American Journal of Epidemiology

Vol. 190, No. 2
DOI: 10.1093/aje/kwaa171
Advance Access publication:
September 2, 2020

Original Contribution

Association Between Midpregnancy Polyunsaturated Fatty Acid Levels and Offspring Autism Spectrum Disorder in a California Population-Based Case-Control Study

Kristen Lyall*, Gayle C. Windham, Nathaniel W. Snyder, Rostislav Kuskovsky, Peining Xu, Anna Bostwick, Lucy Robinson, and Craig J. Newschaffer

* Correspondence to Dr. Kristen Lyall, A.J. Drexel Autism Institute, 3020 Market Street, Suite 560, Drexel University, Philadelphia, PA 19104 (e-mail: kld98@drexel.edu).

Initially submitted January 2, 2020; accepted for publication August 12, 2020.

Polyunsaturated fatty acids (PUFAs) are critical for brain development and have been linked with neurodevelopmental outcomes. We conducted a population-based case-control study in California to examine the association between PUFAs measured in midpregnancy serum samples and autism spectrum disorder (ASD) in offspring. ASD cases ($n = 499$) were identified through the California Department of Developmental Services and matched to live-birth population controls ($n = 502$) on birth month, year (2010 or 2011), and sex. Logistic regression models were used to examine crude and adjusted associations. In secondary analyses, we examined ASD with and without co-occurring intellectual disability (ID; $n = 67$ and $n = 432$, respectively) and effect modification by sex and ethnicity. No clear patterns emerged, though there was a modest inverse association with the top quartile of linoleic acid level (highest quartile vs. lowest: adjusted odds ratio = 0.74, 95% confidence interval: 0.49, 1.11; P for trend = 0.10). Lower levels of total and ω-3 PUFAs were associated with ASD with ID (lowest decile of total PUFAs vs. deciles 4–7: adjusted odds ratio = 2.78, 95% confidence interval: 1.13, 6.82) but not ASD without ID. We did not observe evidence of effect modification by the factors examined. These findings do not suggest a strong association between midpregnancy PUFA levels and ASD. In further work, researchers should consider associations with ASD with ID and in other time windows.

autism; autism spectrum disorder; intellectual disability; maternal diet; polyunsaturated fatty acids

Abbreviations: AOR, adjusted odds ratio; ASD, autism spectrum disorder; CI, confidence interval; DDS, Department of Developmental Services; DHA, docosahexaenoic acid; ID, intellectual disability; LA, linoleic acid; PUFA, polyunsaturated fatty acid.

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition defined by deficits in social communication and the presence of restricted, repetitive behaviors (1). Evidence supports the hypothesis of prenatal origins for ASD (2) and the possibility that both environmental and genetic factors play a role in its etiology (3, 4). Emerging research suggests that certain maternal dietary factors may be inversely associated with ASD. Most of this work has focused on the role of prenatal vitamin supplements, folic acid, and vitamin D. Yet, polyunsaturated fatty acids (PUFAs) also play a critical role in fetal brain development (5). PUFAs are required for neurodevelopmental processes beginning early in gestation, including neurogenesis, differentiation, connectivity, and synaptogenesis (6). There is evidence of disruption of these processes in ASD (7–9). PUFAs also play roles in signal transduction, gene expression and methylation, and placental function and as components of cell membranes (10–13), and they have effects on inflammatory markers and immune responses (14, 15), suggesting a number of potential pathways relevant to ASD (16–21).

PUFAs are fatty acids with multiple double bonds in the hydrocarbon chain; depending on the positioning of the double bond from the methyl end of the molecule, they are classified as either ω-3 or ω-6 fatty acids. Key dietary sources of PUFAs are fish (for ω-3 PUFAs) and nuts, seeds,

and oils (particularly for ω-6 PUFAs). Both linoleic acid (LA; an ω-6 PUFA) and α-linolenic acid (an ω-3 PUFA) are known as essential fatty acids because they cannot be synthesized in the body and must be obtained from the diet. These fatty acids are metabolized into long-chain PUFAs, which can then be further metabolized to both pro- and antiinflammatory molecules or incorporated into membrane lipids (22). Docosahexaenoic acid (DHA) is an ω-3 PUFA of particular interest given its high content in the human brain and evidence for its role in neuronal growth and differentiation processes (6). Given the rapid growth of the brain during gestation, the prenatal period represents a key window for a potential impact of PUFAs on child health outcomes. Furthermore, because the supply of PUFAs to the developing fetus is dependent on maternal diet (23–25), there is the potential for modification if associations are observed.

Although there have been studies reporting differences in circulating PUFA levels in children already diagnosed with ASD (26–29), as well as suggested improvements in certain symptoms in children with ASD following ω-3 PUFA supplementation, these findings do not necessarily address the role of PUFAs in ASD etiology. Existing research considering PUFA levels, or intake, during critical prenatal windows of neurodevelopment in association with ASD has yielded conflicting findings. In the first published study, Lyall et al. (30) found a general pattern of decreased risk of ASD among children of mothers with higher total PUFA and total ω-6 PUFA levels according to reported diet during or surrounding pregnancy, and an increased risk of ASD among those with the very lowest total ω-3 PUFA levels. Two studies suggested protective associations between maternal fish intake and autism or autism symptom scores (31, 32), though in another, Suren et al. (33) reported no association with fish oil supplementation. In one of the only studies with PUFA levels measured during gestation, Steenweg-de Graaff et al. (34) reported an association between higher prenatal plasma ω-6 PUFA levels and more autism-related traits in the child. In the other study, which included only 57 cases from a cohort with high familial risk of ASD, Cohen et al. (35) did not report an association with third-trimester levels and ASD diagnosis, but they did find an inverse association with ω-3 PUFAs according to reported diet. These limited and conflicting findings stand in contrast to a wider body of literature supporting a positive association between PUFAs and broader neurodevelopmental outcomes (36, 37), suggesting the need to clarify associations with ASD specifically.

Our goal in this study was to determine the relationship between levels of PUFAs, measured in prospectively collected midpregnancy samples, and offspring ASD in a large case-control study with participants drawn from the general population. We also sought to further prior work by examining whether results differed by ASD with and without comorbid intellectual disability (ID), by child sex, and by ethnicity.

METHODS

Study population

Study subjects were drawn from women who delivered a live infant in the state of California in 2010 or 2011, participated in routine prenatal screening, and had banked serum samples available and whose infants were not known to have died in the first year of life. Approximately 70% of women in California participate in prenatal screening, with samples from various diverse counties throughout the state being stored as part of the California Biobank Program. (For this study, mothers were residents of Fresno, Madero, Kings, Tulare, Kern, Orange, and San Diego counties at the time of their children's births.) Birth years included here were chosen to allow sufficient time for identification of an ASD diagnosis while optimizing measurement of PUFAs in stored biospecimens. Information on covariates was obtained from California vital statistics data. All study procedures were approved by the institutional review board of Drexel University (Philadelphia, Pennsylvania), as well as the Committee for the Protection of Human Subjects of California.

