Journal of Autism and Developmental Disorders (2022) 52:2801–2811
https://doi.org/10.1007/s10803-021-05110-9

BRIEF REPORT

The Association of Prenatal Vitamins and Folic Acid Supplement Intake with Odds of Autism Spectrum Disorder in a High-Risk Sibling Cohort, the Early Autism Risk Longitudinal Investigation (EARLI)

Katharine K. Brieger[1] · Kelly M. Bakulski[1] · Celeste L. Pearce[1] · Ana Baylin[1,2] · John F. Dou[1] · Jason I. Feinberg[3] · Lisa A. Croen[4] · Irva Hertz-Picciotto[5] · Craig J. Newschaffer[6] · M. Daniele Fallin[3] · Rebecca J. Schmidt[5]

Accepted: 24 May 2021 / Published online: 10 June 2021

Abstract

We examined maternal prenatal vitamin use or supplemental folic acid intake during month one of pregnancy for association with autism spectrum disorder (ASD) in the Early Autism Risk Longitudinal Investigation, an enriched-risk pregnancy cohort. Total folic acid intake was calculated from monthly prenatal vitamins, multivitamins, and other supplement reports. Clinical assessments through age 3 years classified children as ASD (n = 38) or non-ASD (n = 153). In pregnancy month one, prenatal vitamin use (59.7%) was not significantly associated with odds of ASD (OR = 0.70, 95%CI 0.32, 1.53). Sample size was limited and residual confounding was possible. Given the estimated effect sizes in this and previous work, prenatal vitamin intake during early pregnancy could be a clinically useful preventative measure for ASD.

Keywords Prenatal vitamins · Folic acid · Pregnancy cohort · Autism

Introduction

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by persistent deficits in social communication and interaction, as well as restricted, repetitive behaviors (American Psychiatric Association, 2013). In 2018, ASD was observed to affect 1 in 54 children by eight years of age in the United States (Maenner et al., 2020). Perturbations to the delicate in utero neurodevelopmental process likely contribute to the development of ASD. While the etiology of ASD is at least in part genetic, several environmental factors have been associated with increased ASD risk, including prenatal infection with fever (Brucato et al., 2017; Christian et al., 2018; Croen et al., 2019; Hornig et al., 2018), maternal diabetes (Hertz-Picciotto et al., 2018; Xu et al., 2014), perinatal stress (Gardener et al., 2011), chemical toxicants (Ye et al., 2017), and certain medications (Gardener et al., 2009). Understanding modifiable exposures associated with ASD has great public health potential, particularly for high-risk families.

Maternal prenatal nutrition is critical for proper neurodevelopment (Chen et al., 2016; Grant & Soles, 2009; Nuttall, 2017). It is well-established that folic acid is crucial for neural tube development (Berry et al., 1999; Centers for Disease Control & Prevention, 1992; Pitkin, 2007; Rieder, 1994). Pregnant

✉ Kelly M. Bakulski
bakulski@umich.edu

✉ Rebecca J. Schmidt
rjschmidt@ucdavis.edu

1 Department of Epidemiology, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, MI 48109, USA

2 Department of Nutritional Sciences, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, MI 48109, USA

3 Department of Mental Health, Johns Hopkins University, 624 N. Broadway, Hampton House 850, Baltimore, MD 21205, USA

4 Kaiser Permanente Division of Research, 2000 Broadway, Oakland, CA 94612, USA

5 Department of Public Health Sciences and The MIND Institute, School of Medicine, University of California-Davis, UC Davis School of Medicine - Medical Sciences 1C, Suite 123, Davis, CA 95616, USA

6 Department of Biobehavioral Health, Penn State University, State College, 325 Health and Human Development Building, University Park, PA 16802, USA

women should consume at least 400 µg (mcg) folic acid or at least 600 mcg dietary folate equivalents (Bailey, 1998; Centers for Disease Control & Prevention, 1992) from dietary folates (naturally occurring in food), fortified foods (such as cereals), prenatal vitamins, multivitamins, and folic acid supplements. Compared to multivitamins, prenatal vitamins typically contain more folic acid (about 800 mcg in prenatal vitamins versus 400 mcg in multivitamins) and iron (about 28 mg versus 18 mg). Folic acid-specific supplements are prescribed in a range of doses, from 400 to 4000 mcg, with the highest doses often reserved for those with a previous pregnancy affected by a neural tube defect (Centers for Disease Control and Prevention, 1991).

Evidence supports protective associations for intake of prenatal vitamins (Levine et al., 2018; Schmidt et al., 2011, 2019) or folic acid (Levine et al., 2018; Schmidt et al., 2012; Suren et al., 2013) with ASD. However, the size and direction of the reported effects are variable (Braun et al., 2014; DeVilbiss et al., 2017). Two studies observed supplementation versus very low levels was associated with protection, while very high folic acid intake (greater than 1000 mcg) or high maternal plasma folate levels ($\geq$60.3 nmol/L) were associated with risk, suggesting a u-shaped pattern (Raghavan et al., 2016, 2018). It is difficult to compare previous studies with pregnancy exposure periods defined in different and overlapping manners (periconceptionally, first trimester, all of pregnancy). Many studies queried supplement use only once and are subject to exposure misclassification. Replication with prospective prenatal measures of intake doses, dietary and supplement sources, and precise timing is limited.

We evaluated the relationship between prenatal vitamin and folic acid intake (including timing, supplement type, and dose) during early pregnancy and odds of ASD recurrence in younger siblings of persons with ASD. Our analysis was conducted in the Early Autism Risk Longitudinal Investigation (EARLI), a prospective, enriched-risk pregnancy cohort with detailed exposure measurements collected throughout pregnancy and child outcome evaluation at 36-months using a gold-standard clinical assessment protocol for ASD. We hypothesized that prenatal vitamin and folic acid intake during early pregnancy would be associated with reduced odds of ASD in the child.

Methods

Study Design and Overall Sample

EARLI follows families who already have one child (the proband) diagnosed with ASD (autistic disorder, Asperger syndrome, or pervasive developmental disorder not otherwise specified) (Newschaffer et al., 2012). All probands were evaluated during an enrollment clinic visit to confirm ASD diagnosis via the Autism Diagnostic Observation Schedule (ADOS-2 scoring algorithm) (Newschaffer et al., 2012). To allow a broad eligibility window within pregnancy (first two trimesters), mothers were enrolled by the 28th week of a subsequent pregnancy. In total, 249 families were recruited from Southeast Pennsylvania (Drexel University), Northeast Maryland (Johns Hopkins University and Kennedy Krieger Institute), and Northern California (University of California, Davis, the MIND Institute, and Kaiser Permanente Division of Research). Informed consent was obtained from all participants and the Institutional Review Boards of all recruitment sites approved this study.

