Maternal Obesity/Diabetes, Plasma Branched-Chain Amino Acids, and Autism Spectrum Disorder Risk in Urban Low-Income Children: Evidence of Sex Difference

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Maternal metabolic conditions are known risk factors for child autism spectrum disorder (ASD). Branched-chain amino acids (BCAAs) are also associated with ASD. We examined the joint associations of maternal metabolic conditions and BCAAs on the risk of child ASD and whether the associations differed by child's sex. We analyzed 789 mother–infant pairs, a subset of the Boston Birth Cohort, from a predominantly urban, low-income, minority population. Maternal plasma BCAAs were measured by liquid chromatography–tandem mass spectrometry in samples collected 24–72 hr postpartum. A composite BCAA score was created using factor analysis, and prepregnancy obesity and diabetes (ob/DM) were combined into one variable. Logistic regression was used to explore the role of BCAAs as mediators or cofactors with ob/DM and child's sex on ASD risk. BCAA-ob/DM and BCAA-sex interactions were also examined. Maternal BCAAs alone were not associated with ASD and did not mediate the path between ob/DM and ASD. In the presence of maternal ob/DM, BCAA score was significantly associated with ASD (adjusted OR 2.33, 95% CI 1.18, 4.60). Interactions were present for valine with ob/DM and for valine and isoleucine with male sex on ASD risk. The odds ratio (OR) for risk of ASD was the greatest with all three risk factors combined—male sex, above median BCAA score, and ob/DM (OR 10.79, 95% CI 4.40, 26.42). Similar patterns were found for other developmental disorders, though not as strong as for ASD. Additional studies are warranted to clarify the role of maternal BCAAs, ob/DM, and child's sex in ASD. ***Autism Res*** *2019, 00: 1–12.* © 2019 International Society for Autism Research, Wiley Periodicals, Inc.

Lay Summary: This study investigated whether maternal obesity/diabetes and maternal circulating branched-chain amino acids (BCAAs) can jointly affect child ASD risk and whether the associations differ by child's sex. We found that the risk of ASD was greater among mothers with obesity/diabetes who also had elevated concentrations of BCAAs and that this risk was even greater for male children. These findings provide new evidence on fetal origins of ASD and sex difference and warrant additional investigation.

Keywords: autism spectrum disorder; obesity; diabetes mellitus; pre- and perinatal risk factors; sex differences; branched-chain amino acids; metabolomics

Introduction

Autism spectrum disorder (ASD) is an array of neurodevelopmental conditions characterized by deficits in social interaction and communication and by restricted or repetitive interests and behaviors [Lai, Lombardo, & Baron-Cohen, 2014]. The etiology of ASD is complex and poorly understood. Though highly heritable, various environmental factors are also implicated in its pathophysiology [Modabbernia, Velthorst, & Reichenberg, 2017]. This study focuses on two major research gaps. First, maternal obesity and type 2 diabetes mellitus (DM) are important risk factors of ASD as demonstrated by us and others; however, the underlying molecular mechanisms are unclear [Lei, Li, Ou, & Li, 2018; Li et al., 2016; Wan, Zhang, Li, Luan, & Liu, 2018]. Since 20–40% of mothers in developed countries enter pregnancy obese, this is a matter of great urgency [Bahadoer et al., 2015; Kim, Dietz, England, Morrow, & Callaghan, 2007; McIntyre, Gibbons, Flenady, & Callaway, 2012]. Second, though ASD is much more

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common in males, with a male to female ratio of approximately 3:1, there is a lack of understanding of factors responsible for this disproportionate risk [Loomes, Hull, & Mandy, 2017].

Branched-chain amino acids (BCAAs)—leucine, isoleucine, and valine—are essential amino acids mainly found in animal-sourced foods and have important cellular signaling roles. Altered amino acids are known to be associated with obesity and DM [Guasch-Ferre et al., 2016; Rauschert, Uhl, Koletzko, & Hellmuth, 2014]. For example, elevated circulating BCAAs are predictive of incident type 2 DM, especially in an obese state [Newgard, 2012; Tai et al., 2010]. Studies also show links between BCAAs and ASD [Bent et al., 2018; Lussu et al., 2017; Zheng, Wang, Li, Rauw, & Baker, 2017]. However, the role of maternal circulating BCAAs in child ASD remains uncertain as the few metabolomics studies that exist were conducted in individuals with ASD rather than in an intergenerational setting. These studies were also small in sample size and reported inconsistent results. There is a lack of prospective birth cohort studies to assess the temporal relationship of maternal circulating BCAAs on child risk of developing ASD.

In light of persistently high rates of maternal obesity/DM (ob/DM) and growing evidence of BCAA involvement in ob/DM and ASD, we sought to investigate the joint associations of maternal ob/DM and maternal plasma BCAAs on child risk of ASD. Furthermore, we aimed to clarify the role of maternal BCAAs as mediators or cofactors in the pathway from maternal ob/DM to child ASD. Given the striking sex difference in ASD, we were also interested in exploring whether the aforementioned associations differ by the child's sex. We took advantage of the data from the Boston Birth Cohort (BBC), an intergenerational prospective cohort to illuminate the role of the prenatal metabolic environment—as assessed by both clinical measurements of maternal ob/DM and maternal plasma BCAA metabolites—in the development of ASD in children. This cohort was from a largely urban, low-income, minority population in the Boston area.

