrain/midbrain and a number of distinct facial characteristics [29], as well as genitourinary abnormalities [68].

We showed that the association between UFAs and GDVs is particularly relevant to genes that are broadly expressed in all body tissues (*cluster 4* in our analysis). Previous studies indicated that most early prenatal ASD regulatory genes are broadly expressed in multiple organs in addition to the developing prenatal brain, suggesting that broadly expressed regulatory genes might be a major driver of prenatal neural maldevelopment in ASD and might also affect the development and function of other organs and tissues [6, 69–71]. Fifteen of the twenty genes comprising *cluster 4* in our analysis (75%) were also reported in ASD genetic studies to be broadly expressed

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Table 3 Association between UFAs and genetic clusters

UFA type	Mutation type		*N* (%)	Adjusted odds ratio (aOR) [a]	95% CI
Any UFA	Cluster 1	No	42(36.5)	Ref.	
(*N*=43)		Yes	1(9.1)	0.16	0.02–1.32
	Cluster 2	No	37(33.0)	Ref.	
		Yes	6(42.9)	1.64	0.52–5.14
	Cluster 3	No	34(30.9)	Ref.	
		Yes	9(56.3)	2.87	0.98–8.44
	Cluster 4	No	29(29.3)	Ref.	
		Yes	14(51.9)	**2.76**	**1.14–6.68**
Structural anomalies	Cluster 1	No	119(16.5)	Ref.	
(*N*=20)		Yes	1(9.1)	0.50	0.06–4.14
	Cluster 2	No	16(14.3)	Ref.	
		Yes	4(28.6)	2.46	0.68–8.86
	Cluster 3	No	16(14.5)	Ref.	
		Yes	4(25.0)	1.95	0.56–6.81
	Cluster 4	No	11(11.1)	Ref.	
		Yes	9(33.3)	**4.07**	**1.47–11.30**
Soft markers	Cluster 1	No	25(21.7)	Ref.	
(*N*=26)		Yes	1(9.1)	0.32	0.04–2.73
	Cluster 2	No	23(20.5)	Ref.	
		Yes	3(21.4)	1.18	0.30–4.43
	Cluster 3	No	20(18.2)	Ref.	
		Yes	6(37.5)	2.73	0.86–8.62
	Cluster 4	No	21(21.2)	Ref.	
		Yes	5(18.5)	0.89	0.30–2.68
Head and brain	Cluster 1	No	12(10.4)	Ref.	
(*N*=12)		Yes	0(0.0)	NA	NA
	Cluster 2	No	10(8.9)	Ref.	
		Yes	2(14.3)	1.73	0.34–8.92
	Cluster 3	No	9(8.2)	Ref.	
		Yes	3(18.8)	2.58	0.62–10.77
	Cluster 4	No	6(6.1)	Ref.	
		Yes	6(22.2)	**4.50**	**1.31–15.42**
Urinary system	Cluster 1	No	14(12.2)	Ref.	
(*N*=14)		Yes	0(0.0)	NA	NA
	Cluster 2	No	12(10.7)	Ref.	
		Yes	2(14.3)	1.52	0.30–7.76
	Cluster 3	No	10(9.1)	Ref.	
		Yes	4(25.0)	3.31	0.89–12.38
	Cluster 4	No	9(9.1)	Ref.	
		Yes	5(18.5)	2.41	0.72–8.06
Heart	Cluster 1	No	17(14.8)	Ref.	
(*N*=18)		Yes	1(9.1)	0.55	0.07–4.63
	Cluster 2	No	16(14.3)	Ref.	
		Yes	2(14.3)	1.07	0.22–5.31
	Cluster 3	No	16(14.5)	Ref.	
		Yes	2(12.5)	0.82	0.17–3.98
	Cluster 4	No	14(14.1)	Ref.	
		Yes	4(14.8)	1.10	0.33–3.68

Ref. = Reference sample

[a] Logistic regression, adjusted for child's sex

Boldface type indicates statistically significant aOR at a < 0.05.

during prenatal life in the fetal body and the fetal brain, causing disruptions of cell proliferation, neurogenesis, migration, and cell fate, and consequently affecting prenatal brain development [6, 71] These findings suggest that mutations affecting normal organ development are predominantly expressed during prenatal life and lead to abnormal neurodevelopment and organogenesis, which can be detected on prenatal ultrasound.