Determination of case/control status

Information from the California Department of Developmental Services (DDS), which provides services to individuals with autism, ID, and other developmental disabilities, was used to identify children with ASD and potential general population controls. Cases were defined according to the presence of ASD in DDS records at the time of data linkage (mean child age at linkage = 5.24 years; range, 4.1–6.2 years). Following exclusion of DDS clients for any diagnosis, controls were randomly sampled from prenatal screening-program-linked birth and infant death certificates and frequency-matched to ASD cases on sex, birth month, and birth year to create eligible pools to select for biospecimen retrieval. We aimed to include 500 cases and 500 controls; however, because of issues regarding biospecimen retrievability, 499 cases and 502 controls were included in the final analyses (with 1 control not being matched on sex). DDS case status has been utilized in multiple other epidemiologic investigations of ASD (38–41), with previous work supporting a high validity of ASD diagnosis in DDS data. DDS records have also been shown to capture the majority of cases who remain in the state (an estimated 84%, with milder cases being more likely to be missed (38, 41)). In addition to ASD diagnoses, we obtained information on co-occurring ID, defined according to composite scores less than 70 on standardized cognitive and functional tests in DDS records.

Specimens and laboratory analysis of PUFAs

Maternal second-trimester serum specimens were retrieved from the California Department of Public Health's California Biobank Program. The archive includes maternal serum collected for routine prenatal screening at 15–19 weeks' gestation. Maternal specimens were collected in serum separator tubes by obstetrical care service providers; leftover specimens following screening were stored at −20°C. Consent forms for the screening program were distributed at the time of the blood collection, which stipulated that specimens and results from prenatal testing could be used for legitimate research purposes given appropriate institutional

Table 1. Levels and Detection Rates of Polyunsaturated Fatty Acids Measured in Maternal Midpregnancy Serum Samples in a Population-Based Case-Control Study, by Case/Control Status, California, 2010–2011

Class and PUFA	% Below LOQ[a]	Cases (n = 499)		Controls (n = 502)	
		Geometric Mean (SD)	IQR	Geometric Mean (SD)	IQR
ω-3 PUFAs					
α-Linolenic acid	12	0.99 (1.30)	0.59–1.97	0.95 (1.29)	0.53–1.94
Stearidonic acid	0	0.16 (0.06)	0.13–0.20	0.16 (0.06)	0.13–0.19
Eicosapentaenoic acid[b]	0	0.30 (0.52)	0.18–0.49	0.27 (0.36)	0.16–0.45
Docosapentaenoic acid	0	15.60 (7.34)	11.70–20.30	15.50 (7.97)	11.20–20.20
Docosahexaenoic acid	0	1.49 (0.56)	1.20–1.79	1.48 (0.61)	1.20–1.76
Total ω-3 PUFAs[c]		19.10 (8.59)	14.57–24.84	18.80 (9.00)	14.60–24.00
ω-6 PUFAs					
Linoleic acid	0	35.90 (18.50)	26.80–48.10	36.90 (20.70)	27.00–49.40
γ-Linolenic acid	0	2.24 (1.66)	1.50–3.56	2.15 (1.77)	1.50–3.33
Eicosadienoic acid[d]	79				
Dihomo-γ-linolenic acid	0	1.63 (0.88)	1.20–2.24	1.63 (0.94)	1.19–2.31
Arachidonic acid	0	6.50 (1.61)	2.26–5.00	6.43 (1.57)	2.15–4.74
Total ω-6 PUFAs		47.10 (21.00)	36.76–60.80	48.00 (23.30)	36.20–62.40
Total PUFAs[c,e]		66.7 (27.80)	52.0–84.30	67.40 (30.70)	51.80–85.30

Abbreviations: IQR, interquartile range; LOQ, limit of quantification; PUFA, polyunsaturated fatty acid; SD, standard deviation.
[a] The LOQ is not applicable for totals; numbers shown are the percentage of the study population below the LOQ for that fatty acid.
[b] $P < 0.05$ for comparison between case and control levels, according to Student's t test.
[c] Eicosapentaenoic acid was not included because of the low detection rate.
[d] Not included in further analyses because of the low detection rate.
[e] Sum of all individual PUFAs shown.

review board approval unless participants formally opted out.

Nonesterified PUFAs in maternal serum were measured by analysts who were blinded to sample identity using isotope dilution liquid chromatography–high-resolution mass spectrometry following protein precipitation in a randomized order (extraction, analysis, and quality control data are provided in the Web Appendix and Web Table 1, available at https://academic.oup.com/aje). For values below the lower limit of quantification, that value was reported if possible, to provide a value based on a detected peak (42). Where no laboratory-derived value could be provided, values were imputed using multiple-imputation models including all available covariates, assuming sufficient detection of the PUFA in the study population (>60%, so as to not base analyses on primarily imputed levels). Persons with samples that failed quality control for a given PUFA were excluded from analyses. Information on the PUFAs measured here and their detection rates is shown in Table 1.

Statistical methods

PUFA levels and covariates were examined in univariate analyses. Total PUFA levels were calculated by summing levels across all individual measured PUFAs (Table 1); likewise, totals for ω-3 and ω-6 PUFAs were created by summing levels of individual PUFAs measured within these classes. We compared basic demographic and covariate information by case/control status and according to quartiles of total PUFA levels (highest and lowest) in bivariate analyses.

Conditional logistic regression models were used to examine crude and adjusted associations between prenatal PUFA levels and ASD. Unconditional logistic regression, adjusting for matching factors, was used in analyses of effect modifiers (described below) in order to maintain sample size. Covariates examined in adjusted models were selected on the basis of a priori knowledge of associations with maternal diet and ASD status. These included: maternal age (years; continuous), maternal race/ethnicity and educational level (both in categories, as shown in Table 2), an indicator for short interpregnancy interval (defined as having a birth within 2 years prior to the current child under study, relative to all others), and prepregnancy body mass index (calculated as weight (kg)/height $(m)^2$; continuous). Additional variables examined but not retained in the final models because they did not substantially (e.g., <10%) change the estimates included maternal smoking during pregnancy (yes/no), metabolic conditions (gestational diabetes, hypertension, or preeclampsia), parity, maternal birthplace outside the United States, and paternal age.

