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ORIGINAL PAPER

Maternal Dyslipidemia, Plasma Branched-Chain Amino Acids, and the Risk of Child Autism Spectrum Disorder: Evidence of Sex Difference

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Abstract

In contrast to the well-observed associations between obesity, diabetes, and autism spectrum disorder (ASD), the roles of maternal dyslipidemia and sex disparity in ASD have not been well-studied. We examined the joint associations of maternal plasma cholesterols, branched-chain amino acids (BCAAs) and child sex on child ASD risk. We analyzed data from 756 mother-infant pairs (86 ASD) from the Boston Birth Cohort. Maternal plasma cholesterols and BCAAs were measured in samples collected 24–72 h postpartum. We found that in this urban, low-income prospective birth cohort, low maternal high-density lipoprotein cholesterol (HDL-C), above-median maternal plasma BCAA concentrations, and male sex additively or synergistically increased risk of ASD. Additional studies are necessary to confirm our findings.

Keywords Autism spectrum disorder · Maternal cholesterols · Pre- and perinatal risk factors · Sex differences · Branched-chain amino acids · Metabolomics

Autism Spectrum Disorders (ASD) present a wide array of symptoms characterized by impaired social interaction, deficits in communication, and restricted and stereotyped behaviors and interests (Lai et al. 2014). ASD is a complex disorder and its etiology and underlying mechanisms remain unknown. While genetics is believed to play an important role, there are many environmental factors that have been associated with ASD (Modabbernia et al. 2017).

Cholesterols, within normal range, play important roles in brain function, mainly in regulating cell membrane permeability and the formation of synapses. Twenty-five percent of bodily cholesterol is found in the brain with 70% of brain cholesterol incorporated into myelin, the protective sheath for neuronal axons (Saher et al. 2005). Maternal circulating cholesterols can be transported across the placenta and play important roles during fetal neurodevelopment (Wild et al. 2015). For example, they are essential components of cellular membranes and also precursors for steroid hormones in the fetus. Disruptions in the level of certain cholesterols can result in adverse birth outcomes, including pre-term birth (Jiang et al. 2017). Abnormal maternal HDL-C and triglycerides measured post-delivery have been linked to attention deficit hyperactive disorder (ADHD) in offspring, the most common co-occurring condition with ASD (Ji et al. 2018).

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However, there are no studies reporting on the association between maternal dyslipidemia and development of ASD in the children.

We and others have previously shown that maternal obesity and diabetes both contribute to an elevated risk of child development of ASD, especially among male offspring (Lei et al. 2018; Li et al. 2016; Wan et al. 2018). Dyslipidemia is highly associated with obesity and diabetes and contributes to all definitions of the metabolic syndrome (Huang 2009). About a quarter of US women of reproductive age have higher than normal low-density lipoprotein cholesterol (LDL-C) levels (≥ 130 mg/dl) and 13% have lower than normal high-density lipoprotein cholesterol (HDL-C) levels (< 40 mg/dl) (Laz et al. 2013). The goal of this study was to examine the independent and joint effects of maternal dyslipidemia, branched-chain amino acids (BCAAs), and child sex on child risk of ASD.

The BCAAs—leucine, isoleucine, and valine—make up approximately a third of essential amino acids in muscle and are involved in cellular signaling and modulation of glucose homeostasis and body weight (Lu et al. 2013). Elevated levels have also been shown to predict type 2 diabetes mellitus (Newgard 2012). BCAAs are also associated with cholesterol metabolism (Chiarla et al. 1990; Kujala et al. 2016). Plasma cholesterol increased in patients with sepsis when given large dose of BCAAs, while other amino acids, fat, and glucose did not have an impact (Chiarla et al. 1990). However, only a few studies exist on BCAA-ASD associations and with small sample sizes (Bent et al. 2018; Lussu et al. 2017; Zheng et al. 2017). Further, there is a lack of inter-generational studies examining the association of maternal BCAA metabolites with offspring ASD.

We therefore examined whether maternal dyslipidemia is associated with the risk of ASD and sought to clarify whether maternal dyslipidemia can interact with BCAAs to affect child risk of ASD. As there is a sex disparity in the prevalence of ASD, we also investigated whether the associations differed by sex of the child.

Methods

Participants and Data Collection

In the parent study, mother-infant pairs were recruited at birth using a rolling enrollment and followed up from birth up to 21 years of age. The current study includes children followed up between 2004 and 2017 (Fig. S1). Detailed recruitment and follow-up procedures have been previously published (Li et al. 2016, available as a free article in PMC: ID# PMC4732357). The Johns Hopkins Bloomberg School of Public Health and the Boston University Medical Center Institutional Review Boards (IRB) approved the recruitment, follow-up studies, and protocols.

Briefly, at the time of enrolment, 24–72 h post-partum, written informed consent was obtained from all participants. Mothers with multiple deliveries or who became pregnant due to in vitro fertilization were excluded from the analysis as were babies with chromosomal abnormalities or major birth defects as documented in the medical records. These exclusion criteria were applied to all study participants prior to enrollment. Maternal blood was collected at the time of enrolment in a non-fasted state. Maternal and newborn prenatal and perinatal information was obtained via a maternal postpartum questionnaire interview and medical record review. Child postnatal information was obtained via child follow-up questionnaires completed by maternal interviews and child medical record review.