We also noticed a weak, but statistically significant, correlation between the number of UFAs and number of GDVs in our sample. Such a correlation may be indicative of a cumulative effect of genetic variations on fetal development. Multiple genetic mutations may lead to more pronounced disruptions of organogenesis, resulting in multiple UFAs. This conclusion highlights the complexity of genetic factors involved in prenatal organ development and suggests that the risk of UFAs in ASD may be influenced by a combination of genetic factors rather than individual mutations alone. Indeed, previous studies found that ASD children with multiple mutations in known ASD genes demonstrate more severe ASD phenotypes (e.g., worse cognitive ability, symptom impairment, and more seizures), suggesting that multiple gene-disruptive events may co-occur in probands and act synergistically or additively to lead to a more severe phenotype [57, 72, 73]. In addition, a study that explored prenatal-stage regulatory ASD risk genes showed that many of them are upstream regulators of key signaling pathways (e.g., RAS/ERK, PI3K/AKT, WNT/β-catenin) and that the degree of dysregulation in these signaling pathways is associated with the severity of ASD symptoms [6, 74]. Thus, higher dysregulation in the signaling pathways due to multiple genetic mutations may cause more severe ASD alongside higher rates of abnormal organ development.

Limitations
This study has several limitations. First, most (~80%) of the GDVs included in our analyses were inherited from one parent (dominant inheritance). Although the contribution of heritable factors to ASD susceptibility is well established [75, 76], evidence for specific dominantly inherited mutations that are robustly associated with ASD is limited. Nonetheless, we decided to include inherited dominant mutations in our analyses with the aim of increasing the number of GDVs and, subsequently, the statistical power of this study. Of note, the associations found between dominantly inherited variants and UFAs in this study were also seen for de-novo variants and UFAs, but the latter did not reach statistical significance due to their small numbers. Although there is some evidence of the role of inherited dominant mutations in ASD susceptibility, further studies are needed to confirm the clinical relevance of these variants to ASD. Also, as our study focused on rare, GDVs, our findings

apply to a smaller subset of the ASD population harboring rare variants and may not generalize to all children with ASD, since the vast majority of genetic susceptibility is predicted to arise from inherited common variants of low effect size. In addition, the study relied on ultrasound data collected retrospectively from the CHS database. Although these data are based on ultrasound anatomy scans conducted by experienced physicians according to strict clinical guidelines, variation between physicians can still occur. Since we didn't have information about the physician conducting each ultrasound examination, we could not account for such potential bias, in our analyses. Also, since the study is based on the anatomy survey conducted during gestational weeks 20–24, UFAs that evolve after this gestational period would not be seen at the time of this ultrasound and thus, were not included in this study. It should also be noted that while soft markers are examined for and documented by physicians as part of the anatomy survey, they may also be considered as normal variants or transient. Thus, care should be taken in the association between soft markers and ASD. Also, the current study lacked sufficient statistical power to draw conclusions about specific UFAs. Finally, our study is focused on a sample of the Israeli population, which may limit the generalizability of the findings to other populations.

Conclusions
Our findings suggest a potential genetic basis for some fetal anomalies associated with a later diagnosis of ASD, thus shedding light on the complex interplay between rare, genetic variants and fetal development. Notably, since other non-genetic factors (i.e., in-utero viral infection) could also underlie such associations, future studies are needed to address the pathogenetic pathways from the particular causes through their intermediate fetal presentation to the subsequent neurodevelopmental outcomes.

Abbreviations

ANCAN	Azrieli National Center of Autism and Neurodevelopment
ASD	Autism spectrum disorder
CHS	Clalit Health Services
CRL	Crown-rump length
GDV	Gene-disrupting variants
GTEx	Genotype-Tissue Expression
LMP	Last menstrual period
LoF	Loss of function
NGS	Next-generation sequencing
PLS	Preschool Language Scale
SA	Structural anomaly
SM	Soft marker
SUMC	Soroka University Medical Center
UFA	Ultrasonography fetal anomalies
WES	Whole exome sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s11689-024-09573-6.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Data availability

WES data were generated as part of the ASC and are available in dbGaP with study accession: phs000298.v4.p3. The fetal ultrasound results are available upon reasonable request to the corresponding author Prof. Idan Menashe (idanmen@bgu.ac.il).

Declarations

Ethics approval and consent to participate

The study was approved by the SUMC Ethics Committee per the Declaration of Helsinki SOR 295 – 18.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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