In order to allow for comparison with our earlier work based on dietary intake (30), our primary analysis examined PUFAs in quartiles, using the lowest quartile (quartile 1) as

Table 2. Selected Characteristics of Participants in a Study of Midpregnancy Serum Polyunsaturated Fatty Acid Levels and Offspring Autism Spectrum Disorder, by Case/Control Status, California, 2010–2011[a]

Characteristic	ASD Cases (*n* = 499)			Controls (*n* = 502)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Maternal age, years[b]	29.4 (5.8)			28.3 (5.8)		
Paternal age, years[b]	32.6 (6.9)			31.1 (6.7)		
Gestational age, days	274.5 (13.0)			273.8 (13.0)		
Birth weight, g	3,382.1 (496.0)			3,381.8 (538.0)		
Prepregnancy body mass index[b,c]	27.2 (6.9)			25.8 (5.9)		
Parity	1.9 (1.2)			2.0 (1.1)		
Child's sex						
Male		412	83		416	83
Female		87	17		86	17
Maternal education						
Less than high school (no diploma)[b]		86	17		115	23
High school diploma		112	22		109	22
Some college or 2-year degree		161	32		115	23
College degree		79	16		93	19
Graduate degree		44	9		46	9
Missing data		17	3		24	5
Maternal race/ethnicity						
Non-Hispanic White		126	25		128	26
Asian		88	18		67	13
Black		17	3		20	4
Hispanic		251	50		265	53
Other or missing data[d]		17	3		22	4
Maternal birthplace outside United States		228	46		223	44
Heath insurance status at delivery						
Private		216	43		221	44
Government program		274	55		270	54
Other		9	2		11	2
Smoking during pregnancy[e]		12	2		10	2
Short interpregnancy interval (<2 years)		103	21		86	17
Any pregnancy complication		117	23		103	21
Metabolic pregnancy complication[b,f]		48	10		30	6
Preterm birth[g]		33	7		45	9
Low birth weight[h]		21	4		26	5

Abbreviations: ASD, autism spectrum disorder; SD, standard deviation.

[a] Mean values and SDs are shown for continuous variables, numbers and percentages for categorical variables.

[b] Statistically significant *P* value ($P < 0.05$) from Student's *t* test (continuous variables) or a χ^2 test (categorical variables). For parental age and body mass index, $P = 0.001$; for maternal education, $P = 0.01$; for pregnancy complications, $P = 0.03$.

[c] Weight (kg)/height $(m)^2$.

[d] Fewer than 2% of participants were missing information on race/ethnicity.

[e] Defined as any smoking from 3 months prior to conception through pregnancy.

[f] Defined as gestational diabetes, hypertension, or preeclampsia.

[g] Defined as birth at less than 37 weeks' gestation according to California vital statistics data.

[h] Defined as birth weight less than 2,500 g according to California vital statistics data.

the referent. We also examined distributional extremes of levels according to deciles (relative to middle deciles 4–7) and the highest and lowest fifth percentiles of the distribution (relative to the interquartile range). The distribution of values in controls was used to determine category cutpoints. We also considered potential nonlinear associations with continuous PUFA levels, using cubic spline analyses (43, 44) adjusting for covariates.

In order to explore whether PUFAs may be related to phenotypic subgroups within ASD, we conducted secondary analyses of the associations between PUFAs and ASD with and without comorbid ID as recorded in DDS records ($n = 67$ with ID and $n = 432$ without ID). We also examined potential modification by offspring sex, given the skewed sex ratio in ASD and reports of sex-specific findings for certain ASD risk factors (45–47), and by maternal ethnicity (Hispanic vs. non-Hispanic), given potential differences in dietary patterns. Interaction terms (with continuous total PUFAs as well as ω-3 and ω-6 PUFA levels) and stratified models were used to assess differences according to these factors.

RESULTS

With the exception of eicosadienoic acid, all PUFAs were sufficiently detected in the maternal serum samples (Table 1). When PUFA levels were compared by case status, geometric mean levels for all PUFAs, except eicosapentaenoic acid, did not differ. Basic characteristics of the study population are shown in Table 2. Case mothers (and fathers) were slightly older than control mothers and had a higher prepregnancy body mass index. Our study population had a high proportion (∼50%) of Hispanic participants, which did not vary by case status. As would be expected given the sex ratio in ASD and our study's matching, approximately 80% of the children were male. Associations between PUFA levels and demographic covariates differed for total PUFAs and total ω-3 PUFAs; higher levels of ω-3 PUFAs, but not total PUFAs, were more common among women with higher education, while women in the highest quartile of total PUFAs, but not total ω-3 PUFAs, were more likely to be Hispanic (Web Table 2).

In multivariate-adjusted models, overall, we did not find evidence for associations between PUFA levels in quartiles and ASD (Table 3). However, the adjusted odds ratio estimate for the top quartile of LA was below the null value (adjusted odds ratio (AOR) = 0.74, 95% confidence interval (CI): 0.49, 1.11), and a nonsignificant trend (P for trend = 0.10) of decreasing odds of ASD was observed across quartiles. Total PUFA, total ω-6, DHA, and dihomo-γ-linolenic acid levels also demonstrated similar point estimates for quartile 3 or 4 (with confidence intervals overlapping the null) but showed no evidence of a monotonic trend. Evidence of stronger associations for these or other fatty acids was generally not found when examining further extremes of the distribution. While persons with linoleic acid levels in the eighth decile had reduced odds of ASD relative to those with levels in the middle of the distribution (deciles 4–7) (AOR = 0.44, 95% CI: 0.26, 0.75), corresponding decreases were not seen for the highest 2 deciles (Web Table 3). Similar results were observed for total PUFAs in decile 8 (AOR = 0.60, 95% CI: 0.37, 0.98), though the adjusted odds ratio for decile 9 was also below the null. In addition, there was no evidence of potentially stronger associations for persons with the very highest and lowest levels (fifth percentiles) relative to the interquartile range (Web Table 4). When examining associations using cubic splines, we did not find evidence for nonlinearity; the general pattern observed for total PUFA levels was a nonsignificant linear decrease in odds of ASD with increasing total PUFA level (Web Figure 1), with similar findings for LA and total ω-3 PUFAs, and flatter curves with wide confidence intervals for most others.

Examining associations between maternal PUFA levels and ASD with and without comorbid ID, we observed mostly null associations with quartiles of PUFA levels (Table 4). There was some suggestion of differences in ω-3 and ω-6 PUFA associations between these groups, with the highest quartiles of the ω-3 PUFAs DHA and eicosapentaenoic acid being below the null for ASD with ID (but not ASD without ID) and the highest quartiles of total PUFAs and total ω-6 PUFAs being below the null for ASD without ID (but not ASD with ID). However, confidence intervals were wide. When examining deciles, the lowest deciles of docosapentaenoic acid, arachidonic acid, total PUFAs, and total ω-3 PUFAs were all associated with increased odds of ASD with ID (for the lowest decile of total PUFAs vs. deciles 4–7, AOR = 2.78 (95% CI: 1.13, 6.82); similar associations were observed for the other PUFAs), though reductions in odds were generally not observed with higher deciles (Web Table 3). In contrast, a higher decile (decile 8) of total PUFAs and total ω-6 PUFAs was associated with reduced odds of ASD without ID (total PUFAs: AOR = 0.55 (95% CI: 0.33, 0.93); total ω-6 PUFAs: AOR = 0.48 (95% CI: 0.28, 0.83)), though deciles 9 and 10 for these groups were closer to the null (Web Table 3, Web Figure 2). Small numbers in individual categories for the ASD-with-ID group precluded us from examining further distributional extremes.