Prenatal Vitamin and Folic Acid Supplement Assessment

At the first study visit, mothers were asked to recall supplement use (multivitamin, prenatal vitamin, or folic acid) for any previous month of pregnancy. Then, supplement use was reported prospectively by monthly questionnaire. If a supplement was used, mothers were asked about the type, brand, and frequency of use. Average daily total supplemental folic acid intake was estimated from the total in the prenatal vitamins, multivitamins, and any other folic acid-containing supplements (Schmidt et al., 2019). Each brand a mother reported was queried; if the brand was unknown then standard amounts found in that vitamin type were used. Total folic acid intake from supplements was then categorized into low (< 400 mcg), adequate (400–1000 mcg), and high (> 1000 mcg) based on the recommendations of the Centers for Disease Control (Centers for Disease Control & Prevention, 1992). Our primary exposure variable was prenatal vitamin supplement use (yes versus no) during the first month of pregnancy.

ASD Outcome Assessment

Postnatal clinical evaluations were completed four times. Our primary outcome of interest was ASD at the 36-month visit using *DSM-5* criteria (American Psychiatric Association, 2013) and children were grouped into two outcome levels (ASD, non-ASD). Sensitivity analyses considered continuous outcome scores on the Autism Observation Scale for Infants (AOSI) (Bryson et al., 2008) at 12 months, total calibrated severity scores for the ADOS-2 to allow comparison across modules (Gotham et al., 2009; Lord et al., 2000, 2012) at 36 months, and the Mullen Scales of Early Learning (MSEL) at 36 months. In an additional sensitivity analysis, children were grouped into three outcome levels (ASD, non-typically developing, typically developing), separating the non-ASD into those with non-typical development, but not ASD, from those with typical development. These groupings were made according to the Baby Siblings Research

Consortium (BSRC) criteria (Ozonoff et al., 2014), based on definitions of elevated ADOS scores, low MSEL scores, or both.

Demographics and Covariate Ascertainment

Demographic information was collected at the maternal pregnancy interviews. The key covariates considered were: maternal age (years), paternal age (years), maternal education (bachelor's degree or higher), maternal smoking behavior (any), maternal body mass index (BMI) at the first pregnancy study visit, gestational age of the younger sibling at birth (weeks), and sex of the younger sibling.

Exclusion Criteria for Analytic Samples

Of the 249 families enrolled, nine pregnancies did not result in live births. Ten of the remaining 240 pregnancies resulted in twins, and one twin from each family was randomly excluded. Next, 46 families were excluded due to missing ASD diagnosis at 36-months. One family was excluded for missing information for prenatal vitamins and total folic acid intake for the first trimester of pregnancy. Two families were excluded for missing maternal education level. The analytic sample was comprised of 191 mother–child pairs (Supplemental Fig. 1).

Statistical Analyses

We compared demographic information and covariates for families in the analytic sample (n = 191 families) to those excluded (n = 49 families). For categorical variables (maternal race, maternal ethnicity, maternal education, maternal BMI classification, maternal smoking, prenatal vitamin use, prenatal folic acid intake, sibling sex), chi-square tests of proportions were used. For continuous variables (maternal and paternal age, gestational age, AOSI total score at 12 months, and ADOS comparison score at 36 months), t-tests were used.

We conducted bivariate analyses of parental demographics and child characteristics noted above by the primary exposure and outcome variables. We used multivariate logistic regression to estimate odds of ASD for children of mothers who took prenatal vitamins in month one compared to those whose mothers did not, adjusting for (1) maternal education and (2) maternal education and child sex. Maternal education was a confounder (altered the relationship between prenatal vitamins and ASD by > 10%). Inclusion of child sex improved model performance by AIC.

Additional and Sensitivity Analyses

We performed several exposure sensitivity analyses. First, we used logistic regression to estimate the association of categorized folic acid intake (low, adequate, and high) during the first month of pregnancy with ASD. Second, we used spline models to assess the relationship of total folic acid intake in month one of pregnancy to ASD outcomes. Third, we examined prenatal vitamin use across all months (one through nine, each measured separately) of pregnancy with a mixed effects logistic regression model, including a random intercept for each mother and a spline term for timing of prenatal vitamin use.

We also conducted outcome sensitivity analyses, all measured at 36 months. First, we used multinomial logistic regression to assess three-level BSRC groupings (ASD (n = 36) or non-typically developing (n = 71) relative to typically developing (n = 77)). Next, we used a generalized linear model (with a negative binomial distribution and log link function) to assess the relationship between prenatal vitamin use and ADOS calibrated severity score. Finally, we used a generalized linear model approach (with normality assumptions and a linear link function) to assess prenatal vitamin use with the MSEL early learning composite t-score.

Comparison with Prior Studies' Results

We conducted a literature review to identify relevant studies of prenatal nutritional supplements and ASD. Because of the wide variety of types of prenatal supplements and of study designs, it is crucial to put the results of the new study in the context of prior research. The present prospective study has a limited sample size, and comparison with direction and magnitude of prior findings may be important. We classified studies by exposure period (i.e. preconception, first trimester, all of pregnancy) and by exposure type (folic acid, prenatal vitamins, multivitamins). We prioritized studies that examined the relationship between intake of supplements during the first or second trimester and ASD outcomes. From an initial 15 studies, two were excluded for exposure timing or for having only plasma measures (Al-Farsi et al., 2013; Steenweg-de Graaff et al., 2015), two were excluded for missing ASD outcomes (Czeizel & Dobo, 1994; Tamura et al., 2005), and one was excluded for only reporting on the interaction of folic acid with air pollution and not the direct effect of folic acid (Goodrich et al., 2018). Thus, ten studies had tests in at least one supplement type: three of prenatal vitamins (Braun et al., 2014; Schmidt et al., 2011, 2019), five of multivitamins (DeVilbiss et al., 2017; Levine et al., 2018; Raghavan et al., 2018; Suren et al., 2013; Virk et al., 2016), and six of folic acid (DeVilbiss et al., 2017; Levine et al., 2018; Nilsen et al., 2013; Schmidt et al., 2012; Suren et al., 2013; Virk et al., 2016).

Results

Sample Demographic Characteristics

The families in the analytic sample did not differ from those excluded (Supplemental Table 1). Similarly, the siblings excluded were comparable in terms of sex ratio and AOSI total score at 12 months (Supplemental Table 1). In our analytic sample, maternal age at conception ranged from 21 to 44 (mean = 33.59, SD = 4.79) and paternal age ranged from 22 to 55 (mean = 35.66, SD = 5.98). Among mothers in the analytic sample, 60.2% reported White race, 17.3% reported Hispanic ethnicity, and 58.1% reported earning a bachelor's degree or higher (Supplemental Table 1).