Methods

Participants and Data Collection Procedure

This study included 789 mother–infant pairs, a subset of the BBC, an ongoing study at the Boston Medical Center (BMC). The present study includes mothers recruited from 2004 to 2015 and children prospectively followed from 2004 to 2017. Details of BBC recruitment have been previously published [Li et al., 2016]. Both the initial and follow-up studies were approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center.

Briefly, mothers were recruited 24–72 hr post-partum, and written informed consent was obtained from the participants. Exclusion criteria included multiple gestation, pregnancies due to in vitro fertilization, and babies with chromosomal abnormalities or major birth defects. Only mothers with metabolite measurements and children receiving continued care at the BMC without other developmental disorders (DDs) were included in this study (Fig. S1). Maternal and infant medical records were reviewed using standardized abstraction forms and mothers were interviewed face-to-face using a standardized questionnaire. Maternal blood was collected at the time of enrollment during a non-fasted state and fractionated at the BMC. Liquid chromatography–tandem mass spectrometry was used to quantitatively profile maternal plasma metabolites at the Harvard-MIT Broad Institute Metabolite Profiling Laboratory.

Identification of Children with ASD

ASD was defined based on electronic medical records (EMR) containing clinicians' primary and secondary diagnoses using ICD-9 or ICD-10 codes from all child visits at the BMC, including primary care and subspecialty care (Table S1). Based on EMR ICD codes, children who were ever diagnosed with autism (299.00), Asperger syndrome (299.80), and/or pervasive DDs—not otherwise specified (299.90) constituted the ASD cases. Of note, while we did not conduct a systematic screen for ASD, all children with an ASD diagnosis were evaluated by highly trained staff at the BMC autism evaluation program who communicates regularly with the BMC primary care pediatricians. Children with attention deficit hyperactivity disorder (ADHD), developmental delays, or intellectual disabilities without ASD were classified as having other DDs. Children without any diagnosis of ASD or other DDs were classified as typically developing (TD).

Exposures

Maternal body mass index (BMI) was calculated using the prepregnancy weight and height obtained from the standardized questionnaire. Obesity was defined as BMI ≥30 kg/m^2. DM was identified by ICD-9 codes (250.00–250.93 for pregestational DM and 648.00 and 648.03 for gestational diabetes (GDM)). Because obesity and DM are highly correlated, they were combined into one variable and analyzed as a dichotomous variable (no obesity or DM vs obesity and/or DM), or a categorical variable ((a) no obesity or DM; (b) either obesity or DM; (c) both obesity and DM). Each maternal plasma BCAA was dichotomized into "below median" versus "median or above." A composite BCAA score was created using factor analysis and it was dichotomized in the same fashion.

Covariates

Maternal covariates included: age at delivery; race-ethnicity (black, white, Hispanic, or other); smoking during pregnancy ("never smoked," "ever smoked," or

"continuous smoking"); parity (nulliparous vs multiparous), and education ("high school or less" vs "some college or more"). Child covariates included: child's sex (female vs male); and gestational age and birthweight (categorized into four groups: (a) full term (≥37 completed weeks of gestation) and non-low birthweight (non-LBW; ≥2,500 g); (b) full term and LBW; (c) preterm and non-LBW; (d) preterm and LBW).

Statistical Analysis

Our central hypothesis was that maternal plasma BCAA concentrations and maternal metabolic conditions (obesity and/or DM) are jointly associated with child ASD risk. We also queried whether there is a sex difference in the association. As a first step, maternal and child characteristics for the ASD and TD groups were compared using *t*-tests for continuous variables and chi-squared tests for categorical variables. Missing values for categorical variables were incorporated into the largest sized group. The intensity levels of the metabolites were inverse-normally transformed to produce standardized distributions for all subsequent analyses, and any metabolite values below the limit of detection were imputed with one-half the limit of detection. A factor analysis score for the BCAAs (BCAA score) was calculated based on the three BCAAs using the Anderson–Rubin Method [Anderson & Rubin, 1956]. Multinomial logistic regression modeling was conducted to explore the association between ASD and maternal BCAA metabolites as well as the BCAA score.

We further evaluated for mediation and joint effects, including interaction. Mediation analysis of maternal BCAAs for the association of maternal ob/DM and ASD was conducted employing the hierarchical regression method [Baron & Kenny, 1986]. Joint effects of the BCAAs with ob/DM were also analyzed. Based on previous literature, we assigned children with below median maternal BCAA concentrations and without ob/DM as the reference group, and all three other groups (BCAAs below the median and any ob/DM, BCAAs above the median and no ob/DM, and BCAAs above the median and any ob/DM) were compared to it. We tested interactions between the BCAAs and ob/DM and between the BCAAs and child's sex using tests for additive and multiplicative interaction and the relative excess risk due to interaction [Knol et al., 2011]. Some stratified analyses were not adjusted for covariates due to non-convergence. All analyses were conducted in Stata v14.0 (Stata Corporation, College Station, TX) and RStudio v1.1.423 (RStudio, Inc., Boston, MA).

Results

A total of 789 mother–infant pairs from a predominantly urban, low-income, minority population were included in the analysis: 89 ASD children and 700 TD children. Children with ASD had co-occurring, non-mutually exclusive conditions, including ADHD (n = 36) and intellectual disabilities (n = 14). On average, mothers of children diagnosed with ASD were approximately 2 years older than mothers of TD children ($P < 0.01$) (Table 1). Prepregnancy BMI was significantly higher among mothers of ASD children than those of TD children (28.2 kg/m^2 vs 26.4 kg/m^2, $P < 0.05$) as was obesity status (34.8% vs 21.7%, $P < 0.01$). DM was also significantly more prevalent among mothers with ASD children than those with TD children (16.9% vs 9.6%, $P < 0.05$). More ASD children were male (34.5% point difference, $P < 0.0001$), born early preterm (16.5% point difference, $P < 0.0001$), and LBW (9.3% point difference, $P < 0.05$) compared to their TD counterparts. There were no significant differences in maternal parity, race-ethnicity, education, smoking status, or BCAA concentrations. Maternal and child characteristics for included and excluded participants are compared in Table S2.