We did not find evidence for statistically significant interactions of total PUFAs with sex and ethnicity ($P = 0.98$ and $P = 0.78$, respectively; similar results were seen for interactions with ω-3 or ω-6 PUFAs). While a few statistically significant estimates were observed in analyses of PUFA quartiles stratified by these factors, including reduced odds of ASD in Hispanics with higher total ω-6 PUFA levels (quartile 3 vs. quartile 1: AOR = 0.54, 95% CI: 0.30, 0.98) (Web Tables 5 and 6), no clear trends across PUFAs or patterns of association with ethnicity or sex emerged.

DISCUSSION

In this population-based case-control study drawn from pregnant women in California, overall, we did not find strong evidence for an association between PUFA levels measured in midpregnancy and offspring ASD. We examined PUFAs according to several parameterizations and also explored potential effect modification by sex and ethnicity. Secondary analyses did suggest potentially stronger associations between LA and ASD without ID, as well as between some ω-3 PUFAs and ASD with ID, but these findings were

Table 3. Associations Between Maternal Midpregnancy Serum Polyunsaturated Fatty Acid Levels and Offspring Autism Spectrum Disorder in a Population-Based Case-Control Study, California, 2010–2011

PUFA	Median Value, ng/mL	No. of Cases (*n* = 499)	No. of Controls (*n* = 502)	OR[a]	95% CI	AOR[b]	95% CI	*P* for Trend[c]
ALA (18:3)[d]								0.74
Q1 (lowest)	0.30	109	128	1.00	Referent	1.00	Referent	
Q2	0.79	132	119	1.29	0.91, 1.84	1.26	0.87, 1.82	
Q3	1.48	125	125	1.18	0.82, 1.69	1.10	0.76, 1.60	
Q4 (highest)	2.73	124	122	1.21	0.83, 1.75	1.15	0.78, 1.70	
SA (18:4)								0.96
Q1	0.11	90	101	1.00	Referent	1.00	Referent	
Q2	0.14	138	135	1.16	0.79, 1.69	1.08	0.73, 1.60	
Q3	0.17	122	115	1.21	0.81, 1.83	1.13	0.74, 1.73	
Q4	0.22	149	151	1.13	0.76, 1.69	1.03	0.70, 1.56	
EPA (20:5)								0.86
Q1	0.12	107	133	1.00	Referent	1.00	Referent	
Q2	0.22	136	117	1.45	1.02, 2.08	1.28	0.88, 1.87	
Q3	0.34	116	130	1.12	0.78, 1.60	0.88	0.58, 1.31	
Q4	0.71	140	122	1.45	1.00, 2.09	1.16	0.76, 1.77	
DPA (22:5)								0.73
Q1	9.64	125	125	1.00	Referent	1.00	Referent	
Q2	13.50	120	131	0.91	0.64, 1.30	0.81	0.56, 1.17	
Q3	17.70	127	123	1.03	0.72, 1.48	0.88	0.60, 1.28	
Q4	24.50	127	123	1.04	0.72, 1.50	0.88	0.60, 1.30	
DHA (22:6)								
Q1	1.05	118	120	1.00	Referent	1.00	Referent	0.76
Q2	1.31	132	126	1.06	0.75, 1.51	0.96	0.67, 1.38	
Q3	1.58	118	136	0.88	0.61, 1.26	0.79	0.54, 1.15	
Q4	2.11	131	120	1.10	0.77, 1.59	0.95	0.63, 1.43	
LA (18:2)								0.10
Q1	21.90	125	124	1.00	Referent	1.00	Referent	
Q2	31.70	132	120	1.08	0.76, 1.55	0.95	0.65, 1.38	
Q3	42.00	123	126	0.96	0.67, 1.38	0.83	0.56, 1.22	
Q4	58.20	119	132	0.89	0.61, 1.28	0.74	0.49, 1.11	
GLA (18:3)[d]								0.75
Q1	1.10	121	122	1.00	Referent	1.00	Referent	
Q2	1.91	118	137	0.87	0.61, 1.24	0.82	0.57, 1.19	
Q3	2.80	126	123	1.02	0.71, 1.46	0.98	0.67, 1.43	
Q4	4.45	134	117	1.14	0.80, 1.64	0.98	0.67, 1.44	
DGLA (20:3)								0.16
Q1	0.93	121	125	1.00	Referent	1.00	Referent	
Q2	1.42	131	123	1.09	0.77, 1.55	1.01	0.70, 1.45	
Q3	1.92	126	123	1.05	0.73, 1.51	0.88	0.60, 1.29	
Q4	2.76	121	131	0.94	0.65, 1.36	0.77	0.52, 1.16	

Table continues

Table 3. Continued

PUFA	Median Value, ng/mL	No. of Cases (*n* = 499)	No. of Controls (*n* = 502)	OR[a]	95% CI	AOR[b]	95% CI	*P* for Trend[c]
AA (20:4)								0.90
Q1	4.94	118	131	1.00	Referent	1.00	Referent	
Q2	5.92	123	127	1.09	0.76, 1.56	0.96	0.66, 1.40	
Q3	6.94	127	123	1.17	0.81, 1.71	0.97	0.65, 1.44	
Q4	8.44	131	121	1.24	0.85, 1.80	1.02	0.68, 1.51	
Total PUFAs[e]								0.16
Q1	44.10	123	125	1.00	Referent	1.00	Referent	
Q2	60.00	140	126	1.12	0.79, 1.60	1.07	0.74, 1.55	
Q3	75.40	116	125	0.94	0.65, 1.36	0.77	0.52, 1.15	
Q4	101.00	120	126	0.96	0.66, 1.40	0.81	0.54, 1.21	
Total ω-3 PUFAs[f]								0.87
Q1	12.30	124	135	1.00	Referent	1.00	Referent	
Q2	16.70	131	124	0.85	0.59, 1.23	0.76	0.52, 1.11	
Q3	21.20	119	122	0.98	0.68, 1.41	0.83	0.57, 1.21	
Q4	29.00	125	121	1.06	0.73, 1.53	0.90	0.61, 1.33	
Total ω-6 PUFAs[g]								0.18
Q1	30.30	124	127	1.00	Referent	1.00	Referent	
Q2	42.20	142	125	1.25	0.88, 1.79	1.11	0.77, 1.62	
Q3	55.00	118	125	0.91	0.63, 1.32	0.78	0.52, 1.17	
Q4	73.50	115	125	0.97	0.67, 1.40	0.80	0.53, 1.20	

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; AOR, adjusted odds ratio; CI, confidence interval; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; OR, odds ratio; PUFA, polyunsaturated fatty acid; Q, quartile; SA, stearidonic acid.

