

RESEARCH ARTICLE

# Placental gross shape differences in a high autism risk cohort and the general population

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**OPEN ACCESS**

**Citation:** Park BY, Misra DP, Moye J, Miller RK, Croen L, Fallin MD, et al. (2018) Placental gross shape differences in a high autism risk cohort and the general population. PLoS ONE 13(8): e0191276. <https://doi.org/10.1371/journal.pone.0191276>

**Editor:** Jefferson Terry, BC Children's Hospital, CANADA

**Received:** March 16, 2017

**Accepted:** January 2, 2018

**Published:** August 22, 2018

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**Data Availability Statement:** All relevant data are available at: <https://idea.library.drexel.edu/idea7593dataset> and DOI [10.15154/1421920](https://doi.org/10.15154/1421920).

**Funding:** This work was supported in part by NICHD Contracts HHSN275200503414C, HHSN275200503411C, HHSN275200603416C, HHSN275200503415C, HHSN275200503413C, HHSN275200503410C, and HHSN275200503396C NIH- NCS LOI 2-BIO-18. The funder provided support in obtaining placental morphology measures but did not have any

<sup>†</sup> A complete list of consortium members is provided in the Acknowledgments.

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## Abstract

A growing body of evidence suggests that prenatal environment is important in Autism Spectrum Disorder (ASD) etiology. In this study, we compare placental shape features in younger siblings of children with ASD, who themselves are at high ASD risk, to a sample of low risk peers. Digital photographs of the fetal placenta surface and of the sliced placental disk from 129 high ASD risk newborns and from 267 newborns in the National Children's Study Vanguard pilot were analysed to extract comparable measures of placental chorionic surface shape, umbilical cord displacement and disk thickness. Placental thickness measures were moderately higher in siblings of ASD cases. The placentas of ASD-case siblings were also rounder and more regular in perimeter than general population placentas. After stratification by sex, these across-group differences persisted for both sexes but were more pronounced in females. No significant differences were observed in cord insertion measures. Variations in placental shape features are generally considered to reflect flexibility in placental growth in response to changes in intrauterine environment as the placenta establishes and matures. Reduced placental shape variability observed in high ASD risk siblings compared to low-risk controls may indicate restricted ability to compensate for intrauterine changes.

additional role in the study design, data collection and analysis decision to publish or preparation of the manuscript. Dr. Carolyn Salafia was employed by Placental Analytics, LLC. The funder provided support in the form of salaries to Dr. Carolyn Salafia, but did not have any additional role in the study design, data collection and analysis decision to publish or preparation of the manuscript. The specific roles of these authors are articulated in the 'author contributions' section.

**Competing interests:** Dr. Carolyn Salafia is employed by Placental Analytics. Affiliation to Placental Analytics, LLC does not alter our adherence to PLOS ONE policies on sharing data and materials (as detailed online in our guide for authors <http://journals.plos.org/plosone/s/competing-interests>).

## Introduction

Autism Spectrum Disorders (ASD) are neurodevelopmental conditions that manifest as impaired social communication and the presence of restricted/repetitive behaviors and interests [1]. In United States, 1 in 68 eight year old children have ASD [2]. ASD symptoms emerge in early childhood and the associated disabilities are typically lifelong [3]. Converging evidence suggests that the changes in the brain associated with ASD are initiated during prenatal brain development [4–7] but the complete nature of these changes remains unknown.

The placenta is vital to fetal growth and development and plays a key role in maintaining the homeostasis of the intrauterine environment. Fetal and/or placental cell inflammatory response [8], gene expression [9], gene-environment interaction [10], and placental morphology [11–13] have been used as measures of abnormal placental function [14] and have been shown to be associated with reduced fetal growth and adverse neonatal outcomes, including death and neurological morbidities [15]. The human placental formation begins soon after implantation and continually modifies to accommodate the increasing demands of the fetus and can be influenced by factors such as nutrients and smoking [16]. The *ex utero* placenta is a universally accessible organ that is suitable for research into possible indicators of prenatal environment, however, the placenta is generally discarded as medical waste.