We reported maternal and child characteristics by level of maternal plasma BCAAs for the overall sample (Table S3) and among male children only (Table S4). All three BCAAs were highly correlated with each other (Fig. S2), and there were no notable differences in the distribution of maternal BCAA score by child's sex (Fig. S3) nor by maternal ob/DM (Fig. S4).

When BCAAs were considered alone, there was no association between the BCAAs and child risk of ASD (Table S5), nor did they mediate the effect of ob/DM on this risk (Table S6). However, there was a synergistic effect of BCAAs and ob/DM—compared to mothers with no ob/DM and a low BCAA score, mothers with ob/DM and an above median BCAA score had greater than a twofold risk of having a child with ASD (odds ratio (OR) 2.33, 95% CI 1.18, 4.60) (Table 2). Conversely, mothers with ob/DM and below median BCAA concentrations had no significant risk of bearing a child with ASD (BCAA score OR 1.04, 95% CI 0.49, 2.20). Interactions between the BCAAs and ob/DM were significant for valine only on both the additive scale ($P < 0.01$) and multiplicative scale ($P < 0.01$). Stratified ORs are also presented, showing differences in ORs across the strata. Sensitivity analyses were consistent with our results (Tables S7-S10).

We observed similar patterns for the joint effects between maternal BCAA concentrations and child's sex on child ASD risk (Table 3). For the dually exposed category (male fetus and an above median maternal BCAA score) the BCAA score OR was 5.57, 95% CI 2.80, 11.10, while the OR for a male fetus with a below median maternal BCAA score was also significant but not as high (OR 2.99, 95% CI 1.45, 11.10). Conversely, the ORs of ASD risk for female fetuses were not significant, regardless of maternal BCAA level. Furthermore, when stratified by BCAA level, ORs for the association between child's sex and ASD outcome were notably

Table 1. Maternal and Child Characteristics by Child ASD Status (Typically Developing (TD) vs. ASD) in the Boston Birth Cohort

Characteristics	Total (N = 789)	TD (N = 700)	ASD (N = 89)	P-value[a]
Maternal age (years), mean (*SD*)[b]	28.10 (6.52)	27.86 (6.49)	29.95 (6.25)	0.004
Nulliparous, *n* (%)	348 (44.11)	313 (44.71)	35 (39.33)	0.335
Race or ethnicity, *n* (%)[c]				0.128
Black	554 (70.22)	500 (71.43)	54 (60.67)	
White	29 (3.68)	24 (3.43)	5 (5.62)	
Hispanic	150 (19.01)	126 (18.00)	24 (26.97)	
Other	56 (7.10)	50 (7.14)	6 (6.74)	
Maternal education, *n* (%)				0.952
Below college degree	675 (85.55)	600 (85.71)	75 (84.27)	
College degree or above	108 (13.69)	96 (13.71)	12 (13.48)	
Missing	6 (0.76)	4 (0.57)	2 (2.25)	
Maternal BMI, *n* (%)				
Mean (*SD*)	26.57 (6.66)	26.37 (6.46)	28.18 (7.97)	0.020
<25 kg/m^2	375 (47.53)	341 (48.71)	34 (38.20)	0.008
25–<30 kg/m^2	195 (24.71)	177 (25.29)	18 (20.22)	
≥30 kg/m^2	183 (23.19)	152 (21.71)	31 (34.83)	
Missing	36 (4.56)	30 (4.29)	6 (6.74)	
Maternal diabetes[d]				0.034
No diabetes	707 (89.61)	633 (90.43)	74 (83.15)	
Diabetes	82 (10.39)	67 (9.57)	15 (16.85)	
Maternal smoking, *n* (%)[e]				0.364
Never	675 (85.55)	603 (86.14)	72 (80.90)	
Quit	45 (5.70)	38 (5.43)	7 (7.87)	
Continuous	59 (7.48)	50 (7.14)	9 (10.11)	
Missing	10 (1.27)	9 (1.29)	1 (1.12)	
Child's sex, *n* (%)				<0.0001
Male	344 (43.60)	278 (39.71)	66 (74.16)	
Female	445 (56.40)	422 (60.29)	23 (25.84)	
Gestational age, *n* (%)				<0.0001
Term (≥37 weeks)	677 (85.80)	611 (87.29)	66 (74.16)	
Late preterm (34–36 weeks)	59 (7.48)	55 (7.86)	4 (4.49)	
Early preterm (<34 weeks)	53 (6.72)	34 (4.86)	19 (21.35)	
Birthweight				0.035
≥2,500 g	641 (81.24)	576 (82.29)	65 (73.03)	
<2,500 g	148 (18.76)	124 (17.71)	24 (26.97)	
Leucine (above median), *n* (%)	394 (49.94)	344 (49.14)	50 (56.18)	0.211
Isoleucine (above median), *n* (%)	393 (49.81)	347 (49.57)	46 (51.69)	0.707
Valine (above median), *n* (%)	392 (49.68)	340 (48.57)	52 (58.43)	0.080
BCAA score (above median), *n* (%)	388 (49.18)	338 (48.29)	50 (56.18)	0.161

SD, standard deviation.