[a] Crude OR from a conditional logistic regression model accounting for study matching factors only (sex, birth month, and year of birth).

[b] Adjusted OR from a conditional logistic regression model including maternal education, maternal race/ethnicity, prepregnancy body mass index, maternal age, health insurance status at delivery, and an indicator for short interpregnancy interval.

[c] *P* value from a Wald test for trend across the ordinal score variable for the PUFA, defined using the median value within each quartile.

[d] Numbers in ALA and GLA quartiles do not sum to the total sample sizes because of the exclusion of 17 samples that failed quality control for ALA and 3 that failed for GLA.

[e] Sum of all individual PUFAs shown.

[f] Sum of ALA, SA, EPA, DPA, and DHA.

[g] Sum of LA, GLA, DGLA, and AA.

based on very small numbers in the ASD-with-ID group. Taken together, and considering multiple comparisons, the results of this study do not offer much evidence implicating these PUFAs in ASD etiology, though directions for future research are noted below.

A primary goal of this work was to determine whether associations we previously observed (30) on the basis of reported maternal diet in Nurses' Health Study II could be replicated when using measured PUFA levels from midpregnancy. Despite a larger number of cases in our present analysis, we did not replicate the previous statistically significant findings (30) suggesting inverse associations between the highest quartiles of total PUFA level and LA and ASD overall. Potential reasons for differences could be measurement error in estimated PUFA levels according to reported diet or differences in the time periods covered (with the earlier work spanning a broader period overlapping with pregnancy and analyses here timed more specifically to the second trimester). However, it is of some note that the point estimates for the top quartile of LA were quite similar to those of the previous work based on reported diet (30). Taken together, these studies might suggest a potential benefit of higher levels of prenatal LA in child ASD risk. A potential beneficial role of LA in reducing risk of ASD is biologically plausible, perhaps through an immune-mediated pathway; studies in humans have consistently demonstrated reductions in cholesterol levels and in the risk of cardiovascular disease with higher levels of LA and total ω-6 PUFAs (48). However, these findings conflict with those from a Dutch study that found increases in ASD-related traits in association with higher midpregnancy plasma total ω-6 PUFA and LA levels (34) and with those from a high-familial-risk cohort, the Markers of Autism Risk in Babies (MARBLES) cohort (35). In the latter study, Cohen et al.

Table 4. Associations Between Maternal Midpregnancy Serum Polyunsaturated Fatty Acid Levels (Quartiles) and Offspring Autism Spectrum Disorder With and Without Co-Occurring Intellectual Disability in a Population-Based Case-Control Study, California, 2010–2011[a]

PUFA	ASD With ID (*n* = 67)				ASD Without ID (*n* = 432)			
	No. of Cases	AOR[b]	95% CI	*P* for Trend	No. of Cases	AOR[b]	95% CI	*P* for Trend
ALA (18:3)[c]				0.76				0.71
Q1 (lowest)	13	1.00	Referent		96	1.00	Referent	
Q2	20	1.43	0.65, 3.16		112	1.21	0.83, 1.77	
Q3	18	1.33	0.68, 3.01		107	1.06	0.72, 1.56	
Q4 (highest)	14	1.02	0.42, 2.48		110	1.15	0.77, 1.71	
SA (18:4)				0.56				0.93
Q1	16	1.00	Referent		74	1.00	Referent	
Q2	13	0.64	0.28, 1.49		125	1.20	0.80, 1.80	
Q3	22	1.04	0.46, 2.40		100	1.14	0.73, 1.78	
Q4	16	0.69	0.29, 1.66		133	1.10	0.71, 1.70	
EPA (20:5)				0.52				0.39
Q1	17	1.00	Referent		90	1.00		
Q2	17	1.04	0.57, 2.26		119	1.34	0.91, 1.99	
Q3	18	0.85	0.36, 1.70		98	0.92	0.60, 1.40	
Q4	15	0.78	0.32, 1.91		125	1.30	0.83, 2.03	
DPA (22:5)				0.24				0.56
Q1	18	1.00	Referent		107	1.00	Referent	
Q2	15	0.83	0.37, 1.88		105	0.80	0.54, 1.17	
Q3	12	0.67	0.28, 1.62		115	0.92	0.62, 1.36	
Q4	22	1.48	0.67, 3.24		105	0.83	0.56, 1.25	
DHA (22:6)				0.54				0.96
Q1	16	1.00	Referent		102	1.00	Referent	
Q2	21	1.18	0.54, 2.57		111	0.93	0.63, 1.37	
Q3	14	0.62	0.26, 1.45		104	0.82	0.55, 1.22	
Q4	16	0.88	0.37, 2.10		115	1.00	0.65, 1.54	
LA (18:2)				0.94				0.08
Q1	17	1.00	Referent		108	1.00	Referent	
Q2	12	0.67	0.28, 1.62		120	1.00	0.68, 1.48	
Q3	22	1.04	0.47, 2.30		101	0.78	0.52, 1.17	
Q4	16	0.83	0.34, 2.02		103	0.74	0.48, 1.12	
GLA (18:3)				0.76				0.79
Q1	19	1.00	Referent		102	1.00	Referent	
Q2	12	0.55	0.24, 1.27		106	0.90	0.61, 1.32	
Q3	19	1.13	0.52, 2.45		107	0.98	0.77, 1.46	
Q4	17	0.95	0.43, 2.08		117	1.01	0.68, 1.50	
DGLA (20:3)				0.82				0.13
Q1	15	1.00	Referent		106	1.00	Referent	
Q2	19	1.34	0.62, 2.90		112	0.97	0.66, 1.42	
Q3	17	1.01	0.44, 2.34		109	0.86	0.58, 1.29	
Q4	16	1.01	0.42, 2.40		105	0.74	0.49, 1.13	