Distribution of Prenatal Vitamin and Folic Acid Supplement Use

Prenatal vitamin use in the first month of pregnancy was lower among mothers of ASD cases (52.6%) compared to mothers of non-ASD children (61.4%, Table 1). Prenatal vitamin use was significantly associated with maternal education, where a higher proportion of mothers with a bachelor's degree or higher (67.5%) took prenatal vitamins, relative to mothers with less education (44.2%, p = 0.002). Additionally, prenatal vitamin use was associated with total folic acid category, where more mothers who took prenatal vitamins had adequate levels of folic acid (65.8% compared to 14.3%, p < 0.001) (Table 1). Use of prenatal vitamins varied during pregnancy, but by the end of the first trimester, most women (89%) used prenatal vitamins (Fig. 1).

Distribution of ASD Outcomes in Study Sample

One fifth (n = 38, 19.9%) of the children in our analytic sample were diagnosed with ASD at 36-months, according to *DSM-5* criteria (Table 1), as expected (Ozonoff et al., 2011). AOSI total score at 12 months was higher among cases (median = 6.0, IQR: [4.0, 9.0]) compared to non-ASD controls (median = 4.0, IQR: [2.0, 7.0], p = 0.01). Mothers of ASD cases were less educated than those of non-ASD controls (44.7% with a bachelor's degree or higher compared to 61.4% of controls, p = 0.09) (Table 1). Prior to pregnancy,

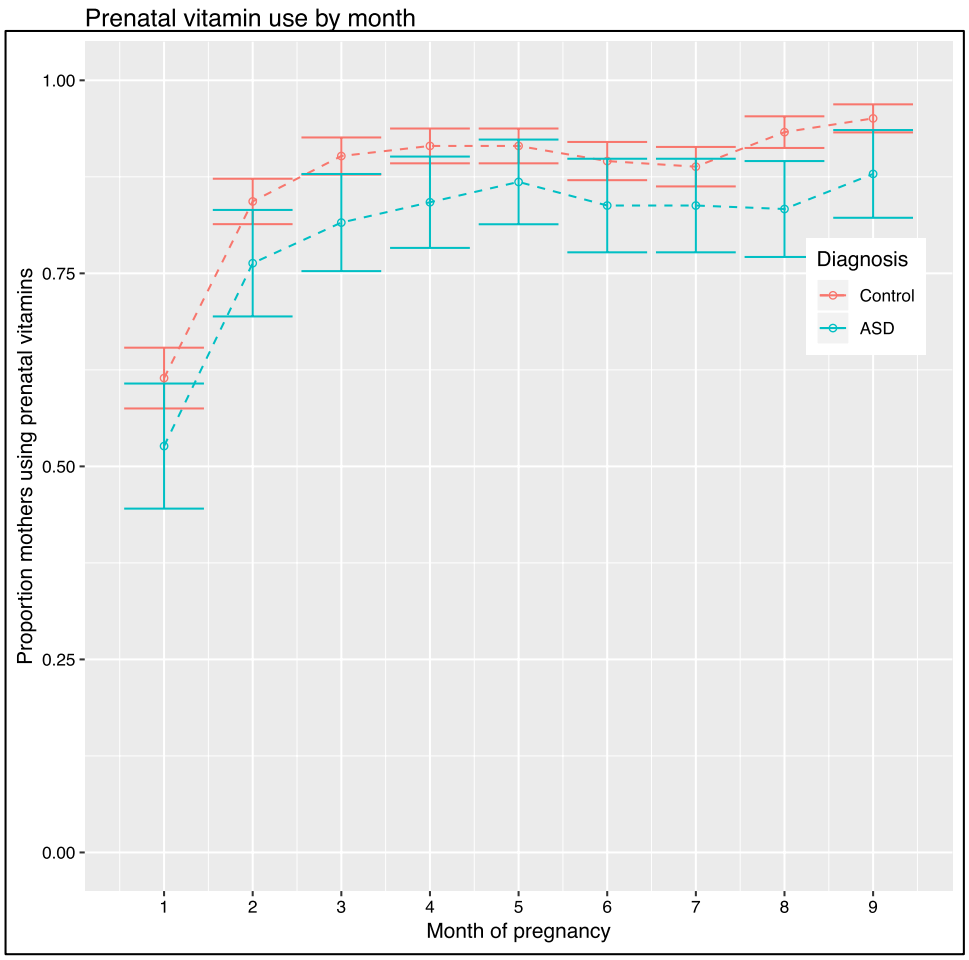

Fig. 1 Prenatal vitamin use across pregnancy in 191 mothers in the Early Autism Risk Longitudinal Investigation pregnancy cohort. Maternal use of prenatal vitamins increased steeply over the first trimester of pregnancy; by month 3 of pregnancy, 88% of mothers took prenatal vitamins. Across pregnancy, a lower proportion of mothers of eventual ASD cases (n = 38) were taking prenatal vitamins compared to mothers of non-ASD controls (n = 153). Errors bars represent the standard error of the mean for proportions; SE = sqrt(p*(1 − p)/n)

Table 1 Characteristics of families in the Early Autism Risk Longitudinal Investigation high-risk pregnancy cohort