Of 3163 mother-infant pairs in the BBC who were enrolled at birth and followed postnatally at the BMC, 756 (86 children with ASD and 670 typically developing (TD) children) who had complete data of the key variables were included in the analyses. Electronic Medical Records (EMR) were used to define ASD case status as per primary and secondary diagnoses using ICD-9 or ICD-10 codes (Table S1). Specifically, ASD was defined for children who were ever diagnosed with autism (299.00), Asperger syndrome (299.80), and/or pervasive developmental disorder—not otherwise specified (299.90). Though a systematic screening for ASD was not conducted, all children were evaluated by highly experienced staff at the BMC autism evaluation program who are in regular communication with primary care physicians at the medical center. Attention deficit hyperactivity disorder (ADHD), developmental delays, or intellectual disabilities without an ASD diagnosis were classified as other developmental disorders. Children with no ASD nor other developmental disorders were classified as typically developing (TD). Given the focus of this study, children with developmental disabilities other than ASD were excluded from the analyses. Our study sample further excluded mothers missing key covariates and cholesterol measurements.

Serum total cholesterol (TC), HDL-C, and triglycerides were measured using standard clinical methods (Ji et al. 2018). LDL-C was calculated using the Friedewald equation (Friedewald et al. 1972). As the accuracy of TC, LDL-C, and TG are dependent on a fasted blood sample, this analysis focused mainly on maternal HDL-C. Quantitative profiling of maternal plasma metabolites, including the BCAAs, was conducted by the Harvard-MIT Broad Institute Metabolite Profiling Laboratory using liquid chromatography tandem mass spectrometry (LC–MS/MS).

Statistical Analysis

Clinical and demographic characteristics for both mother and child were compared for the ASD and TD groups using the *t* test and Chi squared test for continuous and categorical variables, respectively. For major covariates, missing values were combined into the largest group. Metabolite intensity levels were inverse-normally transformed for all subsequent analyses and metabolite values below the limit of detection were imputed with one-half the limit of detection. A BCAA score variable was created based on factor analysis of all three BCAAs using the Anderson–Rubin method (Anderson and Rubin 1956) and was dichotomized at the mean (below median versus at or above median). We explored the association between maternal dyslipidemia and child risk of ASD using logistic regression and further assessed for joint effects with sex and maternal plasma BCAAs, independently and altogether. Plasma HDL-C was evaluated in the main analysis because it is unaffected by the fasting state, unlike the other cholesterols. For joint effects and effect modification analyses, the reference category was the combination of high HDL-C with female sex (or below median maternal BCAA levels) and was compared to three other groups: 2. low HDL-C with female sex (or below median BCAAs) 3. high HDL-C with male sex (or above median BCAAs with), and 4. low HDL-C with male sex (or above median BCAAs). The relative excess risk due to interaction (RERI) was used to show departure from the additive effects of BCAAs and HDL-C or sex (Knol et al. 2011). Tests for multiplicative interaction were also conducted. All analyses were conducted using Stata v14.0 (Stata Corporation, College Station, TX, USA).

To define dyslipidemia, the cholesterol variables were dichotomized by their clinical cut-offs. The cut-off used for HDL-C was < 50 mg/dl, for TC was ≥ 240 mg/dl, and for LDL-C was ≥ 160 mg/dl (Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report.2002; Kilgore et al. 2014). The non-HDL-C variable was created by subtracting HDL-C from TC and using a clinical cut-point of ≥ 190 mg/dl (Kilgore et al. 2014).

Key maternal covariates in this analysis included: age at delivery (< 20, 20–20, 30 and older), race-ethnicity (black, white, Hispanic, or other), smoking during pregnancy ("never smoked," "ever smoked," or "continuous smoking" within 3 months prior to conception), parity (nulliparous vs multiparous), education ("high school or less" vs. "some college or more"). To take into account the correlation between obesity and diabetes, while treating them as separate conditions, and to preserve clinical meaning and statistical power, we categorized the obesity and diabetes variable into three groups as follows: (1. no obesity (BMI ≥ 30 kg/m^2), no type 2 diabetes mellitus or gestational diabetes (DM, collectively) (reference group); 2. either obesity or DM; 3. both obesity and DM). Key child covariates included: sex (female vs male), and gestational age and birthweight (categorized into 1. full term (≥ 37 weeks of gestation) and non-low birth weight (non-LBW; ≥ 2500 g); 2. full term and LBW; 3. preterm and non-LBW; 4. preterm and LBW). Additional sensitivity analysis also adjusted for breastfeeding, which was categorized into 1. formula fed only (reference group); 2. both breastfed and formula fed; 3. exclusively breastfed.

Results

In total, 756 mother-infant pairs were included in this study consisting of 86 children with ASD and 670 TD children. Children with ASD had co-occurring, non-mutually exclusive conditions, including ADHD (n = 35) and intellectual disabilities (n = 13). Table 1 presents maternal and child characteristics by child ASD status. Mothers with ASD children were approximately two years older (p = 0.01) and more obese (p = 0.02) than mothers with TD children. They were also more likely to have diabetes (p = 0.04). There was a 3:1 male to female ratio in the prevalence of ASD (p < 0.001) and ASD children had shorter gestation (p < 0.001) and lower birth weight (p = 0.04) than their TD counterparts. When considered by themselves, maternal cholesterol levels, BCAA levels, smoking, race/ethnicity, parity, or education were not associated with child ASD risk. To evaluate potential selection bias of the study sample, Table S2 compares maternal and child characteristics for included and excluded participants; there were significantly more Black mothers included in the study than other races and fewer mothers who had ever smoked or continued to smoke. A significantly greater number of infants included in the study were female, born full term (≥ 37 weeks), and at a normal weight (> 2500 g). There were no significant in maternal and child characteristics by level of maternal HDL-C (Table S3) and maternal cholesterols did not significantly vary by ASD case status (Fig. S2).