A typical placenta is described as round or oval but can have various shapes [17]. Variant shapes, cord insertion sites and placental disk thickness (reflecting later villous branching arborization) have been associated with reduced placental efficiency [12]. Irregular placental chorionic surface shapes have been linked to low birthweight [18], however, it is also rare to have perfectly round placentas with central umbilical cord insertion [19] and placental shape variability may be the norm [12]. Examination of placental morphology has also yielded insights into mechanisms of pre-eclampsia, prematurity and fetal growth restriction [13, 20], which are conditions also associated with increased ASD risk [21]. Increased ASD risk have been associated with indicators of decreased placental function, such as abnormal placental pathology (trophoblast inclusion) [22] and obstetric complications (preeclampsia/eclampsia) [23].

Here we compare placental shape features in a cohort of children at higher risk for ASD, by virtue of the fact that they have older ASD-affected siblings, to a population of typical-risk peers.

## Materials and methods

### Sample collection

The National Children's Study (NCS) Vanguard recruited pregnant women during 2009–2010 in 7 locations in the United States (Orange County, California; Queens County, New York; Duplin County, North Carolina; Montgomery County, Pennsylvania; Salt Lake and Cache County, Utah; Waukesha County, Wisconsin; multiple counties in South Dakota/Minnesota) using a multistage area probability sampling design to identify study locations (generally, counties) for house-hold based recruitment [24]. As such, the children in this sample would be expected to experience the typical ASD risk of the general U.S. population. The latest CDC prevalence studies suggest that 1.5% of US children have an ASD [2].

Formative research projects were conducted based on the NCS Vanguard study, one of which was focused on collection and analysis of placental samples. Of 831 enrolled and eligible pregnant women, 267 mothers consented and provided placental samples. However, in the pilot phase of placental collection, there was no attempt to collect demographic and perinatal data, which left a subgroup of 180 mothers with both placenta and linked demographic and

perinatal data. However, in not all instances were all perinatal data available. Gestational age information, which had to be abstracted from medical records, was available for 31 participants.

The Early Autism Risk Longitudinal Investigation (EARLI) network of research sites (Southeast Pennsylvania: Drexel University, The Children’s Hospital of Philadelphia and the University of Pennsylvania; Northeast Maryland: Johns Hopkins University and Kennedy Krieger Institute; Northern California: University of California Davis) enrolled a group of mothers of children with ASD diagnoses at the start of a subsequent pregnancy and is documenting the development of the newborn child (sibling of the ASD case) through three years of age [25]. EARLI singleton birth placentas from 133 mothers were available. Four subjects were excluded because placenta morphology measures could not be obtained and 129 placentas were included in the analysis. The study was approved by multiple Institutional Review Boards (IRB) listed below:

Mt. Sinai Institutional Review Boards, University of Rochester IRB, UC Davis IRB, Medical College of Wisconsin IRB, University of North Carolina IRB, University of Utah IRB, UC Irvine IRB, South Dakota State University IRB, University of Pennsylvania IRB, Northwestern University IRB, Columbia University IRB, University of Illinois at Chicago IRB, Baylor University IRB, University of Arkansas IRB, University of Iowa IRB, University of Massachusetts IRB, Duke University IRB, and Drexel University IRB.

### Placenta morphology measures

Placentas were fixed with formalin in uniform sealable bags for one week. A uniform protocol of placental gross measurement developed by the pathology group collected a range of placental gross measures that reflect three facets of placental shape: the chorionic plate surface shape and irregularity; umbilical cord displacement; and disk thickness (Table 1). Digital photographs were obtained of the placental chorionic surface shape which included the site of the umbilical cord insertion, and of the sliced placental disk. The chorionic surface shape and cord insertion, or the edge of the placental disk slice, were marked as a “layer” in GIMP (GNU Image Manipulation Program). These layers were then analyzed using dedicated algorithms as