	Case status			Prenatal vitamin use month 1		
	Non-ASD (n = 153)	ASD (n = 38)	p[a]	No (n = 77)	Yes (n = 114)	p[a]
Child characteristics						
Male sex (%)	71 (46.4)	30 (78.9)	0.001	36 (46.8)	65 (57.0)	0.213
Gestational age at birth, weeks (mean (sd))	39.12 (1.45)	38.70 (1.51)	0.143	39.06 (1.18)	39.02 (1.62)	0.85
Missing (%)	23 (15.0)	6 (15.8)		14 (18.2)	15 (13.2)	
AOSI[b] total score, 12 months (median [IQR])	4.0 [2.0, 7.0]	6.0 [4.0, 9.0]	0.012	4.0 [2.0, 6.25]	4.0 [2.0, 8.0]	0.803
Missing (%)	9 (5.9)	1 (2.6)		5 (6.5)	5 (4.4)	
ADOS[c] calibrated severity score (total), 36 months (median [IQR])	2.0 [1.0, 3.0]	7.0 [6.0, 8.0]	< 0.001	2.0 [1.0, 5.0]	2.0 [1.0, 5.0]	0.354
Missing (%)	4 (2.6)	2 (5.3)		0 (0.0)	6 (5.3)	
ASD[d] case (%)	–	–	–	18 (23.4)	20 (17.5)	0.42
Parental characteristics						
Maternal age (mean (sd))	33.61 (4.91)	33.50 (4.31)	0.901	33.40 (5.15)	33.71 (4.54)	0.664
Paternal age (mean (sd))	35.85 (6.00)	34.92 (5.95)	0.394	35.12 (6.34)	36.04 (5.73)	0.3
Missing (%)	1 (0.7)	0 (0.0)		0 (0.0)	1 (0.9)	
Maternal race (%)			0.180			0.550
Asian	19 (12.4)	5 (13.2)		8 (10.4)	16 (14.0)	
Black, African American	16 (10.5)	5 (13.2)		10 (13.0)	11 (9.6)	
White	94 (61.4)	21 (55.3)		46 (59.7)	69 (60.5)	
Other/multiple	13 (8.5)	1 (2.6)		5 (6.5)	9 (7.9)	
Missing	11 (7.2)	6 (15.8)		8 (10.4)	9 (7.9)	
Maternal ethnicity (%)			0.538			0.966
Hispanic	24 (15.7)	9 (23.7)		15 (19.5)	18 (15.8)	
Non-Hispanic	115 (75.2)	25 (65.8)		54 (70.1)	86 (75.4)	
Missing	14 (9.2)	4 (10.5)		8 (10.4)	10 (8.8)	
Maternal education, bachelor's degree or higher (%)	94 (61.4)	17 (44.7)	0.092	34 (44.2)	77 (67.5)	0.002
Maternal BMI[e] (%)			0.005			0.274
Underweight	1 (0.7)	1 (2.6)		0 (0.0)	2 (1.8)	
Normal	63 (41.2)	14 (36.8)		27 (35.1)	50 (43.9)	
Overweight	48 (31.4)	4 (10.5)		25 (32.5)	27 (23.7)	
Obese	37 (24.2)	19 (50.0)		25 (32.5)	31 (27.2)	
Missing	4 (2.6)	0 (0.0)		0 (0.0)	4 (3.5)	
Maternal pregnancy behaviors						
Prenatal vitamin use, Mo. 1 pregnancy (%)	94 (61.4)	20 (52.6)	0.42	–	–	–
Folic acid, Mo. 1 pregnancy			0.662			< 0.001
Low (< 400 mcg)	71 (46.4)	16 (42.1)		65 (84.4)	22 (19.3)	
Adequate (400–1000 mcg)	69 (45.1)	17 (44.7)		11 (14.3)	75 (65.8)	
High (> 1000 mcg)	13 (8.5)	5 (13.2)		1 (1.3)	17 (14.9)	
Prenatal smoking (%)			0.031			> 0.99
No	122 (79.7)	25 (65.8)		57 (74.0)	90 (78.9)	
Yes	3 (2.0)	4 (10.5)		3 (3.9)	4 (3.5)	
Missing	28 (18.3)	9 (23.7)		17 (22.1)	20 (17.5)	
Study enrollment gestational week	18.8 (6.9)	19.1 (7.2)	0.810	18.6 (6.0)	19.1 (7.5)	0.678

[a]Continuous variables tested with a t-test for difference in means and categorical variables tested with a chi-square test of equal proportions. Missing category excluded for categorical variables before testing for differences

[b]AOSI = Autism Observation Scale for Infants. The AOSI assessment consists of 18 items and is administered to infants age 6 to 18 months. An AOSI total score of 13 or greater indicates a child is likely to go on to develop ASD

[c]ADOS = Autism Diagnostic Observation Schedule. This is a semi-structured interview conducted by a trained examiner to assess communication, social interaction, and play. It is valid for ages 12 months through adulthood. Higher scores indicate higher severity of autism symptoms; threshold level for a total score indicating ASD varies by module and age level. The calibrated severity score is standardized across modules

[d]ASD = autism spectrum disorder. Primary analyses will use diagnosis as defined by the *Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5).*

[e]Pre-pregnancy self-report

mothers of ASD cases were more likely to have BMI levels categorized as obese (50.0%) than mothers of non-ASD controls (24.2%, p = 0.005).

Association of Prenatal Vitamin Use with ASD Outcomes

Prenatal vitamin use in month one of pregnancy was not associated with ASD before adjustment (OR = 0.70, 95%CI 0.34, 1.43) or after adjusting for maternal education and child sex (OR = 0.70, 95%CI 0.32, 1.53) (Table 2, Fig. 2). Results were consistent after additional adjustment for pre-pregnancy maternal BMI (OR = 0.67, 95%CI 0.29, 1.55) among the 187 mother-infant pairs with available information.

Additional and Sensitivity Analyses

High folic acid intake (> 1000 mcg) in month one compared to adequate (400–1000 mcg) was not associated with odds of ASD (OR = 1.64, 95%CI 0.44, 5.47), adjusted for child sex and maternal education (Table 2). In spline modeling of continuous total folic acid intake, we observed a suggestive increase in risk at the high end of intake values, but the confidence limit ratio was large (Supplemental Fig. 3).

When examining timing of prenatal vitamin use across pregnancy, in every month mothers whose child eventually developed ASD were less likely to be taking prenatal vitamins (p = 0.074) (Fig. 1).

Sensitivity analyses using three-level BSRC groupings revealed that prenatal vitamin use was not associated with odds of ASD versus typically developing, nor for odds of non-typically developing compared to typically developing (Supplemental Table 2). We did not observe significant differences in MSEL scores between children whose mothers who took prenatal vitamins in month one of pregnancy versus those whose mothers did not (increase in t-score of 5.24, 95%CI − 8.10, 18.6). There was no significant difference in ADOS calibrated severity score between children whose mothers took prenatal vitamins and those whose mothers did not (Table 1).

Discussion

In the EARLI prospective, enriched-risk pregnancy cohort, we did not observe a statistically significant association between prenatal vitamin intake in the first month of pregnancy and odds of ASD, although the direction and magnitude of association were consistent with previous findings (Schmidt et al., 2011, 2019). In these families at high risk of ASD, it is especially important to understand modifiable environmental risk factors. To our knowledge, this is only the second study reporting on prenatal vitamins' association with odds of recurrence in younger siblings. We observed a protective association of maternal education, consistent with prior studies (Delobel-Ayoub et al., 2015; Emerson, 2012; Rai et al., 2012). We also observed a higher rate of ASD among male children (sex ratio: 3.75 male to 1 female),

Table 2 Association of month 1 prenatal vitamin use and total folic acid supplementation with ASD outcomes in the Early Autism Risk Longitudinal Investigation high-risk pregnancy cohort

	Outcome		OR (95% CI)		
	ASD[a] (n = 38)	Non-ASD (n = 153)	Unadjusted	Adjusted[b] for education	Adjusted[b] for education and sex
Prenatal vitamins					
No (%)	18 (23.4)	59 (76.6)	1 (reference)	1 (reference)	1 (reference)
Yes (%)	20 (17.5)	94 (82.5)	0.70 (0.34, 1.43)	0.81 (0.38, 1.68)	0.70 (0.32, 1.53)
Folic acid					
CDC guidelines:					
Adequate[c]	17 (44.7)	69 (45.1)	1 (reference)	1 (reference)	1 (reference)
Low (< 400 mcg) (%)	16 (42.1)	71 (46.4)	0.91 (0.43, 1.96)	0.75 (0.34, 1.67)	0.75 (0.32, 1.69)
High (> 1000 mcg) (%)	5 (13.2)	13 (8.50)	1.56 (0.45, 4.79)	1.47 (0.45, 4.78)	1.64 (0.44, 5.47)