Maternal cholesterols had joint effects with child sex on child risk of ASD, even after adjustment for pertinent covariables, including maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight (Table 2). The effect of HDL-C, non-HDL-C, and LDL-C on child risk of ASD all differed by child sex. Males whose mothers had low HDL-C were at highest risk of ASD (odds ratio (OR) 5.86; 95% CI 2.76, 12.46), followed by males whose mothers had high HDL-C (OR 4.14; 95% CI 2.28, 7.51), compared to females whose mothers had high HDL-C. Further, the risk of child ASD was higher for males whose mothers had low HDL-C than for those whose mothers had high HDL-C (crude OR 7.05; 95% CI

Table 1 Maternal and child characteristics by child autism spectrum disorder status [typically developing (TD) vs. ASD] in the Boston Birth Cohort

Maternal characteristics	Total (N = 756)	TD (N = 670)	ASD (N = 86)	P-value[a]
Age, mean (SD)[b]	28.20 (6.49)	27.97 (6.47)	30.00 (6.34)	0.006
Nulliparous, n (%)	331 (43.78)	296 (44.18)	35 (40.70)	0.540
Race or ethnicity, n (%)[c]				0.080
Black	530 (70.11)	478 (71.34)	52 (60.47)	
White	27 (3.57)	22 (3.28)	5 (5.81)	
Hispanic	145 (19.18)	121 (18.06)	24 (27.91)	
Other	54 (7.14)	49 (7.31)	5 (5.81)	
Education, n (%)				0.955
Below college degree	643 (85.05)	571 (85.22)	72 (83.72)	
College degree or above	107 (14.15)	95 (14.18)	12 (13.95)	
Missing	6 (0.79)	4 (0.60)	2 (2.33)	
Pre-pregnancy BMI, n (%)				
Mean (SD)	26.57 (6.65)	26.40 (6.46)	27.96 (7.91)	0.047
< 25 kg/m^2	359 (47.49)	325 (48.51)	34 (39.53)	0.017
25 to < 30 kg/m^2	187 (24.74)	170 (25.37)	17 (19.77)	
≥ 30 kg/m^2	175 (23.15)	146 (21.79)	29 (33.72)	
Missing	35 (4.63)	29 (4.33)	6 (6.98)	
Diabetes, n (%)[d]				0.037
No diabetes	674 (89.15)	603 (90.00)	71 (82.56)	
Diabetes	82 (10.85)	67 (10.00)	15 (17.44)	
Smoking, n (%)				0.437
Never	648 (85.71)	578 (86.27)	70 (81.40)	
Quit	44 (5.82)	37 (5.52)	7 (8.14)	
Continuous	55 (7.28)	47 (7.01)	8 (9.30)	
Missing	9 (1.19)	8 (1.09)	1 (1.16)	
Total cholesterol, mean (SD)	218.72 (59.40)	218.94 (59.89)	216.97 (55.74)	0.773
High cholesterol (≥ 240 mg/dl), n (%)	241 (31.88)	216 (32.24)	25 (29.07)	0.553
LDL cholesterol, mean (SD)	125.94 (40.19)	125.99 (40.52)	125.55 (37.75)	0.924
High LDL (≥ 160 mg/dl), n (%)	146 (17.61)	134 (18.03)	12 (13.95)	0.347
HDL cholesterol, mean (SD)	63.11 (17.99)	63.51 (18.29)	60.02 (15.16)	0.090
Low HDL (≤ 50 mg/dl), n (%)	176 (23.28)	154 (22.99)	22 (25.58)	0.529
Leucine ($\geq$ median), n (%)	374 (49.47)	326 (48.66)	48 (55.81)	0.211
Isoleucine ($\geq$ median), n (%)	381 (50.40)	372 (50.15)	45 (52.33)	0.704
Valine ($\geq$ median), n (%)	381 (50.40)	330 (49.25)	51 (59.30)	0.079
BCAA score ($\geq$ median), n (%)	410 (49.46)	362 (48.72)	48 (55.81)	0.213
Child characteristics	Total (N = 756)	TD (N = 670)	ASD (N = 86)	P-value[a]
Sex, n (%)				< 0.001
Male	330 (43.65)	266 (39.70)	64 (74.42)	
Female	426 (56.35)	404 (60.30)	22 (25.58)	
Gestational age, n (%)				< 0.001
Term (≥ 37 weeks)	651 (86.11)	587 (87.61)	64 (74.42)	
Late preterm (34–36 weeks)	55 (7.28)	51 (7.61)	4 (4.65)	
Early preterm (< 34 weeks)	50 (6.61)	32 (4.78)	18 (20.93)	
Birth weight				0.037
≥ 2500 g	616 (81.54)	613 (82.50)	63 (73.26)	
< 2500 g	140 (18.46)	130 (17.50)	23 (26.74)	