**Table 1. Placenta morphology measure definitions.**

Morphology	Definition
Perimeter (cm)	perimeter of the placenta from the traced 2D fetal surface image
Area (cm <sup>2</sup> )	area of the placenta from the traced 2D fetal surface image
Radius (cm)	radius from geometric center to the perimeter of the placental disk measured per degree
Median	median radius of the placenta based on 360 radii
Minimum	minimum radius of the placenta based on 360 radii
Maximum	maximum radius of the placenta based on 360 radii
Umbilical distance from center (cm)	distance between umbilical cord insertion and geometric center of the placenta from the traced 2D fetal surface image
Eccentricity	
Umbilical cord	maximum radius/minimum radius from cord insertion point of the disk
Geometric center	maximum radius/minimum radius from geometric center of the disk
Thickness (cm)	thickness of the placenta f each vertical pixel pair of the disk slice
Maximum	maximum thickness of the disk slice
Mean	mean thickness of the disk slice
Standard deviation	standard deviation of thickness of the disk slice

<https://doi.org/10.1371/journal.pone.0191276.t001>

described previously [12, 26, 27]. Thickness measures could not be obtained in three EARLI and 84 NCS samples. All measurements were obtained by a trained pathologist blinded to the study group.

Several placental morphology measures, such as placental disk eccentricity, were skewed. Wilcoxon rank-sum test was used to compare all perinatal characteristics and placental morphology between EARLI and NCS. Spearman’s rho was used to examine the correlation between placental shapes and perinatal characteristics. Chi squared test was used to test for difference between categorical variables. An alpha-error tolerance of 5% ( $P < 0.05$ ) was used to determine statistical significance of comparisons. Wilcoxon rank-sum test was also used to determine if differences in EARLI and NCS placentas varied significantly within sex. Two way ANOVA was used to examine whether any association between placental morphology and cohort differed by the sex of the subject. Birthweight and gestational age have been associated with placental size and ASD risk. Birthweight was not available in 8 (6%) EARLI subjects and gestational age was not available in 88% of NCS subjects. Sex was missing in one EARLI subject and was excluded from the sex stratified analysis. Spearman’s correlation was used to determine correlation between birthweight, gestational age and placental morphology. All analyses were performed using STATA 12 [28].

## Results

Siblings of children diagnosed with ASD were similar in sex ratio, birthweight, and gestational age compared to NCS subjects in the subset with perinatal data (Table 2). Correlations between birthweight and placenta morphologies were in same direction across sexes except for the umbilical distance from center (Spearman’s rho -0.12 to 0.47) with strongest correlation observed with area and birthweight among females (Spearman’s rho 0.47 [0.39, 0.58]) (Table 3). Gestational age and placental shapes correlations showed more variation in direction and magnitude across sexes (Spearman’s rho -0.16 to 0.33) with strongest correlation observed between gestational week and median radius among males (Spearman’s rho 0.33 [0.21, 0.43]) (Table 3).

EARLI placentas had smaller, but non-statistically significant differences in, median radius, umbilical cord distance from center, and eccentricity from umbilical cord compared to NCS placentas (Table 4). We did not observe statistically significant differences between the average placental disk thickness across EARLI and NCS placentas ( $P = 0.71$ ), although maximum thickness ( $P = 0.01$ ) and standard deviation of thickness ( $P = 0.01$ ) were significantly greater in EARLI subjects. Finally, eccentricity, the placenta measures that capture shape deviation from a circle, based on the geometric center of the placenta, were significantly lower in EARLI than in NCS placentas ( $P = 0.04$ ). The eccentricity using umbilical cord insertion point showed the same directionality but were not statistically significant ( $P = 0.12$ ).

Table 5 shows the same comparisons stratified by sex. The minimum placental radius was greater in EARLI placentas compared to NCS among females ( $P = 0.05$ ) but not in males. The

**Table 2. Perinatal characteristics by study.**

	EARLI (n = 129)					NCS (n = 267)					P
	N	Mean	SD	Min	Max	n	Mean	SD	Min	Max	
Birthweight (g)	121	3454	532.7	2296	6039	180	3498	430.8	2190	4760	0.49
Gestational week	127	39.46	1.3	34	42	31	39.48	1.1	37	41	0.97
Male (%)	128	49%				180	50%				0.89

SD = Standard deviation; Min = minimum; Max = maximum.