[a]According to *DSM-5* diagnostic criteria

[b]Education refers to maternal education (less than bachelor's versus bachelor's degree or higher) and sex refers to child sex

[c]Adequate intake used as reference category

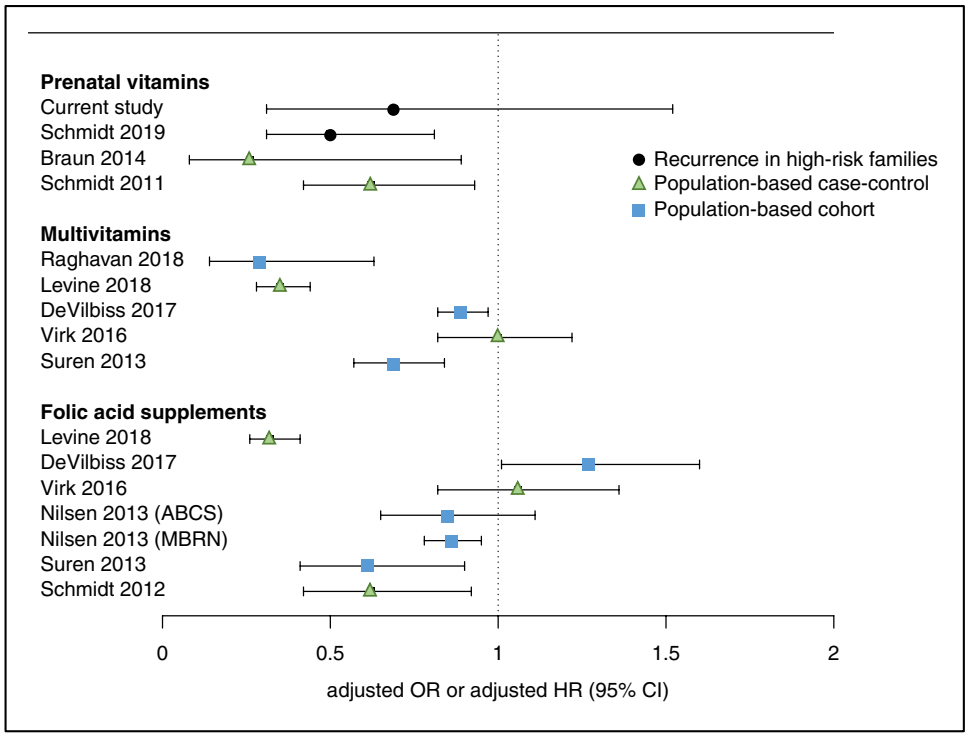

Fig. 2 The protective effect of prenatal vitamin use in month 1 of pregnancy among mothers in the EARLI cohort is consistent in magnitude and direction when compared to prior studies, though the estimate is less precise. *ABCS* Autism Birth Cohort Study, *MBRN* Medical Birth Registry of Norway

consistent with the literature (Loomes et al., 2017). The higher rate of pre-pregnancy obesity among mothers of children with ASD was also consistent with previous reports (Bilder et al., 2013; Krakowiak et al., 2012; Li et al., 2016).

The effect size of prenatal vitamin use during the first month of pregnancy (OR 0.70, 95%CI 0.32, 1.53) was similar in magnitude and direction to previously reported estimates (Schmidt et al., 2011, 2019), but with a wider confidence interval and a non-significant test of association. Our precision was limited by sample size. Although statistical significance is an important consideration, observed effect sizes are valuable for clinical utility (Schober et al., 2018). In our study, mothers of children that later developed ASD were consistently less likely to be taking prenatal vitamins throughout pregnancy; future studies should investigate combinations of time points and possible time intervals. Our findings appeared specific to ASD, as prenatal vitamins did not show associations with other non-typical development in this sample. A previous study of prenatal vitamin use in the first month of pregnancy in a prospective U.S. high-risk sibling cohort found a protective OR of 0.50 (95%CI 0.31, 0.81) for ASD recurrence (Schmidt et al., 2019). Likewise, a U.S. case–control study of prenatal vitamin use in either the three months prior to pregnancy or the first month of pregnancy found an OR for ASD of 0.62 (95%CI 0.42, 0.93) (Schmidt et al., 2011). A large prospective general population study in Norway that examined prenatal folic acid supplement use (400 mcg) during the 4 weeks before and 8 weeks after conception also observed an OR for ASD of 0.61 (95%CI 0.40, 0.91), although these supplements differ from those used in the U.S. (Suren et al., 2013). Given the magnitude of the effect estimates observed in this and previous work, prenatal vitamin intake during early pregnancy could be a highly clinically useful preventative measure for ASD.

Several studies from other countries have examined associations between prenatal vitamin use and neurodevelopmental outcomes (DeVilbiss et al., 2017; Levine et al., 2018; Suren et al., 2013). A key difficulty in comparing or meta-analyzing studies of supplements from other countries is that "multivitamin" and "prenatal vitamin" indicate different nutrient compositions by country (Blumberg et al., 2018). Additionally, prescription and non-prescription products differ; in the U.S., prescription products typically contain higher levels of folic acid, while non-prescription products tend to have higher levels of vitamins A and D, iodine, and calcium (Saldanha et al., 2017). Studies define adequacy in different ways, such as using the CDC recommendation of 400 to 1000 µg per day (Centers for Disease Control and Prevention, 1991), the Institute of Medicine definition of at least 600 dietary folate equivalents per day (Bailey, 1998), or defining their own cut point. Additionally, some of the largest studies of folic acid during pregnancy and ASD were conducted in countries without mandatory grain fortification, such as Norway (Suren et al., 2013), Sweden (DeVilbiss et al., 2017), and Israel (Levine et al., 2018). The consumption of whole grains may differ across countries, further complicating the comparison of supplemental folic acid. Further work is still needed to understand the

components or combinations of nutrients most related to the protection afforded by prenatal vitamins. It is possible that the protection offered by prenatal vitamins is in part due to synergistic effects of the many component vitamins and minerals and that examining individual nutrients will not yield comparable results.