[a]P-values were obtained from chi-square or t-test; missing values for categorical variables were incorporated with largest sized group.

[b]Maternal age at time of delivery.

[c]Black includes self- reported Black, African American, Haitian, Cape Verdean, and Caribbean race and ethnicities; other includes Asian and Pacific Islander races.

[d]Type II diabetes mellitus and/or gestational diabetes mellitus.

[e]Never smokers were defined as mothers with no history of smoking six months prior to conception or during pregnancy; some smoking includes mothers who smoked at some point in the window of six months prior to conception to delivery but did not smoke throughout that window; continuous is defined as mothers that smoked starting six months prior to and throughout pregnancy.

disparate, indicating effect modification by maternal BCAA level. Tests for interaction between the BCAAs and child's sex were significant on the additive scale, with the strongest BCAA score–ASD associations found for male children ($P < 0.05$). There was also evidence of multiplicative interaction for above median maternal isoleucine and male sex ($P < 0.05$).

The effect of maternal ob/DM on child risk of ASD also differed by child's sex. Compared to females with no maternal ob/DM, males with any maternal ob/DM had over a sevenfold increased risk for developing ASD when adjusting for key covariates (Table S11). Figure 1 further illustrates the association of BCAA score on ASD risk, overall and by sex (a.-c.) and when stratified by ob/DM status, overall and by sex (d.-f.). These associations are also shown for the individual BCAAs (Fig. S5). The crude risk of the child developing ASD was 10.8 times higher (95% CI 4.40, 26.42) with all three risk factors—above

Table 2. Association of Maternal Plasma Branch-Chained Amino Acids (BCAAs) and Risk of Child ASD—Joint Effect With Maternal Obesity/Diabetes (ob/DM)

	Leucine below median		Leucine above median		
Maternal obesity/DM	*N* ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	26/274	1.00 (Reference)	28/290	0.96 (0.53, 1.73)	1.02 (0.58, 1.79)
Any ob/DM	13/121	0.97 (0.46, 2.07)	22/104	**2.18 (1.15, 4.50)**	**2.23 (1.06, 4.69)**
OR (95% CI) ob/DM within strata of BCAA[a]		1.15 (0.57, 2.32)		**2.51 (1.36, 4.62)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (−0.003, 0.19); *P* = 0.059					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.44 (0.90–6.60); *P* = 0.080					
	Isoleucine below median		Isoleucine above median		
	N ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	28/285	1.00 (Reference)	26/279	0.86 (0.48, 1.56)	0.94 (0.54, 1.65)
Any ob/DM	15/111	1.16 (0.56, 2.39)	20/114	1.77 (0.89, 3.48)	1.36 (0.66, 2.82)
OR (95% CI) ob/DM within strata of BCAA[a]		1.43 (0.73, 2.80)		**2.07 (1.10, 3.88)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.06 (−0.04, 0.15); *P* = 0.247					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.77 (0.66–4.74); *P* = 0.258					
	Valine below median		Valine above median		
	N ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	27/283	1.00 (Reference)	27/281	0.95 (0.52, 1.72)	1.01 (0.58, 1.77)
Any ob/DM	10/114	0.72 (0.32, 1.62)	25/111	**2.65 (1.37, 5.14)**	**3.02 (1.38, 6.64)**
OR (95% CI) ob/DM within strata of BCAA[a]		0.91 (0.43, 1.95)		**2.73 (1.51, 4.97)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.14 (0.04, 0.23); *P* = 0.005					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 3.90 (1.38–11.06); *P* = 0.006					
	BCAA score below median		BCAA score above median		
	N ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI) BCAA within strata of ob/DM[a]
No ob/DM	26/281	1.00 (Reference)	28/283	1.05 (0.58, 1.89)	1.08 (0.61, 1.89)
Any ob/DM	13/120	1.04 (0.49, 2.20)	22/105	**2.33 (1.18, 4.60)**	**2.18 (1.04, 4.59)**
OR (95% CI) ob/DM within strata of BCAA[a]		1.19 (0.59, 2.41)		**2.41 (1.31, 4.45)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.08 (−0.01, 0.18); *P* = 0.096					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.15 (0.80–5.83); *P* = 0.131					

Note. ORs adjusted for maternal age, race/ethnicity, education, parity, smoking status, child's sex, and gestational age/birthweight unless otherwise noted. Bolded values are significant (P < 0.05).[a]

[a]Stratified ORs unadjusted.

median maternal BCAA concentrations, maternal ob/DM, and a male fetus—combined, compared to the reference group of females with below median maternal BCAA concentrations, and no maternal ob/DM (Fig. 2).

Similar to ASD, maternal BCAAs alone were not associated with other DDs (data not shown), and ob/DM was significantly associated with other DD ($P < 0.05$, data not shown). A trend was also observed in analysis of the association between joint risk factors—ob/DM and BCAAs—and other DD (BCAA score OR 1.52, 95% CI 1.06, 2.18, *P* for interaction = 0.06) (Table S12). Of note, this effect was mainly driven by leucine. As found in ASD, there was a sex difference observed for other DD as well (Tables S13-S14), with males at a higher risk than females (ob/DM OR among mothers with above median BCAA score: 1.88 95% CI 1.09, 3.24, *P* for interaction <0.05). Furthermore, the risk for other DD was also the highest with all three risk factors combined—male sex, maternal ob/DM, and above median maternal BCAAs—adjusted for key covariates (Fig. S6).