Table continues

Table 4. Continued

PUFA	ASD With ID (*n* = 67)				ASD Without ID (*n* = 432)			
	No. of Cases	AOR[b]	95% CI	*P* for Trend	No. of Cases	AOR[b]	95% CI	*P* for Trend
AA (20:4)				0.79				0.89
Q1	18	1.00	Referent		100	1.00	Referent	
Q2	18	0.91	0.42, 1.97		105	0.94	0.63, 1.38	
Q3	13	0.74	0.31, 1.77		114	1.01	0.67, 1.52	
Q4	18	1.12	0.48, 2.64		113	1.00	0.66, 1.52	
Total PUFAs[d]				0.92				0.12
Q1	17	1.00	Referent		106	1.00	Referent	
Q2	15	0.87	0.38, 2.01		125	1.12	0.76, 1.65	
Q3	17	0.85	0.37, 1.96		99	0.76	0.50, 1.16	
Q4	18	1.01	0.43, 2.38		102	0.79	0.52, 1.21	
Total ω-3 PUFAs[e]				0.35				0.74
Q1	21	1.00	Referent		103	1.00	Referent	
Q2	18	0.75	0.32, 1.76		113	0.76	0.51, 1.13	
Q3	11	0.71	0.30, 1.71		108	0.85	0.57, 1.26	
Q4	17	1.28	0.58, 2.80		108	0.87	0.58, 1.30	
Total ω-6 PUFAs[f]				0.90				0.07
Q1	17	1.00	Referent		107	1.00	Referent	
Q2	15	1.05	0.45, 2.41		127	1.14	0.77, 1.67	
Q3	17	0.89	0.38, 2.07		101	0.76	0.50, 1.16	
Q4	18	1.10	0.45, 2.69		97	0.77	0.51, 1.18	

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; AOR, adjusted odds ratio; ASD, autism spectrum disorder; CI, confidence interval; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; ID, intellectual disability; LA, linoleic acid; PUFA, polyunsaturated fatty acid; Q, quartile; SA, stearidonic acid.

[a] Cases were identified in California Department of Developmental Services records.

[b] AOR from a conditional logistic regression model (as in Table 3) accounting for study matching factors (sex, birth month, and year of birth), maternal education, maternal race/ethnicity, prepregnancy body mass index, maternal age, health insurance status at delivery, and an indicator for short interpregnancy interval.

[c] Numbers for ALA do not sum to the total sample sizes because some samples failed quality control.

[d] Sum of all individual PUFAs shown.

[e] Sum of ALA, SA, EPA, DPA, and DHA.

[f] Sum of LA, GLA, DGLA, and AA.

found no association with ω-6 PUFA and LA levels but suggestive findings for ω-3 PUFAs according to measured levels of eicosapentaenoic acid and total ω-3 PUFAs based on reported diet (35). However, the study's small sample size limited statistical power, and it is not known whether or how associations may differ in a high-familial-risk setting. The Dutch investigation (34) considered only a subset of items from a common ASD trait parent-report measure, the Social Responsiveness Scale, and it may be that associations differ by phenotypic aspects of ASD (as potentially suggested by our results for ASD with and without ID). Differences in fish intake, overall diet, and levels of other environmental exposures potentially interacting with PUFAs across the different study populations may also account for discrepant findings.

A wealth of literature suggests neurocognitive benefits of ω-3 PUFAs; contrary to our hypotheses, our findings were generally not supportive of strong associations with DHA or other ω-3 PUFAs. We did observe somewhat stronger findings for an association with the lowest deciles of total PUFAs and ω-3 PUFAs in association with ASD with ID; however, we did not observe corresponding associations with DHA specifically, nor were patterns clear across all deciles or PUFAs. In contrast, there were several ω-6 PUFAs for which reductions in odds of ASD were suggested with higher (but not the highest) deciles. These results should be interpreted with caution given the small number of persons with ASD with ID in our study population and the potential underrepresentation in the DDS records of ID in the presence of ASD. These findings should be examined in other, larger study populations with more complete information on ID status. Because of the roles of ω-3 PUFAs in neurodevelopmental processes like synaptogenesis, neurogenesis, and differentiation, future mechanistic work might also consider

whether ω-3 mechanisms act more directly on fetal brain development and whether ω-6 mechanisms might be mediated through immune system pathways.

Lack of strong associations with maternal levels here does not preclude a potential role of PUFAs in ASD etiology. Abnormalities in placental structure and function (for which there is some emerging evidence in ASD (49, 50)) may impact placental fatty acid transfer to the developing fetus (51), and we were not able to measure fetal levels. In addition, PUFAs may serve as modifiers of other ASD risk factors; for example, recent work suggested that PUFAs may mitigate the adverse associations of the pesticide dichlorodiphenyltrichloroethane (DDT) with child neurodevelopment as measured by the Bayley Scales (52). It may also be that other time windows of rapid brain growth and PUFA uptake (including the late third trimester or infancy) represent critical windows for a relationship between PUFAs and ASD. If diet changed over the course of this time, such relationships might be missed. Future work with repeated measures should consider the role of PUFAs in these additional time periods, as neither diet nor development is static. Finally, it is possible that PUFAs relate to specific phenotypic aspects of ASD, given associations with other developmental outcomes (8, 22, 53) (and suggestive findings for differences by co-occurring ID here). Each of these areas represents potential directions for future research.

This study had a number of strengths representing advances over prior work, most notably the use of measured PUFA levels in samples collected during a time frame suspected to be biologically relevant. Additional strengths include examination of associations in approximately 1,000 mother-child pairs drawn from the general population with representation of ethnic diversity, use of a reliable source to obtain child diagnostic information, and examination of not just classes of PUFAs or total PUFAs but also individual fatty acids. However, a number of limitations should be noted. As stated, our ASD-with-ID case group was smaller than would be expected given comorbidity estimates (54); milder ID in particular is often not recognized until offspring reach an age older than that of the children included here (55) and therefore may not have been accounted for in DDS records at the time of data linkage. We did not have information on levels of mercury, which co-occurs with PUFAs in some fish and may have opposing effects on neurodevelopment (56). In future work, researchers should consider combined impacts of nutrient and environmental toxicant levels on ASD outcomes. We also did not have the ability to examine dietary sources of PUFAs or adjust for other dietary factors. However, adjustment for short interpregnancy interval (which has been associated with ASD in prior work (57–61)) may serve as a proxy for nutrient depletion. Although prior work supports the stability of PUFAs stored under our conditions (62, 63), we cannot rule out potential degradation of PUFAs, though this would not have been differential by case status because of the matching on time of birth. Low fish intake in the United States may have affected our ability to examine potential benefits with high levels of ω-3 PUFAs like DHA. Finally, we cannot rule out potential chance findings observed in secondary analyses.

Given the known importance of PUFAs in neurodevelopment and their involvement in multiple pathways relevant to ASD (6, 13, 64), this class of nutrients should be further considered for associations with ASD-related outcomes and phenotypes. Even in the face of no or limited direct associations with ASD, future work should also consider the potential modifying effects these fatty acids may exert on the impact of other ASD risk factors that may act through the same pathways. Because ASD is currently estimated to occur in 1 out of every 58 children, identifying modifiable factors that have the potential to ameliorate risks and associated disabilities is critical.