SD standard deviation, *BMI* body mass index, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *BCAA score* branched-chain amino acid score

[a]P-values were obtained from χ^2 tests or t-tests; missing values for categorical variables were incorporated into the largest 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

Table 1 (continued)

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

Table 2 Joint association of maternal plasma cholesterols and child's sex on the risk of child ASD

Child's sex[a]	HDL-C (≥ 50 mg/dl)		HDL-C (< 50 mg/dl)		OR (95% CI) HDL-C within strata of sex[b]
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	
Female	18/328	1.00 (Reference)	4/98	0.67 (0.21, 2.09)	0.73 (0.24, 2.22)
Male	46/252	**4.14 (2.28, 7.51)**	18/78	**5.86 (2.76, 12.46)**	1.34 (0.73, 2.49)
OR (95% CI) sex within strata of HDL-C[b]		**3.85 (2.17, 6.82)**		**7.05 (2.28, 21.84)**	
	Non-HDL-C (< 190 mg/dl)		Non-HDL-C (≥ 190 mg/dl)		OR (95% CI) Non-HDL-C within strata of sex[b]
	N ASD/total	OR (95% CI)	N ASD/Total	OR (95% CI)	
Female[c]	19/341	1.00 (Reference)	3/85	0.63 (0.18, 2.24)	0.62 (0.18, 2.15)
Male	54/265	**4.90 (2.74, 8.75)**	10/65	**3.18 (1.33, 7.64)**	0.71 (0.34, 1.48)
OR (95% CI) sex within strata of non-HDL-C[b]		**4.34 (2.50, 7.52)**		**4.97 (1.31, 18.88)**	
	LDL-C (< 160 mg/dl)		LDL-C (≥ 160 mg/dl)		OR (95% CI) LDL-C within strata of sex[b]
	N ASD/Total	OR (95% CI)	N ASD/Total	OR (95% CI)	
Female[d]	19/350	1.00 (Reference)	3/76	0.77 (0.22, 2.71)	0.72 (0.21, 2.48)
Male	55/272	**5.01 (2.81, 8.94)**	9/58	**3.44 (1.41, 8.42)**	0.72 (0.34, 1.57)
OR (95% CI) sex within strata of LDL-C[b]		**4.42 (2.55, 7.65)**		**4.47 (1.15, 17.34)**	

HDL-C high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *ORs* adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birth weight

[a]Measure of interaction on additive scale: RERI (95% CI) = 0.07 (−0.04, 0.18); P = 0.227; measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 2.13 (0.57, 7.99); P = 0.264

[b]Stratified ORs not adjusted

[c]Measure of interaction on additive scale: RERI (95% CI) = −0.04 (−0.14, 0.07); P = 0.491; Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 1.03 (0.23, 4.53); P = 0.968

[d]Measure of interaction on additive scale: RERI (95% CI) = −0.04 (−0.15, 0.07); P = 0.501; Measure of interaction on multiplicative scale: ratio of ORs (95% CI) = 0.88 (0.20, 3.95); P = 0.873

2.28, 21.84 and crude OR 3.85; 95% CI 2.17, 6.82, respectively), compared to females under the same circumstances. Similar patterns were found for LDL-C and non-HDL-C. Assessment of maternal cholesterol-infant sex interactions on additive and multiplicative scales were not significant for any of these cholesterol measures.

Maternal HDL-C also modified the effect of maternal plasma BCAAs on child risk of ASD, especially valine (Table 3). Compared to mothers with high HDL-C and below median valine concentrations, mothers with low HDL-C and above median valine concentrations had a two-fold greater risk of having a child with ASD (adjusted OR 2.02; 95% CI 1.04, 3.93). Among mothers with low HDL-C levels, the effect of an above median BCAA score on ASD risk was over four-fold (OR 4.62; 95% CI 1.31, 16.29). For isoleucine and valine, this effect was close to seven-fold. Assessment of BCAA-HDL-C interactions were significant on the additive scale (BCAA score RERI 0.13; 95% CI 0.03, 0.22) and multiplicative scale (BCAA score OR: 5.47; 95% CI 1.29, 23.21) across all three BCAAs. Upon additionally adjusting for breastfeeding, however, the joint effect of low HDL-C with above median valine concentrations lost significance (Table S4). All other sensitivity analyses showed consistent results (Tables S5–S7). Analyses on TC, LDL-C, and non-HDL-C are also presented (Tables S8–S16), and manifested similar trends, though not as robust as HDL-C.