Discussion

Main Findings

Examined alone, maternal plasma BCAAs were not significantly associated with child ASD risk and did not mediate the risk conferred by maternal metabolic conditions. However, BCAAs did have significant joint effects with

Table 3. Association of Maternal Plasma Branch-Chained Amino Acids (BCAAs) and Risk of Child ASD—Joint Effect With Child's Sex

	Leucine below median		Leucine above median		
Child's sex	*N* ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI)BCAA within strata of sex[a]
Female	13/229	1.00 (Reference)	10/216	0.78 (0.32, 1.87)	0.81 (0.35, 1.88)
Male	26/166	**3.21 (1.55, 6.64)**	40/178	**5.52 (2.77, 11.00)**	1.56 (0.90, 2.70)
OR (95% CI) sex within strata of BCAA[a]		**3.09 (1.53, 6.21)**		**5.97 (2.89, 12.34)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (−0.002, 0.18); *P* = 0.056					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.21 (0.77, 6.31); *P* = 0.140					
	Isoleucine below median		Isoleucine above median		
	N ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI)BCAA within strata of sex[a]
Female	15/222	1.00 (Reference)	8/223	0.45 (0.18, 1.11)	0.51 (0.21, 1.24)
Male	28/174	**2.74 (1.37, 5.44)**	38/170	**4.22 (2.17, 8.19)**	1.50 (0.87, 2.58)
OR (95% CI) sex within strata of BCAA[a]		**2.65 (1.37, 5.13)**		**7.74 (3.50, 17.09)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.10 (0.01, 0.19); *P* = 0.033					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 3.43 (1.17, 10.07); *P* = 0.025					
	Valine below median		Valine above median		
	N ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI)BCAA within strata of sex[a]
Female	11/223	1.00 (Reference)	12/222	0.96 (0.40, 2.30)	1.10 (0.48, 2.55)
Male	26/174	**3.36 (1.57, 7.21)**	40/170	**6.42 (3.09, 13.31)**	**1.75 (1.01, 3.03)**
OR (95% CI) sex within strata of BCAA[a]		**3.39 (1.62, 7.06)**		**5.38 (2.72, 10.64)**	
Measure of interaction on additive scale: RERI (95% CI) = 0.09 (0.002, 0.18); *P* = 0.045					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.98 (0.69, 5.65); *P* = 0.202					
	BCAA score below median		BCAA score above median		
	N ASD/total	OR (95% CI)	*N* ASD/total	OR (95% CI)	OR (95% CI)BCAA within strata of sex[a]
Female	13/229	1.00 (Reference)	10/216	0.72 (0.30, 1.74)	0.81 (0.35, 1.88)
Male	26/172	**2.99 (1.45, 11.10)**	40/172	**5.57 (2.80, 11.10)**	1.70 (0.98, 2.94)
OR (95% CI) sex within strata of BCAA[a]		**2.96 (1.47, 5.95)**		**6.24 (3.02, 12.91)**	
Measure of interaction on additive scale: RERI (95% CI) = **0.10 (0.01, 0.19); *P* = 0.032**					
Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.58 (0.90, 7.42); *P* = 0.079					

Note. ORs adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, and gestational age/birthweight unless otherwise noted. Bolded values are significant (P < 0.05).

[a]Stratified ORs unadjusted.

maternal ob/DM and child male sex on increasing the risk of ASD. The presence of additive interactions between BCAAs and child's sex and between BCAAs and maternal ob/DM was consistent with our hypothesis regarding biological mechanisms. The combination of maternal ob/DM and below median BCAA concentrations was not associated with child risk of ASD, highlighting the strong influence of BCAA concentrations in mothers with ob/DM. While child male sex and maternal ob/DM are previously known risk factors of child ASD, our data suggest that elevated BCAA concentrations are associated with further increases in this risk. When examined altogether, the three risk factors conferred a nearly ninefold higher risk of child ASD. These effects were also observed with other DD though they were not as strong compared to ASD.

Interpretation

Over the past several years, multiple studies have brought into focus the associations between maternal ob/DM and child ASD [Buffington et al., 2016; Lei et al., 2018; Wan et al., 2018]. Other DDs, including ADHD and developmental delay, have also been associated with ob/DM [Ornoy, Reece, Pavlinkova, Kappen, & Miller, 2015; Sanchez et al., 2018]. While the exact biological mechanisms are not clear, metabolic conditions, like obesity and DM, are associated with chronic, systemic inflammation. This is reflected by increased