We noted that very high folic acid (> 1000 mcg total supplemental from any source) relative to adequate (400–1000 mcg) was not associated with odds of ASD, but no dose-related trend was observed and this was a very unstable finding, with a confidence limit ratio > 12 and only four ASD cases in this category. At least one other study has noted a relationship between odds of ASD and high maternal folate levels (Raghavan et al., 2018), though their folate measures were taken at the time of delivery. There is also the possibility of confounding by indication in this highest supplemental intake category, since high doses of folic acid can be indicated for at-risk pregnancies, such as mothers who have already had a pregnancy resulting in a neural tube defect (Centers for Disease Control and Prevention, 1991), other birth defects (Botto et al., 2000; Huhta & Linask, 2015; Wilson et al., 2015), or maternal anemia (Green & Miller, 1999; Sanghvi et al., 2010). For all of these reasons, the elevated risk in those with high intake should be viewed cautiously.

In addition to the prenatal vitamin association with ASD diagnosis, we tested the specificity for continuous neurodevelopment measures and subscales. In the study sample, MSEL scores at 36-months were not associated with prenatal vitamin use, though a trend was observed in the expected direction, with higher development scores (motor skills, visual perception, expressive and receptive language) among those with prenatal vitamin use. Because our non-ASD group included children with low cognitive scores, there is concern that the controls were enriched with children who had overall lower folic acid exposure in early pregnancy. ADOS calibrated severity scores at 36-months were not different among children whose mothers took prenatal vitamins. Given that this is a very high-risk group as siblings of affected children, we observed a relatively high proportion of non-typically developing children in the sample, which would cause higher ADOS averages even in the absence of ASD diagnoses.

One limitation of this study is the modest sample size, limiting the precision of effect estimates. Small numbers in strata prevented analysis stratified by sex. A further limitation was that data on very early life (before age 3) interventions was not available in this study. All participating children are infant siblings of a person with ASD, so potential increased services at very young ages compared to the general population may have been similarly likely across the whole cohort. There could also be residual confounding and confounding by indication; because prenatal vitamin use is associated with other healthy behaviors, there is some concern that other health-related behaviors were not measured or controlled for. Confounding by indication may have affected results for total supplemental folic acid, as women who are prescribed folic acid have a different set of risk factors. We recruited women in the first or second trimester of pregnancy, hence much of their supplement use during pregnancy was captured prospectively, a major strength of this study. We also retrospectively queried their supplement intake prior to pregnancy, which may introduce some measurement error, however, recall of pregnancy use of multivitamins and over-the-counter medications, including initiation and duration of use, has been shown to be accurate up to five years following birth of the child (Bosco et al., 2010; Werler et al., 1989), whereas in this study, the recall was over a period of months, not years and we did not observe differences in response by enrollment week.

In summary, taken together with prior research, prenatal vitamins and prenatal care are important for pregnant people, especially those with a prior child with ASD. The current study's prospective design allowed for accurate exposure collection during the biologically relevant period, including details about type and dose that allow for specificity in examining risk factors. Additionally, the detailed phenotyping of the children allowed for assessment of not just outcome classification but also markers of severity. Future work should examine other months of pregnancy; incorporate dietary nutrient information; seek larger sample sizes while still achieving well-characterized phenotypes; and include parental and child folate metabolism along with ASD genetic risk.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10803-021-05110-9.

Acknowledgements Funding for the EARLI study was provided by the National Institutes of Health (R01ES016443, PI: Newschaffer) and Autism Speaks (003,953 PI: Newschaffer). The nutrient measures in this study were supported by the National Institutes of Health (R01 ES025574, PI: Schmidt). Support for this research was also provided by the National Institutes of Health (P30ES017885). Mr. Dou and Dr. Bakulski were supported by grants (R01 ES025531, PI: Fallin; R01 AG055406, MPI: Bakulski; R01 MD013299). Ms. Brieger was supported by the Genome Sciences Training Program (T32 HG000040), the Medical Scientists Training Program (T32 GM007863), and the University of Michigan Department of Epidemiology David Schottenfeld, M.D. Endowed Scholarship. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Contributions KKB performed the analysis and wrote the original draft. KMB and CLP guided the analytic methodology used and supervised. JIF and JFD curated the dataset and performed code review. LAC, IHP, CJN, and MDF developed the cohort and acquired funding to support the project. RJS and KKB conceptualized the goal of the paper. KKB, KMB, CLP, AB, MDF, and RJS provided interpretation, review, and editing of the manuscript. RJS and MDF provided funding for this analysis.

References

Al-Farsi, Y. M., Waly, M. I., Deth, R. C., Al-Sharbati, M. M., Al-Shafaee, M., Al-Farsi, O., Al-Khaduri, M. M., Gupta, I., Ali, A., Al-Khalili, M., Al-Adawi, S., Hodgson, N. W., & Ouhtit, A. (2013). Low folate and vitamin B12 nourishment is common in Omani children with newly diagnosed autism. *Nutrition, 29*(3), 537–541. https://doi.org/10.1016/j.nut.2012.09.014

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders : DSM-5* (5th ed.). American Psychiatric Association.

Bailey, L. B. (1998). Dietary reference intakes for folate: The debut of dietary folate equivalents. *Nutrition Reviews, 56*(10), 294–299. https://doi.org/10.1111/j.1753-4887.1998.tb01662.x

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., … Dietz, P. M. (2020). Prevalence of autism spectrum disorder among children aged 8 Years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. *MMWR Surveillance Summaries, 69*(4), 1–12. https://doi.org/10.15585/mmwr.ss6904a1

Berry, R. J., Li, Z., Erickson, J. D., Li, S., Moore, C. A., Wang, H., Mulinare, J., Zhao, P., Wong, L. Y., Gindler, J., Hong, S. X., & Correa, A. (1999). Prevention of neural-tube defects with folic acid in China China-US Collaborative Project for Neural Tube Defect Prevention. *New England Journal of Medicine, 341*(20), 1485–1490. https://doi.org/10.1056/NEJM199911113412001

Bilder, D. A., Bakian, A. V., Viskochil, J., Clark, E. A., Botts, E. L., Smith, K. R., Pimentel, R., McMahon, W. M., & Coon, H. (2013). Maternal prenatal weight gain and autism spectrum disorders. *Pediatrics, 132*(5), e1276-1283. https://doi.org/10.1542/peds.2013-1188

Blumberg, J. B., Cena, H., Barr, S. I., Biesalski, H. K., Dagach, R. U., Delaney, B., Frei, B., Moreno Gonzalez, M. I., Hwalla, N., Lategan-Potgieter, R., McNulty, H., van der Pols, J. C., Winichagoon, P., & Li, D. (2018). The use of multivitamin/multimineral supplements: A modified Delphi consensus panel report. *Clinical Therapeutics, 40*(4), 640–657. https://doi.org/10.1016/j.clinthera.2018.02.014