Finally, we examined the joint effect of HDL-C, BCAA, and child sex on child ASD risk. Figure 1 illustrates the association of maternal HDL-C levels with offspring risk of ASD, stratified by maternal plasma BCAA status as defined by the BCAA score, among the overall sample, and among males and females, respectively. Most notably, in mothers with high BCAA scores, the risk of ASD was higher with lower HDL-C concentrations, decreasing

Table 3 Joint association of maternal plasma branch-chained amino acids (BCAAs) and high density lipoprotein cholesterol (HDL-C) on the risk of child ASD

Maternal HDL-C	Leucine below median		Leucine above median		OR (95% CI) BCAA within strata of HDL-C[b]
	N ASD/total	OR (95% CI)	N ASD/total	OR (95% CI)	
High HDL-C[a]	35/315	1.00 (ref)	29/265	0.97 (0.55–1.71)	0.98 (0.58–1.66)
Low HDL-C	3/67	0.35 (0.10–1.24)	19/109	1.72 (0.89–3.36)	**4.50 (1.28–15.86)**
OR (95% CI) HDL-C within strata of BCAA[b]		0.38 (0.11–1.26)		1.71 (0.91–3.22)	
	Isoleucine below median		Isoleucine above median		OR (95% CI) BCAA within strata of HDL-C[b]
	N ASD/total	OR (95% CI)	N ASD/total	OR (95% CI)	
High HDL-C[c]	39/310	1.00 (ref)	25/270	0.68 (0.38–1.21)	0.71 (0.42–1.21)
Low HDL-C	2/65	**0.21 (0.05–0.92)**	20/111	1.53 (0.80–2.94)	**6.92 (1.56–30.67)**
OR (95% CI) HDL-C within strata of BCAA[b]		**0.22 (0.05–0.94)**		**2.15 (1.14–4.07)**	
	Valine below median		Valine above median		OR (95% CI) BCAA within strata of HDL-C[b]
	N ASD/total	OR (95% CI)	N ASD/total	OR (95% CI)	
High HDL-C[d]	33/310	1.00 (ref)	31/270	1.31 (0.64-2.00)	1.09 (0.65-1.83)
Low HDL-C	2/65	0.23 (0.05–1.03)	20/111	**2.02 (1.04–3.93)**	**6.92 (1.56–30.67)**
OR (95% CI) HDL-C within strata of BCAA[b]		0.27 (0.06–1.14)		1.69 (0.92-3.12)	
	BCAA score below median		BCAA score above median		OR (95% CI) BCAA within strata of HDL-C[b]
	N ASD/total	OR (95% CI)	N ASD/total	OR (95% CI)	
High HDL-C[e]	35/314	1.00 (ref)	29/266	0.96 (0.55–1.69)	0.98 (0.58-1.64)
Low HDL-C	3/68	0.34 (0.10–1.19)	19/108	1.77 (0.91–3.46)	**4.62 (1.31–16.29)**
OR (95% CI) HDL-C within strata of BCAA[b]		0.37 (0.11–1.23)		1.74 (0.93–3.27)	

Note: low HDL-C < 50 mg/dl

HDL-C high-density lipoprotein cholesterol, *ORs* adjusted for maternal age, race/ethnicity, education, parity, obesity/diabetes, smoking status, gestational age, and birthweight unless otherwise noted

[a]Measure of interaction on additive scale: RERI (95% CI) = **0.12 (0.03–0.22); P = 0.011;** measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **5.10 (1.20–21.70); P = 0.027**

[b]Stratified ORs unadjusted

[c]Measure of interaction on additive scale: RERI (95% CI) = **0.17 (0.08–0.26); P < 0.001;** measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **10.95 (2.07–57.89); P = 0.005**

[d]Measure of interaction on additive scale: RERI (95% CI) = **0.14 (0.05-0.23); P = 0.003;** measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **7.75 (1.49–40.26); P = 0.015**

[e]Measure of interaction on additive scale: RERI (95% CI) = **0.13 (0.03–0.22); P = 0.008;** measure of interaction on multiplicative scale: ratio of ORs (95% CI) = **5.47 (1.29-23.21); P = 0.021**

steadily as HDL-C increased. This pattern was more exaggerated among male children. Figure 2 displays similar patterns for the other cholesterol measures. The group with all three exposures combined—males whose mothers had a high BCAA score and low HDL-C—had the greatest risk of ASD, compared to the reference group (crude OR: 8.06; 95% CI 3.29, 19.76) (Fig. S3).

Discussion

Main Findings

To our knowledge, this is the first study of this kind in a US urban, low income prospective birth cohort. We

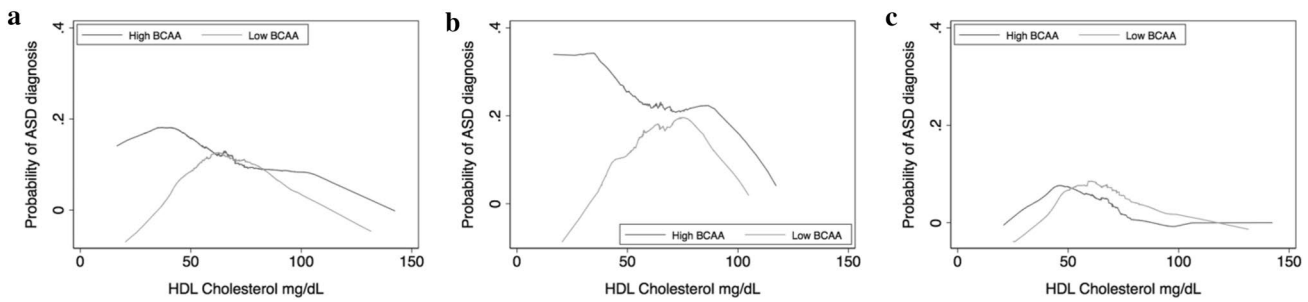

Fig. 1 Association of maternal HDL levels with offspring risk of ASD, stratified by maternal plasma branch-chained amino acid (BCAA) status defined by BCAA score, among overall sample, and among males and females, respectively. **a** Overall, **b** male, **c** female