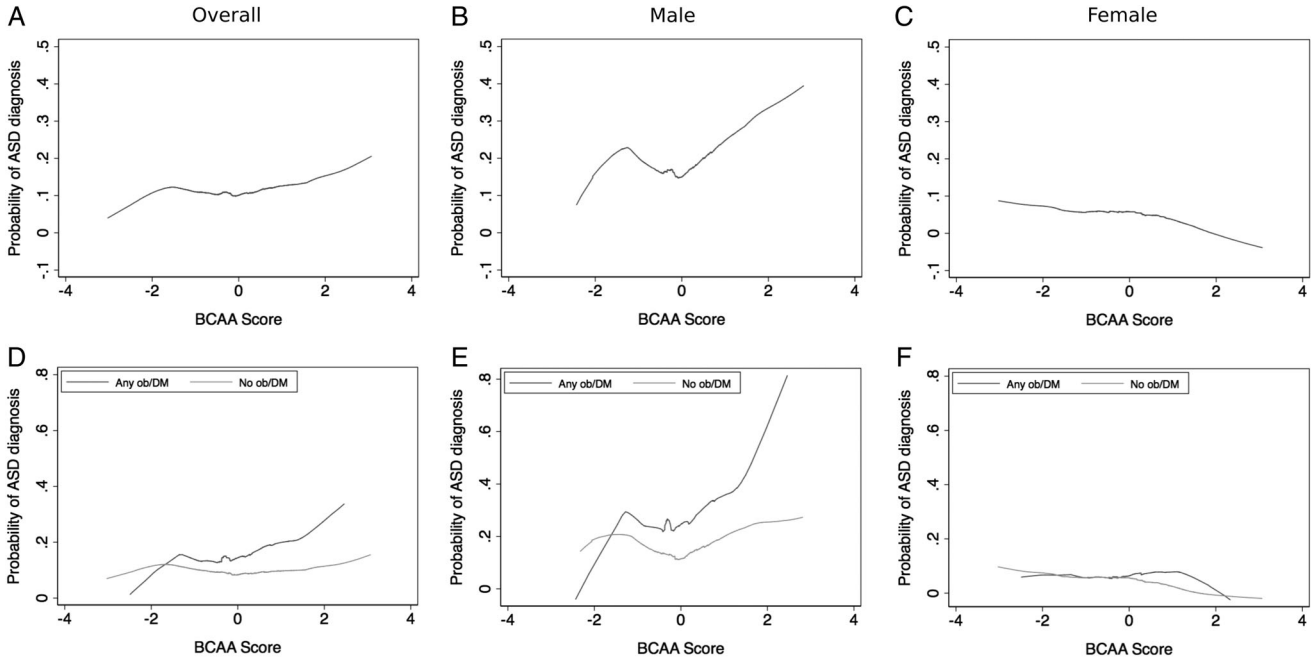

Figure 1. Association of maternal plasma branch-chained amino acid (BCAA) score with risk of child ASD and by obesity/diabetes (ob/DM) status, overall and by sex.

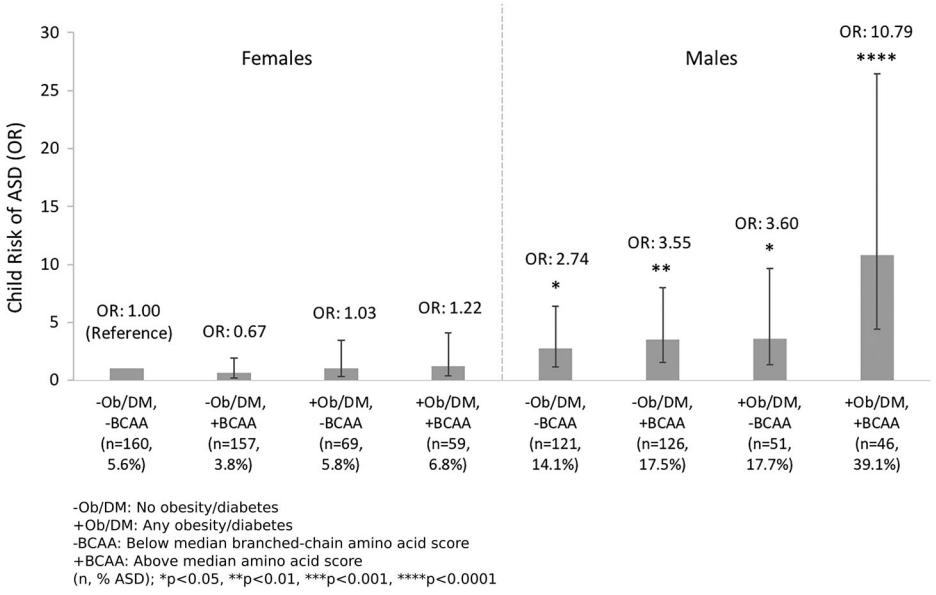

Figure 2. Joint association of maternal plasma branch-chained amino acid (BCAA) score, obesity/diabetes status, and child's sex on child ASD risk.

concentrations of circulating cytokines and proinflammatory markers, which can pass through the placenta and adversely affect the development of the fetal brain [Bilbo, Block, Bolton, Hanamsagar, & Tran, 2018; Jawerbaum & Gonzalez, 2006]. Insulin also plays a role in neurodevelopment, and maternal DM can cause dysregulation of insulin signaling in the fetal brain [Hami et al., 2015]. BCAAs are intricately involved in the cellular mechanisms underpinning obesity and DM [Chen, Francis, Hu, & Chen, 2018; Newgard, 2012]. For example, homeostasis model assessment-insulin resistance index is more strongly correlated with BCAAs than with fatty acids [Newgard, 2012]. Insulin resistance leads to a

decrease in BCAA catabolism and consequently an increase in circulating BCAAs. Conversely, elevated concentrations of BCAAs promote accumulation of fatty acids, which can result in insulin resistance [Chen et al., 2018].

There are a few studies showing the link between BCAA and ASD in children [Bent et al., 2018; Lussu et al., 2017; Zheng et al., 2017]. While exact mechanisms underlying the role of maternal BCAAs and BCAAs in children with ASD remain to be investigated, previous literature has raised several possibilities. One of these possibilities surrounds the mammalian target of rapamycin (mTOR) pathway, which mediates the relationship between BCAAs and insulin. mTOR is a kinase within a vast signaling network that regulates several key cellular functions, including cellular growth and energy balance [Zoncu, Efeyan, & Sabatini, 2011]. As the mTOR signaling pathway promotes synaptic protein synthesis, its dysregulation has been implicated in the etiology of ASD, in which pruning of neurons during development is inhibited [Liu, Talalay, & Fahey, 2016]. Chronic activation of mTOR can inhibit the cellular uptake of glucose and lead to insulin resistance, and BCAAs, especially leucine, are known inducers of mTOR [Gürke et al., 2015; Zoncu et al., 2011]. Though the studies on BCAAs and ASD were conducted on individuals with ASD, it is possible that elevated BCAA concentrations in mothers may induce mTOR activity in both the mother and fetus.