<https://doi.org/10.1371/journal.pone.0191276.t002>

**Table 3. Spearman’s correlation (rho) between birthweight, gestational week and placental morphology by child sex.**

	Birthweight (g)				Gestational week			
	Males		Females		Males		Females	
	Rho	95% CI	rho	95% CI	rho	95% CI	rho	95% CI
Perimeter (cm)	0.34	[0.20, 0.43]	0.42	[0.33, 0.53]	0.26	[0.14, 0.37]	-0.09	[-0.21, 0.04]
Area (cm <sup>2</sup> )	0.37	[0.25, 0.46]	0.47	[0.39, 0.58]	0.26	[0.14, 0.37]	-0.12	[-0.23, 0.01]
Radius (cm)								
Median	0.22	[0.09, 0.32]	0.31	[0.21, 0.43]	0.33	[0.21, 0.43]	-0.11	[-0.23, 0.01]
Minimum	0.16	[0.04, 0.28]	0.15	[0.06, 0.3]	-0.02	[-0.14, 0.1]	0.09	[-0.03, 0.21]
Maximum	0.06	[-0.08, 0.17]	0.22	[0.12, 0.35]	0.22	[0.1, 0.34]	-0.16	[-0.28, -0.04]
Umbilical distance from center (cm)	-0.07	[-0.21, 0.03]	0.04	[-0.08, 0.17]	0.14	[0.02, 0.26]	-0.13	[-0.25, -0.003]
Eccentricity								
Umbilical cord	-0.12	[-0.24, -0.002]	-0.04	[-0.2, 0.05]	0.07	[-0.05, 0.19]	-0.16	[-0.27, -0.03]
Geometric center	-0.05	[-0.20, 0.04]	-0.03	[-0.2, 0.05]	0.07	[-0.05, 0.19]	0.02	[-0.1, 0.14]
Thickness (cm)								
Maximum	0.06	[-0.05, 0.19]	0.14	[0.05, 0.29]	0.13	[0.01, 0.25]	-0.05	[-0.17, 0.08]
Mean	0.05	[-0.06, 0.19]	0.10	[0.05, 0.29]	0.15	[0.02, 0.26]	-0.06	[-0.18, 0.06]
Standard deviation	0.06	[-0.07, 0.18]	0.06	[-0.07, 0.18]	0.06	[-0.02, 0.22]	-0.02	[-0.14, 0.11]

<https://doi.org/10.1371/journal.pone.0191276.t003>

eccentricity was greater in NCS among both males and females (smaller in EARLI placentas), but the difference remained statistically significant only for eccentricity from geometric center in females ( $P = 0.04$ ). Two placental thickness measures, maximum and standard deviation, were greater in EARLI placentas compared to NCS with each sex, however, only the maximum thickness showed significant difference among females ( $P = 0.02$ ). Two way ANOVA test of EARLI-NCS differences across sexes for any measure were not statistically significant.

**Table 4. Comparison of placental shape measures between EARLI and NCS using the Wilcoxon rank-sum test.**

	EARLI (n = 129)				NCS (n = 267)				P*
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Perimeter (cm)	61.85	7.68	45.00	92.40	61.54	6.59	48.60	93.00	0.73
Area (cm <sup>2</sup> )	274.40	53.04	156.00	440.00	272.10	49.76	175.00	496.00	0.62
Radius (cm)									
Median	10.09	1.23	7.40	13.90	10.13	1.27	6.97	15.80	0.85
Minimum	5.25	2.08	0.42	8.95	4.98	1.90	0.34	9.61	0.13
Maximum	13.55	2.31	9.46	19.50	13.75	2.27	8.51	22.50	0.37
Umbilical distance from center (cm)	3.48	2.07	0.23	8.95	3.68	1.96	0.04	10.50	0.27
Eccentricity									
Umbilical cord	4.14	5.84	1.23	40.67	4.45	7.01	1.23	51.88	0.12
Geometric center	1.40	0.26	1.11	2.64	1.45	0.33	1.11	3.51	0.04
Thickness (cm)**									
Maximum	2.34	0.34	1.71	3.76	2.24	0.33	1.52	3.52	0.01
Mean	1.74	0.27	1.26	2.78	1.72	0.27	1.08	2.87	0.71
SD	0.45	0.10	0.22	0.77	0.41	0.10	0.21	0.72	0.01

\*Determined using the Wilcoxon rank-sum test.