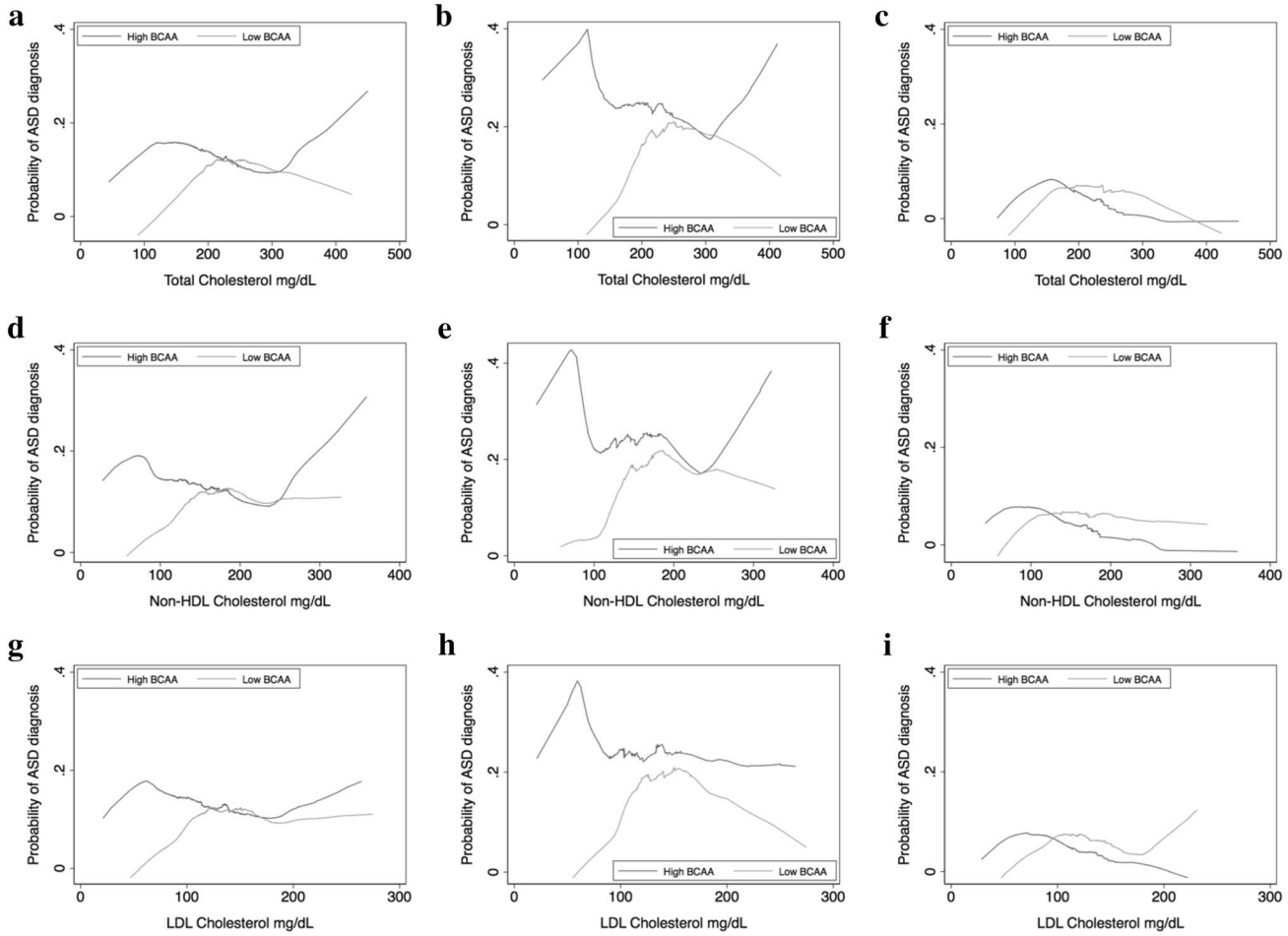

Fig. 2 Association of maternal total cholesterol, non-HDL cholesterol, and LDL levels with offspring risk of ASD, stratified by maternal plasma branch-chained amino acid (BCAA) status defined by BCAA score, among overall sample, and among males and females, respectively. **a** Association of maternal TC with risk of ASD in offspring by maternal plasma BCAA score in the overall sample, **b** association of maternal TC with risk of ASD in male offspring by maternal plasma BCAA score, **c** association of maternal TC with risk of ASD in female offspring by maternal plasma BCAA score, **d** association of maternal non-HDL cholesterol with risk of ASD in offspring by maternal plasma BCAA score in the overall sample, **e** association of maternal non-HDL cholesterol with risk of ASD in male offspring by maternal plasma BCAA score, **f** association of maternal non-HDL cholesterol with risk of ASD in female offspring by maternal plasma BCAA score, **g** association of maternal LDL cholesterol with risk of ASD in offspring by maternal plasma BCAA score in the overall sample, **h** association of maternal LDL cholesterol with risk of ASD in male offspring by maternal plasma BCAA score, **i** association of maternal LDL cholesterol with risk of ASD in female offspring by maternal plasma BCAA score

found that low maternal HDL-C, high maternal plasma BCAA concentrations, and male sex can additively or multiplicatively increase offspring risk of ASD. This study extends previous studies by us and others on the role of maternal metabolic factors in offspring ASD risk.