Another possibility is that elevated BCAA concentrations tip the balance to increased BCAA catabolism, which can overload mitochondria and lead to defective energy metabolism [Yoon, 2016]. This is especially true with an existing inflammatory condition such as obesity or DM. Disruption of beta oxidation is a precursor to mitochondrial dysfunction, a condition more prevalent in individuals with ASD and thought to be linked to its etiology [Frye & James, 2014; Liu et al., 2016]. ASD is more common among patients with propionic acidemia, a metabolic disorder marked by a deficiency in propionyl-CoA carboxylase (PCC), the enzyme responsible for the catabolism of BCAAs and other amino acids [de la Batie et al., 2018]. Insufficient PCC leads to disruptions in tricarboxylic acid cycle intermediates that feed into the mitochondrial respiratory chain. Altered BCAA concentrations are also associated with other mental health disorders. For example, treatment for major depressive disorder was shown to be more effective in individuals with lower BCAA concentrations, allowing for possible prediction of response to treatment via metabolic profile analysis [Kaddurah-Daouk et al., 2013]. Furthermore, supplementation with BCAAs in mice resulted in anxiety-like behavior, a common characteristic in several neurocognitive disorders [Coppola et al., 2013]. With both BCAAs and metabolic conditions linked to ASD via overlapping inflammatory pathways in utero, it is plausible that in combination they may contribute to a compounded risk of the disorder.

Male preponderance in ASD prevalence is well-established in the literature [Loomes et al., 2017]. Our findings suggest that sexual dimorphism in ASD is influenced by maternal obesity and DM in conjunction with maternal plasma BCAA concentrations and thus may begin in utero. A recent study showed a sex-specific effect in GDM-altered maternal metabolites in second trimester amniotic fluid [O'Neill et al., 2018]. The authors reported while other amino acids are reduced in the amniotic fluid milieu, leucine, along with methionine and tyrosine, was elevated with maternal GDM. Furthermore, leucine metabolites concentrations shifted more in the amniotic fluid of female fetuses than in their male counterparts. Our findings on sex differences may be explained by the theory that the female brain is better protected from inflammation in utero by higher concentrations of estradiol, leaving the male brain more vulnerable to inflammatory insults [Crider & Pillai, 2017; Patrick & Ames, 2014]. There may also be a higher threshold for genetic mutations in female fetuses before the disorder manifests [Jacquemont et al., 2014].

Strengths and Limitations

A particular strength of this study is its prospective, longitudinal, and intergenerational design. Additionally, we explored targeted maternal circulating metabolites specifically related to obesity and DM via metabolomic profiling. No other published work has reported on the association between maternal metabolites and child risk of ASD. More importantly, our study sheds light on potential maternal biomarkers in conjunction with obesity and DM and fetal sex on child risk of ASD.

As this study is the first of its kind to explore the association between maternal metabolites and child ASD, there were some limitations. The primary limitation of our study was that BCAAs were only measured at one timepoint using maternal blood samples collected 24–72 hr postdelivery. Along with the physical stress associated with delivery, mothers experience changes in protein and hormone homeostasis, as well as medications during the peripartum period. Amino acid concentrations at this time may not be reflective of a steady-state condition. Additionally, the measurement was taken in a non-fasted state. It is uncertain to what degree our observed maternal BCAA association with ASD was affected by the timing and the events peripartum. While it is known that children with ASD were more likely to be born preterm, the associations remained after adjusting for preterm birth.

Other limitations include the lack of data as to the BCAA status of the children at the time of ASD diagnosis, whether there was a family history of ASD, as well as additional medical histories from both parents. The BBC enrolment also spanned across the transition from the American Psychiatric Association's Diagnostic and Statistical Manual

fourth edition to the fifth edition and from ICD-9 to ICD-10. Since the definition of ASD changed during this time, there may be inconsistencies in diagnoses between these two periods. While we adjusted for known risk factors, we cannot exclude the possibility of residual confounding.

The results from this study may not be generalizable to other populations since our study population consisted of predominantly urban, low-income minorities. However, our study helps fill a major knowledge gap since this population is mostly underrepresented in ASD research. A larger sample size would have also facilitated further sex-specific and more detailed dose–response analyses. It would have also allowed for teasing apart the effects of obesity and DM alone since though their mechanistic pathways run in parallel and influence one another, they are distinct. Our grouping of BCAAs was based on the exploration of the functional relationship between the maternal BCAA score and child ASD risk shown in Figure 1. Our data suggest an overall monotonic positive association, and thus our cut off at the median was a compromise between the desire for precision and the limitation of sample size.