ACKNOWLEDGMENTS

Author affiliations: A.J. Drexel Autism Institute, Drexel University, Philadelphia, Pennsylvania (Kristen Lyall, Nathaniel W. Snyder, Rostislav Kuskovsky, Peining Xu, Anna Bostwick, Craig J. Newschaffer); Environmental Health Investigations Branch, California Department of Public Health, Richmond, California (Gayle C. Windham); Department of Epidemiology and Biostatistics, Dornsife School of Public Health, Drexel University, Philadelphia, Pennsylvania (Lucy Robinson); and Department of Biobehavioral Health, College of Health and Human Development, Pennsylvania State University, State College, Pennsylvania (Craig J. Newschaffer).

This work was funded by the US Army Medical Research Acquisition Activity through Autism Spectrum Disorder Research Program IDEA Development Award AR150143 (K.L.), as well as by National Institutes of Health grants K22 ES026235 and R03 CA211820 (N.W.S.).

The opinions, interpretations, conclusions, and recommendations presented in this article are those of the authors and are not necessarily endorsed by the US Army Medical Research Acquisition Activity. The biospecimens and data used in this study were obtained from the California Biobank Program (Screening Information Systems Biobank Request identification number 656), Section 6555(b), 17CCR. The California Department of Public Health is not responsible for the results or conclusions drawn by the authors of this publication, nor do they represent the official position of the California Department of Public Health.

Conflict of interest: none declared.

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington, DC: American Psychiatric Association; 2013.
2. Rice D, Barone S, Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect*. 2000;108(suppl 3): 511–533.

3. Lyall K, Croen L, Daniels J, et al. The changing epidemiology of autism spectrum disorders. *Annu Rev Public Health*. 2017;38:81–102.
4. Lyall K, Schmidt RJ, Hertz-Picciotto I. Maternal lifestyle and environmental risk factors for autism spectrum disorders. *Int J Epidemiol*. 2014;43(2):443–464.
5. Haggarty P. Effect of placental function on fatty acid requirements during pregnancy. *Eur J Clin Nutr*. 2004; 58(12):1559–1570.
6. McNamara RK, Vannest JJ, Valentine CJ. Role of perinatal long-chain omega-3 fatty acids in cortical circuit maturation: mechanisms and implications for psychopathology. *World J Psychiatry*. 2015;5(1):15–34.
7. Liu JJ, Green P, John Mann J, et al. Pathways of polyunsaturated fatty acid utilization: implications for brain function in neuropsychiatric health and disease. *Brain Res*. 2015;1597:220–246.
8. Schuchardt JP, Huss M, Stauss-Grabo M, et al. Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *Eur J Pediatr*. 2010; 169(2):149–164.
9. Wegiel J, Kuchna I, Nowicki K, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol*. 2010;119(6): 755–770.
10. Casper RC. Nutrients, neurodevelopment, and mood. *Curr Psychiatry Rep*. 2004;6(6):425–429.
11. Deckelbaum RJ, Worgall TS, Seo T. n-3 fatty acids and gene expression. *Am J Clin Nutr*. 2006;83(6 suppl):1520S–1525S.
12. Lee HS, Barraza-Villarreal A, Biessy C, et al. Dietary supplementation with polyunsaturated fatty acid during pregnancy modulates DNA methylation at IGF2/H19 imprinted genes and growth of infants. *Physiol Genomics*. 2014;46(23):851–857.
13. Jones ML, Mark PJ, Waddell BJ. Maternal dietary omega-3 fatty acids and placental function. *Reproduction*. 2014; 147(5):R143–R152.
14. Kiecolt-Glaser JK, Belury MA, Porter K, et al. Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. *Psychosom Med*. 2007;69(3):217–224.
15. Simopoulos AP. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed Pharmacother*. 2006;60(9):502–507.
16. Elovitz MA, Brown AG, Breen K, et al. Intrauterine inflammation, insufficient to induce parturition, still evokes fetal and neonatal brain injury. *Int J Dev Neurosci*. 2011; 29(6):663–671.
17. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol*. 2001; 120(1-2):170–179.
18. Smith SE, Li J, Garbett K, et al. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*. 2007;27(40):10695–10702.
19. Braunschweig D, Ashwood P, Krakowiak P, et al. Autism: maternally derived antibodies specific for fetal brain proteins. *Neurotoxicology*. 2008;29(2):226–231.
20. Croen LA, Grether JK, Yoshida CK, et al. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Arch Pediatr Adolesc Med*. 2005;159(2):151–157.
21. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. *Br J Psychiatry*. 2009; 195(1):7–14.
22. Agostoni C, Nobile M, Ciappolino V, et al. The role of omega-3 fatty acids in developmental psychopathology: a systematic review on early psychosis, autism, and ADHD. *Int J Mol Sci*. 2017;18(12):2608.
23. Alessandri JM, Poumés-Ballihaut C, Langelier B, et al. Incorporation of docosahexaenoic acid into nerve membrane phospholipids: bridging the gap between animals and cultured cells. *Am J Clin Nutr*. 2003;78(4):702–710.
24. Chung WL, Chen JJ, Su HM. Fish oil supplementation of control and (n-3) fatty acid-deficient male rats enhances reference and working memory performance and increases brain regional docosahexaenoic acid levels. *J Nutr*. 2008; 138(6):1165–1171.
25. Green P, Glozman S, Kamensky B, et al. Developmental changes in rat brain membrane lipids and fatty acids. The preferential prenatal accumulation of docosahexaenoic acid. *J Lipid Res*. 1999;40(5):960–966.
26. Brigandi SA, Shao H, Qian SY, et al. Autistic children exhibit decreased levels of essential fatty acids in red blood cells. *Int J Mol Sci*. 2015;16(5):10061–10076.
27. Mankad D, Dupuis A, Smile S, et al. A randomized, placebo controlled trial of omega-3 fatty acids in the treatment of young children with autism. *Mol Autism*. 2015;6:Article 18.
28. Mostafa GA, El-Khashab HY, Al-Ayadhi LY. A possible association between elevated serum levels of brain-specific auto-antibodies and reduced plasma levels of docosahexaenoic acid in autistic children. *J Neuroimmunol*. 2015;280:16–20.
29. Voigt RG, Mellon MW, Katusic SK, et al. Dietary docosahexaenoic acid supplementation in children with autism. *J Pediatr Gastroenterol Nutr*. 2014;58(6):715–722.
30. Lyall K, Munger KL, O'Reilly EJ, et al. Maternal dietary fat intake in association with autism spectrum disorders. *Am J Epidemiol*. 2013;178(2):209–220.
31. Gao L, Cui SS, Han Y, et al. Does periconceptional fish consumption by parents affect the incidence of autism spectrum disorder and intelligence deficiency? A case-control study in Tianjin, China. *Biomed Environ Sci*. 2016;29(12): 885–892.
32. Julvez J, Mendez M, Fernandez-Barres S, et al. Maternal consumption of seafood in pregnancy and child neuropsychological development: a longitudinal study based on a population with high consumption levels. *Am J Epidemiol*. 2016;183(3):169–182.
33. Suren P, Roth C, Bresnahan M, et al. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA*. 2013;309(6): 570–577.
34. Steenweg-de Graaff J, Tiemeier H, Ghassabian A, et al. Maternal fatty acid status during pregnancy and child autistic traits: the Generation R Study. *Am J Epidemiol*. 2016;183(9): 792–799.
35. Huang Y, Iosif AM, Hansen RL, et al. Maternal polyunsaturated fatty acids and risk for autism spectrum disorder in the MARBLES high-risk study. *Autism*. 2020; 24(5):1191–1200.
36. Jensen CL. Effects of n-3 fatty acids during pregnancy and lactation. *Am J Clin Nutr*. 2006;83(6 suppl):1452s–1457s.
37. Croen LA, Grether JK, Hoogstrate J, et al. The changing prevalence of autism in California. *J Autism Dev Disord*. 2002;32(3):207–215.
38. Croen LA, Grether JK, Selvin S. Descriptive epidemiology of autism in a California population: who is at risk? *J Autism Dev Disord*. 2002;32(3):217–224.
39. Lyall K, Croen LA, Sjödin A, et al. Polychlorinated biphenyl and organochlorine pesticide concentrations in maternal