Interpretation

Though the role of maternal obesity and diabetes in child ASD has received increasing attention due to accumulating evidence, there is a lack of inter-generational studies linking maternal cholesterols to child ASD. However, as reviewed in the introduction and below, cholesterols have important biological functions and it is biologically plausible that abnormal maternal cholesterols may affect the risk of child ASD. While cholesterols are formed de novo by the fetus, maternal cholesterols are also transported via placenta, at least during the first trimester, and enter fetal circulation (Wild et al. 2015). We previously showed in the BBC that low maternal plasma HDL-C concentrations were associated with risk of ADHD in the offspring, especially among males (Ji et al. 2018). In the current study, over 40% of children with ASD also had co-occurring ADHD. However, there were no discernable differences in the estimates when children with co-occurring ASD and ADHD were removed from the analysis (Table S7). Nevertheless, there may be common underlying mechanisms between these conditions linked to maternal lipids that should be further explored. A recent meta analyses of maternal dyslipidemia reported that both low and high levels of cholesterol were associated with preterm birth (Jiang et al. 2017), which is also highly associated with ASD (Joseph et al. 2017; Limperopoulos et al. 2008). Low maternal cholesterol has also been linked with low birthweight and microcephaly (Edison et al. 2007). In cases of maternal hypercholesterolemia, fetal cholesterols also become elevated, and this increased exposure has been shown to predispose the offspring to atherosclerosis later in life (Napoli et al. 1997). Elevated maternal cholesterol has been consistently linked with adverse adult offspring outcomes, including atherosclerosis and cardiovascular disease (Jin et al. 2016), which are co-occurring conditions in individuals with ASD (Mouridsen et al. 2016). It is possible that selective diets and food sensitivities in ASD could partially account for the pathophysiology of these inflammatory conditions (Mari-Bauset et al. 2014).

Adequate levels of cholesterol are necessary for proper membrane myelination during fetal development. Reduced levels of myelin have been seen in individuals with Fragile X syndrome, a disorder closely related to ASD (Gillberg et al. 2017). However, there is inconsistent evidence for altered cholesterol levels in ASD individuals; some studies have found higher total and LDL-C levels among ASD individuals while others report normal or lower cholesterol compared to controls (Dziobek et al. 2007; Tierney et al. 2006). This may be due to the differing subtypes and etiologies of ASD.

Genetic defects of cholesterol biosynthesis have severe impacts on development and survival of the fetus (Tierney et al. 2006). Smith–Lemli–Opitz syndrome (SLOS) is a milder genetic disorder characterized by a defect in cholesterol synthesis and is associated with autistic traits. One study reported around 20% of children with ASD had hypocholesterolemia (Aneja and Tierney 2008). Abnormal cholesterol metabolism in SLOS and ASD may lead to dysfunction in serotonin signaling, as cholesterol modulates the activity of serotonin, especially in its transport. Serotonin has been implicated in altered social behaviors characteristic of SLOS and ASD. A mouse model for SLOS supports this finding (Waage-Baudet et al. 2003). A recent study also found that adult rats prenatally exposed to valproic acid to induce autistic-like traits had sex-specific differences in brain cholesterol metabolism compared to unexposed rats (Cartocci et al. 2019).

With the exception of vitamin D, all steroid hormones are formed from cholesterol precursors. Steroids have been implicated in the etiology of anxiety and mood disorders as well as ASD (Aneja and Tierney 2008). There is growing literature on maternal vitamin D deficiency during pregnancy and ASD (Garcia-Serna and Morales 2019; Patrick and Ames 2014). Serotonin, oxytocin, and vasopressin all contain a vitamin D response element (VDRE) (Patrick and Ames 2014). The genes encoding production of each require vitamin D to activate them. All three of these hormones are also modulated by cholesterol. Although cholesterol is not a precursor for vitamin D, they are both derived from the same molecule, 7-dehydrocholesterol (7DHC) (Gillberg et al. 2017). The 7DHC reductase gene (DHCR7) is the same as that implicated in SLOS. Gillberg et al. (2017) hypothesized there may be an association between ASD and this "branching point," possibly explaining the links between ASD and cholesterol and ASD and vitamin D (Gillberg et al. 2017). Pregnant women with inadequate levels of 25-hydroxy vitamin D during the first trimester had significantly increased TC, LDL-C, and TC/HDL-C ratio compared to pregnant women with sufficient vitamin D levels (Lepsch et al. 2017).

It is a well-observed phenomenon that there is a profound sex disparity in ASD, with a male to female ratio of 3:1 (Loomes et al. 2017). However, the underlying reasons and mechanisms for the sex difference are poorly understood. In the present study, for the first time, we showed that the effect of low maternal HDL-C on child ASD risk was most pronounced in male children. This link between cholesterol and sex is supported by a study that reported estrogen is able to compensate for insufficient maternal vitamin D, potentially rescuing female fetuses from developing psychiatric disorders, including ASD (Patrick and Ames 2014). Thus, our finding puts forth new indications for future research.