\*\*Thickness was available in 123 EARLI and 183 NCS samples.

SD = Standard deviation; Min = minimum; Max = maximum.

<https://doi.org/10.1371/journal.pone.0191276.t004>

Table 5. Comparison of placental morphology measures between EARLI and NCS by sex using the Wilcoxon rank-sum test.

	Male				P*	Female				P*
	EARLI (n = 64)		NCS (n = 90)			EARLI (n = 64)		NCS (n = 90)		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Perimeter (cm)	61.00	6.26	61.56	6.35	0.86	62.89	8.93	60.80	6.12	0.19
Area (cm <sup>2</sup> )	268.3	44.4	274.4	51.5	0.92	282.0	60.6	267.8	47.1	0.23
Radius (cm)										
Median	10.08	1.30	10.24	1.40	0.52	10.13	1.19	9.94	1.12	0.30
Minimum	4.91	2.15	4.88	1.92	0.82	5.55	1.99	5.05	1.70	0.05
Maximum	13.83	2.39	13.96	2.16	0.53	13.34	2.22	13.56	2.22	0.54
Umbilical distance from center (cm)	3.82	2.24	3.90	2.02	0.73	3.19	1.86	3.57	1.83	0.16
Eccentricity										
Umbilical cord	5.31	7.91	4.73	7.38	0.65	3.07	2.26	3.71	5.46	0.12
Geometric center	1.40	0.22	1.42	0.31	0.84	1.39	0.30	1.41	0.27	0.04
Thickness (cm)**										
Maximum	2.32	0.28	2.27	0.33	0.32	2.37	0.39	2.23	0.34	0.02
Mean	1.71	0.22	1.74	0.26	0.58	1.77	0.30	1.71	0.28	0.31
SD	0.46	0.11	0.42	0.09	0.02	0.43	0.10	0.41	0.10	0.12

\*Determined using the Wilcoxon rank-sum test.

\*\*Thickness was available among 62 male and 64 female EARLI samples.

SD = Standard deviation; Min = minimum; Max = maximum.

<https://doi.org/10.1371/journal.pone.0191276.t005>

## Discussion

Proper placental function is essential to normal fetal development and the shape and function of the placenta can be affected by variations in maternal vascular supply, location of uterine implantation, regional variation in the decidua, and structure of underlying placental vascular tree [11]. The relationship between placental morphology and fetal neurodevelopment is largely unknown.

As calculated from a large modern birth cohort with extensive photography images, the typical placental shape is circular with a center at the umbilical insertion point [12] and irregular placental shapes have been associated with lower birthweight [27]. However, perfectly round placentas with central umbilical cord insertion are uncommon [19] and some variability of placental shapes may be the norm [12]. Interestingly, our study of placental morphology suggested reduced variability in placental chorionic surface shapes in EARLI as compared to NCS placentas. We also observed that EARLI placentas had greater maximum thickness and standard deviation compared to NCS placentas. It is important to emphasize that the observed significant differences across EARLI and NCS placental morphology were modest in magnitude compared to the observed variation of each measures. Furthermore, our understanding of connections between placental morphology and neurodevelopment is still in the formative stages and we need additional studies to determine whether these differences are epiphenomena of a poor intratuterine environment or etiologically important mechanisms in neurodevelopment.

The placenta's morphology at the time of delivery may provide information on etiologically significant events occurring earlier in pregnancy. Comparisons of placental morphology from detailed ultrasonography data collected at 11–14 weeks gestation and placental measures obtained at term delivery show correlations from 0.14 (Pearson's r of umbilical cord displacement at 11–14 weeks and at term) to 0.25 (Pearson's r of maximal placental disk thickness at 11–14 weeks and the at term) [29]. Observed differences between siblings of ASD individuals



to general population in placental shapes may be reflecting differences in the maternal intra-uterine environments. Towards the end of the first trimester extensive villous remodeling takes place with onset of the maternal arterial circulation. Later in pregnancy, a wide variety of stressors are capable of affecting placental growth, the most common being nutrient deprivation and vascular compromise [30]. Mothers of ASD individuals may be exposed to more gestational stressors including inflammation and oxidative stress and/or carry genetic risk factors that interferes with proper response to changes in intrauterine environment.