Of note, BCAA metabolite concentrations have mostly been reported as reduced in ASD subjects compared to TD controls [Lussu et al., 2017; Zheng et al., 2017]. While this may seem to contradict our findings, our study was different from previous studies in that we focused on maternal BCAAs rather than the child's postnatal BCAAs. Extrapolating from the "Barker Hypothesis" of early origins of disease, it is possible that high concentrations of BCAAs in the womb may cue the developing fetus to prepare for a similar external environment—an unlikely scenario [Barker, Eriksson, Forsen, & Osmond, 2002]. Another possible mechanism for reduced BCAAs in individuals with ASD could be a mutation in the branched-chain ketoacid dehydrogenase kinase, the rate-limiting enzyme in BCAA catabolism [West et al., 2014]. Additionally, sensitivity to certain tastes and textures often results in poor diet quality among the ASD population and may explain the altered concentrations [Mari-Bauset, Zazpe, Mari-Sanchis, Llopis-Gonzalez, & Morales-Suarez-Varela, 2014]. Another link between BCAAs and ASD is via the gut microbiome; specific bacteria in the gut are able to synthesize BCAAs de novo. Individuals with ASD are known to have a distinctly atypical gut microflora composition, and this could contribute to altered BCAA concentrations [Perng et al., 2014]. Conversely, others have demonstrated a twofold increase in concentrations of BCAA and greater mTOR activity in rabbit embryos of dams with insulin resistance and elevated circulating BCAAs compared to embryos of healthy dams [Gürke et al., 2015]. This research team concluded the BCAA composition of the blastocyst was reflective of maternal plasma and urine BCAA concentrations.

This study raises more questions than it could answer, and it will hopefully stimulate more clinical and mechanistic research in this area. Future studies may consider additional time-points for metabolite measurement, including preconception and early pregnancy to avoid the influence of factors associated with the stress of delivery. It would be ideal to simultaneously examine paired maternal and child cord blood metabolome in relation to child ASD risk. The examination of plasma sampled at additional time-points during early childhood will also provide us with a greater understanding of the changes in BCAA concentrations as the disorder manifests.

The findings of this study provide important insight into the role of the BCAAs in the pathway between maternal ob/DM and child risk of ASD. Mothers with obesity or DM with abnormal cholesterol levels or other metabolic conditions may be at an even greater risk for development of child ASD when combined with elevated BCAA concentrations. However, BCAAs almost certainly will not tell the entire story. As illustrated in Figure S7, BCAA levels in the plasma are significantly associated with those of several other amino acids. Further study is required to explore the role of these additional amino acids in the pathway from maternal ob/DM to child risk of ASD. Of course, other pathways are tightly linked to amino acid metabolism as well. For example, lipid metabolites may also play an integral role in these pathways. Thus, this present study is a powerful entry point into the metabolomic exploration of ASD pathophysiology.

This study also lends further support that ASD is potentially predictable and preventable. For example, investigators from a large-scale metabolomics study recently reported dysregulation of amino acid/BCAA metabolism in close to 17% of their ASD subjects and were also able to identify metabotypes of ASD with over 90% sensitivity and specificity [Smith et al., 2019]. Thus, there is potential for similarly identifying maternal metabotypes at risk for a child with ASD, especially those with the added risk factors of ob/DM and a male fetus. This will greatly enhance our ability to detect children at high risk of ASD at the earliest possible developmental windows, when interventions may be most cost-effective.

Conclusions

In this prospective birth cohort study, we found joint associations between maternal ob/DM and elevated maternal plasma BCAAs and between child male sex and elevated maternal plasma BCAAs on child risk of ASD. These synergistic effects suggest elevated maternal BCAA concentrations may further increase the risk of child ASD in the setting of maternal ob/DM and/or a male fetus. Additional metabolomics studies on maternal and cord plasma BCAAs are warranted to confirm and better understand mechanistic pathways underlying these joint associations. Such new insight may inform early prediction and early intervention strategies for ASD.

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Conflict of Interest

The authors do not have any conflicts of interest to disclose.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Flowchart of study sample included and excluded in the analyses

Figure S2. Correlations between maternal plasma branch-chained amino acids (BCAAs)

Caption: a. correlation between leucine and valine, b. correlation between leucine and isoleucine, c. correlation between valine and isoleucine

Figure S3. Distribution of maternal plasma branch-chained amino acids (BCAA) score overall and by child's sex

Caption: a. overall distribution of BCAA, b. distribution of BCAA among males, c. distribution of BCAA among females

Figure S4. Maternal plasma BCAAs stratified by maternal obesity/diabetes (ob/DM) status
Caption: a. leucine by ob/DM status, b. isoleucine by ob/DM status, c. valine by ob/DM status
Figure S5. Association of maternal plasma branch-chained amino acids (BCAAs) with risk of child ASD, overall and by child's sex
Caption: a. association between relative abundance in maternal plasma leucine and probability of child ASD diagnosis, b. association between relative abundance in maternal plasma leucine and probability of ASD diagnosis by child's sex, c. association between relative abundance in maternal plasma isoleucine and probability of ASD diagnosis, d. association between relative abundance in maternal plasma isoleucine and probability of ASD diagnosis by child's sex, e. association between relative abundance in maternal plasma valine and probability of ASD diagnosis, f. association between relative abundance in maternal plasma valine and probability of ASD diagnosis by child's sex
Figure S6. Joint association of maternal plasma branch-chained amino acid (BCAA) score, obesity/diabetes status, and child's sex on child other DD risk
Figure S7. Manhattan plot of associations between branched-chain amino acid (BCAA) score and other amino acids and derivatives
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Table S12. Association of maternal plasma branch-chained amino acids (BCAAs) and risk of other child developmental disorders (DD) - joint effect with maternal obesity/diabetes (ob/DM)
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Table S14. Association of maternal plasma branch-chained amino acids (BCAAs) and risk of other child developmental disorders (DD) - joint effect with maternal obesity/diabetes (ob/DM), among female children