This study demonstrated that maternal cholesterols by themselves were not associated with child risk of ASD. However, HDL-C, non-HDL-C, and LDL-C did modify maternal effect of BCAAs on offspring ASD risk with significant additive and multiplicative interactions. BCAAs are highly correlated with obesity and T2DM and predictive of T2DM (Newgard 2012; Perng et al. 2014). Yet, there is limited evidence linking cholesterol and BCAAs. One study showed plasma cholesterol levels increased in septic patients given a large dose of BCAAs, while other amino acids, fat, and glucose were not impacted (Chiarla et al. 1990). This suggests BCAA metabolites contribute to cholesterol synthesis in an inflammatory state. Both BCAA and lipid metabolism involve the tricarboxylic acid (TCA) cycle in the mitochondria and are thus dependent on the proper functioning of each other. Disturbances in lipid metabolism have been shown to yield elevated serum concentrations of BCAAs (Kujala et al. 2016). These disturbances in energy metabolism have the potential to cause oxidative stress and inflammation. BCAAs, especially leucine, are inducers of mammalian target of rapamycin (mTOR), an important kinase involved in cell growth, protein synthesis, and energy balance (Zoncu et al. 2011). Thus, together with low maternal HDL-C levels and child male sex, elevated BCAAs have the potential to increase the risk of child ASD. Our results showed that male children whose mothers had low HDL-C levels and low BCAA concentrations, were not at significant risk compared to the reference group (female children whose mothers had high HDL-C levels and low BCAA concentrations) (Fig. S3). However, male children whose mothers had low HDL-C levels and *high* BCAA concentrations had a significantly increased risk, suggesting elevated BCAA concentrations is a key factor in increasing the risk under these conditions. This specific analysis was unadjusted for key covariates and conducted as exploratory research.

It is well-documented that breastfeeding is protective against infant obesity (Marseglia et al. 2015). However, a recent meta-analysis supports the association between breastfeeding and a reduced likelihood of child ASD (Tseng et al. 2019). Our results showed that upon adjusting for breastfeeding, the joint effect of low maternal HDL-C and an above median valine concentration became insignificant. Thus, our findings support the notion that breastfeeding may be a protective factor against ASD and may also counteract the effects of low maternal HDL-C and high BCAA concentrations.

Strengths and Limitations

This is the first prospective, longitudinal, and inter-generational study to examine the joint effects of maternal biomarkers of cholesterols and metabolites on the risk of her child developing ASD. This study also brought to light potential biomarkers associated with sex differences observed in ASD. Limitations of this study include a one-time post-delivery measurement of maternal BCAAs and cholesterols in a non-fasted state. Apart from HDL-C, cholesterol measurements in a non-fasted state are not as reliable, especially triglycerides, which we did not analyze. Though our sample size was among the largest for a prospective study of its kind, we were not able to examine stratified odds ratios adjusted for pertinent covariables. We caution the number of cases is very small in certain categories, and the observed associations may not be reliable. Furthermore, our study was conducted in a predominantly urban, low income US population with African ancestry. Thus, the authors caution in generalizing these findings to other populations with different environmental and genetic backgrounds. For example, SLOS has a higher prevalence in families of Northern European, and especially Polish descent. As such, our findings should be regarded as hypothesis-generating and warrant further investigation and confirmation.

Implications for Future Research

While most current research on BCAAs in ASD has shown reduced levels in cases versus controls, we are not aware of any that have examined the effect of maternal BCAAs and child risk of ASD. The developmental origins of health and disease model asserts that in a womb environment of plenty, the fetus adapts to expect a similar postnatal environment (Barker et al. 2002). Thus, fetuses exposed to excess levels of BCAAs in the womb may express less circulating concentrations postnatally. Other possible explanations of lower concentrations of BCAAs among individuals with ASD include food sensitivities and poor diet quality leading to insufficient intake of certain nutrients (Mari-Bauset et al. 2014), and possible mutations in the branched chain ketoacid dehydrogenase kinase (BCKDK), a key enzyme in BCAA catabolism (West et al. 2014). Future studies including both maternal plasma measurements during specific trimesters of pregnancy as well as cord blood and postnatal child plasma cholesterols and BCAAs may help clarify the inter-generational link of cholesterols and BCAAs with child ASD risk.

Conclusions

The results of this longitudinal, prospective birth cohort study showed that while maternal plasma cholesterols were not associated with the risk of child ASD, they had a joint effect with the child's sex on ASD risk. Low HDL-C and elevated maternal plasma BCAAs jointly increased the risk as well. These joint associations also differed by sex. The three factors together (low HDL-C, high BCAAs and male sex) had the greatest effect on the risk of child development

of ASD. Future studies with larger sample sizes and additional time points for HDL-C and BCAAs throughout pregnancy, from fetal cord blood through early childhood, may further elucidate the role of maternal and fetal/child cholesterols and BCAAs in the risk of offspring ASD.

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Author contributions AAP conceptualized the study, performed the statistical analysis, participated in the interpretation of the data, and drafted the manuscript. YJ conceptualized the study and assisted in statistical analysis and interpretation of the data. JWF and AP conceptualized the study and participated in the interpretation of the data. GW and XH participated in the design and coordination of the study and data cleaning. BZ oversaw and managed participant recruitment, follow-up, and data collection. XW is the founder and principal investigator of the Boston Birth Cohort and oversaw participant recruitment, follow-up and data collection, conceptualized the study, and provided critical input on study design, data analyses, interpretation of data, and initial draft of the manuscript. All authors critically reviewed and approved the final manuscript.

Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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