Bosco, J. L., Tseng, M., Spector, L. G., Olshan, A. F., & Bunin, G. R. (2010). Reproducibility of reported nutrient intake and supplement use during a past pregnancy: A report from the Children's Oncology Group. *Paediatric and Perinatal Epidemiology, 24*(1), 93–101. https://doi.org/10.1111/j.1365-3016.2009.01070.x

Botto, L. D., Mulinare, J., & Erickson, J. D. (2000). Occurrence of congenital heart defects in relation to maternal mulitivitamin use. *American Journal of Epidemiology, 151*(9), 878–884. https://doi.org/10.1093/oxfordjournals.aje.a010291

Braun, J. M., Froehlich, T., Kalkbrenner, A., Pfeiffer, C. M., Fazili, Z., Yolton, K., & Lanphear, B. P. (2014). Brief report: Are autistic-behaviors in children related to prenatal vitamin use and maternal whole blood folate concentrations? *Journal of Autism and Developmental Disorders, 44*(10), 2602–2607. https://doi.org/10.1007/s10803-014-2114-x

Brucato, M., Ladd-Acosta, C., Li, M., Caruso, D., Hong, X., Kaczaniuk, J., Stuart, E. A., Fallin, M. D., & Wang, X. (2017). Prenatal exposure to fever is associated with autism spectrum disorder in the boston birth cohort. *Autism Research, 10*(11), 1878–1890. https://doi.org/10.1002/aur.1841

Bryson, S. E., Zwaigenbaum, L., McDermott, C., Rombough, V., & Brian, J. (2008). The Autism Observation Scale for Infants: Scale development and reliability data. *Journal of Autism and Developmental Disorders, 38*(4), 731–738. https://doi.org/10.1007/s10803-007-0440-y

Centers for Disease Control and Prevention. (1991). Effectiveness in disease and injury prevention use of folic acid for prevention of Spina Bifida and other neural tube defects—1983–1991. *MMWR Weekly, 40*(30), 513–516.

Centers for Disease Control and Prevention. (1992). Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *MMWR Surveillance Summary, 41*(No. RR-14).

Chen, J., Xin, K., Wei, J., Zhang, K., & Xiao, H. (2016). Lower maternal serum 25(OH) D in first trimester associated with higher autism risk in Chinese offspring. *Journal of Psychosomatic Research, 89*, 98–101. https://doi.org/10.1016/j.jpsychores.2016.08.013

Christian, M. A., Samms-Vaughan, M., Lee, M., Bressler, J., Hessabi, M., Grove, M. L., Shakespeare-Pellington, S., Coore Desai, C., Reece, J. A., Loveland, K. A., Boerwinkle, E., & Rahbar, M. H. (2018). Maternal exposures associated with autism spectrum disorder in Jamaican children. *Journal of Autism and Developmental Disorders, 48*(8), 2766–2778. https://doi.org/10.1007/s10803-018-3537-6

Croen, L. A., Qian, Y., Ashwood, P., Zerbo, O., Schendel, D., Pinto-Martin, J., Daniele Fallin, M., Levy, S., Schieve, L. A., Yeargin-Allsopp, M., Sabourin, K. R., & Ames, J. L. (2019). Infection and fever in pregnancy and autism spectrum disorders: Findings from the study to explore early development. *Autism Research, 12*(10), 1551–1561. https://doi.org/10.1002/aur.2175

Czeizel, A. E., & Dobo, M. (1994). Postnatal somatic and mental development after periconceptional multivitamin supplementation. *Archives of Disease in Childhood, 70*(3), 229–233. https://doi.org/10.1136/adc.70.3.229

Delobel-Ayoub, M., Ehlinger, V., Klapouszczak, D., Maffre, T., Raynaud, J. P., Delpierre, C., & Arnaud, C. (2015). Socioeconomic disparities and prevalence of autism spectrum disorders and intellectual disability. *PLoS ONE, 10*(11), e0141964. https://doi.org/10.1371/journal.pone.0141964

DeVilbiss, E. A., Magnusson, C., Gardner, R. M., Rai, D., Newschaffer, C. J., Lyall, K., Dalman, C., & Lee, B. K. (2017). Antenatal nutritional supplementation and autism spectrum disorders in the Stockholm youth cohort: population based cohort study. *BMJ, 359*, 4273. https://doi.org/10.1136/bmj.j4273

Emerson, E. (2012). Deprivation, ethnicity and the prevalence of intellectual and developmental disabilities. *Journal of Epidemiology and Community Health, 66*(3), 218–224. https://doi.org/10.1136/jech.2010.111773

Gardener, H., Spiegelman, D., & Buka, S. L. (2009). Prenatal risk factors for autism: Comprehensive meta-analysis. *British Journal of Psychiatry, 195*(1), 7–14. https://doi.org/10.1192/bjp.bp.108.051672

Gardener, H., Spiegelman, D., & Buka, S. L. (2011). Perinatal and neonatal risk factors for autism: A comprehensive meta-analysis. *Pediatrics, 128*(2), 344–355. https://doi.org/10.1542/peds.2010-1036

Goodrich, A. J., Volk, H. E., Tancredi, D. J., McConnell, R., Lurmann, F. W., Hansen, R. L., & Schmidt, R. J. (2018). Joint effects of prenatal air pollutant exposure and maternal folic acid supplementation on risk of autism spectrum disorder. *Autism Research, 11*(1), 69–80. https://doi.org/10.1002/aur.1885

Gotham, K., Pickles, A., & Lord, C. (2009). Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *Journal of Autism and Developmental Disorders, 39*(5), 693–705. https://doi.org/10.1007/s10803-008-0674-3

Grant, W. B., & Soles, C. M. (2009). Epidemiologic evidence supporting the role of maternal vitamin D deficiency as a risk factor for the development of infantile autism. *Dermatoendocrinol, 1*(4), 223–228.

Green, R., & Miller, J. W. (1999). Folate deficiency beyond megaloblastic anemia: hyperhomocysteinemia and other manifestations

of dysfunctional folate status. *Seminars in Hematology, 36*(1), 47–64.