This study is a comparison of placentas from mothers of children at higher risk of ASD to placentas from a general population of mothers and not a comparison of placentas from mothers of children with and without ASD. It is important to note that we are comparing high and low risk population and that the NCS children, although they are at low risk at developing ASD, may not all be typically developing children. The increased recurrence risk of ASD in younger siblings of older affected children mentioned above has been estimated to range from 3 to 25% [31, 32]. The latest estimate from the ASD Baby Sibs Research Consortium, a multi-site study with a similar case-confirmation approach to EARLI, is at the upper end of this range [32]. Recently, evidence has been presented from a small nested case-control comparison of 52 ASD cases and 161 controls from the ALSPAC (Avon Longitudinal Study of Parents and Children) general population pregnancy cohort study that suggests reduced eccentricity from cord insertion and reduced villous branching growth in those children who will receive an ASD diagnosis when compared to sex and gestational age matched peers [33]. The reduced variability in both ASD individuals and siblings of ASD may indicate a shared disadvantage of lesser adaptive capacity and greater vulnerability to gestational stressors.

Several obstetric complications that may reflect or induce an altered intrauterine environment, have been associated with ASD [23, 34–37]. Two of these ASD risk factors have been associated with both decreased placental thickness in pre-eclampsia [38] and increased thickness among intrauterine growth restriction [39]. If we assume that differences we observed in placentas from the at-risk cohort reflect differences that are relevant to ASD etiology, differences we observe in this study could be the result of obstetric complications. However, data on obstetric complications and other possible confounders in the NCS population were not available at the time of the current study and were not included in the analysis. This is a major limitation of this study and these findings must be considered as preliminary and need to be replicated.

Our findings suggest that there may be some gross morphological differences between general population and high ASD risk placentas and provide an initial indication that the ASD placenta might be less able to compensate for intrauterine variability. Morphological changes could be merely a marker for vulnerability to risk factors or it could be mechanistically important in ASD etiology if reduced compensatory capacity leaves the fetus more vulnerable to other stressors. Results need to be cautiously interpreted as this is a small initial study and multiple comparisons were done in an exploratory manner which could lead to false-positive findings. Future work should explore whether these placental morphological differences are associated with the ASD phenotype. Moreover, investigation on whether obstetric complications are associated with placental morphology would help clarify the placental morphology differences that are observed in the current study.

## Acknowledgments

**Disclaimer:** The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Children's Study, the National Institutes of Health, or U.S. Department of Health and Human Services.

The National Children's Study Placenta Consortium represents more than 100 investigators from twenty different institutions: University of Rochester (Lead Scientific Institution; Richard K Miller, PhD), Icahn College of Medicine at Mount Sinai (Philip Landrigan, MD), University of Illinois at Chicago (An Li, PhD), Columbia University (Ronald Wapner, MD), University of Utah (Edward Bowersox Clark, MD/Michael Varner, MD), University of California at Davis (Cheryl Walkers, MD), South Dakota State University (Bonnie Specker, PhD), The Children's Hospital of Philadelphia (Jane Culhane), University of North Carolina (Nancy Dole PhD, University of California at Irvine (James, University of Massachusetts/Duke University (Thomas Darrah, PhD), Baylor College of Medicine (Kirsti Aagaard, MD, PhD), University of Iowa (James Murrar, MD), University of Arkansas (Charlotte Hobbs, MD), Medical College of Wisconsin (Stevn Leuthner, MD /Sarah Szabo, MD), University of Wisconsin (Maureen Durkin, PhD), National Institutes of Health (John Moye, MD), Placenta Analytics (Carolyn Salafia, MD, MS), and Westat (Barbara O'Brien).

Special note of thanks for data set contributions to Christopher Stodgell, PhD, Philip Katzman, MD, Terri Gerardi, Ruchit Shah, MS, Luis Ruffalo, MD and Philip Weindenborner.

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