mid-pregnancy serum samples: association with autism spectrum disorder and intellectual disability. *Environ Health Perspect*. 2017;125(3):474–480.
40. Windham GC, Anderson MC, Croen LA, et al. Birth prevalence of autism spectrum disorders in the San Francisco Bay area by demographic and ascertainment source characteristics. *J Autism Dev Disord*. 2011;41(10): 1362–1372.
41. Hertz-Picciotto I, Croen LA, Hansen R, et al. The CHARGE Study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect*. 2006;114(7):1119–1125.
42. Succop PA, Clark S, Chen M, et al. Imputation of data values that are less than a detection limit. *J Occup Environ Hyg*. 2004;1(7):436–441.
43. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med*. 1989;8(5):551–561.
44. Li R, Hertzmark ME, Louie M, et al. The SAS LGTPHCURV9 Macro. 2011. https://cdn1.sph.harvard.edu/wp-content/uploads/sites/271/2012/09/lgtphcurv9_7-3-2011.pdf. Accessed November 25, 2019.
45. Huen K, Yousefi P, Bradman A, et al. Effects of age, sex, and persistent organic pollutants on DNA methylation in children. *Environ Mol Mutagen*. 2014;55(3):209–222.
46. Lyall K, Croen LA, Weiss LA, et al. Prenatal serum concentrations of brominated flame retardants and autism spectrum disorder and intellectual disability in the Early Markers of Autism study: a population-based case control study in California. *Environ Health Perspect*. 2017; 125(8):087023.
47. Roberts A, Lyall K, Hart JE, et al. Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. *Environ Health Perspect*. 2013;121(8):978–984.
48. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation*. 2009; 119(6):902–907.
49. Park BY, Misra DP, Moye J, et al. Placental gross shape differences in a high autism risk cohort and the general population. *PLoS One*. 2018;13(8):e0191276.
50. Walker CK, Anderson KW, Milano KM, et al. Trophoblast inclusions are significantly increased in the placentas of children in families at risk for autism. *Biol Psychiatry*. 2013; 74(3):204–211.
51. Lewis RM, Childs CE, Calder PC. New perspectives on placental fatty acid transfer. *Prostaglandins Leukot Essent Fatty Acids*. 2018;138:24–29.
52. Ogaz-González R, Mérida-Ortega A, Torres-Sánchez L, et al. Maternal dietary intake of polyunsaturated fatty acids modifies association between prenatal DDT exposure and child neurodevelopment: a cohort study. *Environ Pollut*. 2018;238:698–705.
53. Strain JJ, Davidson PW, Thurston SW, et al. Maternal PUFA status but not prenatal methylmercury exposure is associated with children's language functions at age five years in the Seychelles. *J Nutr*. 2012;142(11):1943–1949.
54. Maski KP, Jeste SS, Spence SJ. Common neurological co-morbidities in autism spectrum disorders. *Curr Opin Pediatr*. 2011;23(6):609–615.
55. Committee to Evaluate the Supplemental Security Income Disability Program for Children With Mental Disorders; Board on the Health of Select Populations; Board on Children, Youth, and Families; Institute of Medicine; Division of Behavioral and Social Sciences and Education; National Academies of Sciences, Engineering, and Medicine. Clinical characteristics of intelletual disabilities. In: Boat TF, Wu JT, eds. *Mental Disorders and Disabilities Among Low-Income Children*. Washington, DC: National Academies Press; 2015:169–178.
56. Oken E, Bellinger DC. Fish consumption, methylmercury and child neurodevelopment. *Curr Opin Pediatr*. 2008;20(2): 178–183.
57. Cheslack-Postava K, Liu K, Bearman PS. Closely spaced pregnancies are associated with increased odds of autism in California sibling births. *Pediatrics*. 2011;127(2):246–253.
58. Cheslack-Postava K, Suominen A, Jokiranta E, et al. Increased risk of autism spectrum disorders at short and long interpregnancy intervals in Finland. *J Am Acad Child Adolesc Psychiatry*. 2014;53(10):1074–1081.e4.
59. Coo H, Ouellette-Kuntz H, Lam YM, et al. The association between the interpregnancy interval and autism spectrum disorder in a Canadian cohort. *Can J Public Health*. 2015; 106(2):e36–e42.
60. Gunnes N, Surén P, Bresnahan M, et al. Interpregnancy interval and risk of autistic disorder. *Epidemiology*. 2013; 24(6):906–912.
61. Zerbo O, Yoshida C, Gunderson EP, et al. Interpregnancy interval and risk of autism spectrum disorders. *Pediatrics*. 2015;136(4):651–657.
62. Fox H, Ross BM, Tocher D, et al. Degradation of specific polyunsaturated fatty acids in red blood cells stored at −20°C proceeds faster in patients with schizophrenia when compared with healthy controls. *Prostaglandins Leukot Essent Fatty Acids*. 2003;69(5):291–297.
63. Powers CD, Chen H, Sternberg M, et al. Stability of 24 plasma fatty acids stored at 20°C [abstract]. *Presented at the Annual Scientific Meeting of the American Association of Clinical Chemistry*, Atlanta, Georgia, July 24–28, 2011.
64. Dyall SC. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. *Front Aging Neurosci*. 2015;7:Article 52.