Hertz-Picciotto, I., Schmidt, R. J., & Krakowiak, P. (2018). Understanding environmental contributions to autism: Causal concepts and the state of science. *Autism Research, 11*(4), 554–586. https://doi.org/10.1002/aur.1938

Hornig, M., Bresnahan, M. A., Che, X., Schultz, A. F., Ukaigwe, J. E., Eddy, M. L., Hirtz, D., Gunnes, N., Lie, K. K., Magnus, P., Mjaaland, S., Reichborn-Kjennerud, T., Schjolberg, S., Oyen, A. S., Levin, B., Susser, E. S., Stoltenberg, C., & Lipkin, W. I. (2018). Prenatal fever and autism risk. *Molecular Psychiatry, 23*(3), 759–766. https://doi.org/10.1038/mp.2017.119

Huhta, J. C., & Linask, K. (2015). When should we prescribe high-dose folic acid to prevent congenital heart defects? *Current Opinion in Cardiology, 30*(1), 125–131. https://doi.org/10.1097/HCO.0000000000000124

Krakowiak, P., Walker, C. K., Bremer, A. A., Baker, A. S., Ozonoff, S., Hansen, R. L., & Hertz-Picciotto, I. (2012). Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. *Pediatrics, 129*(5), e1121-1128. https://doi.org/10.1542/peds.2011-2583

Levine, S. Z., Kodesh, A., Viktorin, A., Smith, L., Uher, R., Reichenberg, A., & Sandin, S. (2018). Association of maternal use of folic acid and multivitamin supplements in the periods before and during pregnancy with the risk of autism spectrum disorder in offspring. *JAMA Psychiatry, 75*(2), 176–184. https://doi.org/10.1001/jamapsychiatry.2017.4050

Li, Y. M., Ou, J. J., Liu, L., Zhang, D., Zhao, J. P., & Tang, S. Y. (2016). Association between maternal obesity and autism spectrum disorder in offspring: A Meta-analysis. *Journal of Autism and Developmental Disorders, 46*(1), 95–102. https://doi.org/10.1007/s10803-015-2549-8

Loomes, R., Hull, L., & Mandy, W. P. L. (2017). What Is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry, 56*(6), 466–474. https://doi.org/10.1016/j.jaac.2017.03.013

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Leventhal, B. L., DiLavore, P. C., Pickles, A., & Rutter, M. (2000). The Autism Diagnostic Observation Schedule-Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30*(3), 205–223. https://doi.org/10.1023/A:1005592401947

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K., & Bishop, S. L. (2012). *Autism Diagnostic Observation Schedule (ADOS-2)* (2nd ed.). Western Psychological Services.

Newschaffer, C. J., Croen, L. A., Fallin, M. D., Hertz-Picciotto, I., Nguyen, D. V., Lee, N. L., Berry, C. A., Farzadegan, H., Hess, H. N., Landa, R. J., Levy, S. E., Massolo, M. L., Meyerer, S. C., Mohammed, S. M., Oliver, M. C., Ozonoff, S., Pandey, J., Schroeder, A., & Shedd-Wise, K. M. (2012). Infant siblings and the investigation of autism risk factors. *Journal of Neurodevelopmental Disorders, 4*(1), 7. https://doi.org/10.1186/1866-1955-4-7

Nilsen, R. M., Suren, P., Gunnes, N., Alsaker, E. R., Bresnahan, M., Hirtz, D., Hornig, M., Lie, K. K., Lipkin, W. I., Reichborn-Kjennerud, T., Roth, C., Schjolberg, S., Smith, G. D., Susser, E., Vollset, S. E., Oyen, A. S., Magnus, P., & Stoltenberg, C. (2013). Analysis of self-selection bias in a population-based cohort study of autism spectrum disorders. *Paediatric and Perinatal Epidemiology, 27*(6), 553–563. https://doi.org/10.1111/ppe.12077

Nuttall, J. R. (2017). The plausibility of maternal toxicant exposure and nutritional status as contributing factors to the risk of autism spectrum disorders. *Nutritional Neuroscience, 20*(4), 209–218. https://doi.org/10.1080/1028415X.2015.1103437

Ozonoff, S., Young, G. S., Belding, A., Hill, M., Hill, A., Hutman, T., Johnson, S., Miller, M., Rogers, S. J., Schwichtenberg, A. J., Steinfeld, M., & Iosif, A. M. (2014). The broader autism phenotype in infancy: When does it emerge? *Journal of the American Academy of Child & Adolescent Psychiatry, 53*(4), 398–407. https://doi.org/10.1016/j.jaac.2013.12.020

Ozonoff, S., Young, G. S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., Bryson, S., Carver, L. J., Constantino, J. N., Dobkins, K., Hutman, T., Iverson, J. M., Landa, R., Rogers, S. J., Sigman, M., & Stone, W. L. (2011). Recurrence risk for autism spectrum disorders: A Baby Siblings Research Consortium study. *Pediatrics, 128*(3), e488-495. https://doi.org/10.1542/peds.2010-2825

Pitkin, R. M. (2007). Folate and neural tube defects. *American Journal of Clinical Nutrition, 85*(1), 285S-288S. https://doi.org/10.1093/ajcn/85.1.285S

Raghavan, R., Fallin, M. D., & Wang, X. (2016). Maternal plasma folate, vitamin B12 levels and multivitamin supplementation during pregnancy and risk of Autism Spectrum Disorder in the Boston Birth Cohort. *The FASEB Journal*. https://doi.org/10.1096/fasebj.30.1_supplement.151.6

Raghavan, R., Riley, A. W., Volk, H., Caruso, D., Hironaka, L., Sices, L., Hong, X., Wang, G., Ji, Y., Brucato, M., Wahl, A., Stivers, T., Pearson, C., Zuckerman, B., Stuart, E. A., Landa, R., Fallin, M. D., & Wang, X. (2018). Maternal multivitamin intake, plasma folate and vitamin B12 levels and autism spectrum disorder risk in offspring. *Paediatric and Perinatal Epidemiology, 32*(1), 100–111. https://doi.org/10.1111/ppe.12414

Rai, D., Lewis, G., Lundberg, M., Araya, R., Svensson, A., Dalman, C., Carpenter, P., & Magnusson, C. (2012). Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(5), 467–476. https://doi.org/10.1016/j.jaac.2012.02.012

Rieder, M. J. (1994). Prevention of neural tube defects with periconceptional folic acid. *Clinics in Perinatology, 21*(3), 483–503.

Saldanha, L. G., Dwyer, J. T., Andrews, K. W., Brown, L. L., Costello, R. B., Ershow, A. G., Gusev, P. A., Hardy, C. J., & Pehrsson, P. R. (2017). Is nutrient content and other label information for prescription prenatal supplements different from nonprescription products? *Journal of the Academy of Nutrition and Dietetics, 117*(9), 1429–1436. https://doi.org/10.1016/j.jand.2017.04.002

Sanghvi, T. G., Harvey, P. W., & Wainwright, E. (2010). Maternal iron-folic acid supplementation programs: Evidence of impact and implementation. *Food and Nutrition Bulletin, 31*(2 Suppl), S100-107. https://doi.org/10.1177/15648265100312S202

Schmidt, R. J., Hansen, R. L., Hartiala, J., Allayee, H., Schmidt, L. C., Tancredi, D. J., Tassone, F., & Hertz-Picciotto, I. (2011). Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. *Epidemiology, 22*(4), 476–485. https://doi.org/10.1097/EDE.0b013e31821d0e30

Schmidt, R. J., Iosif, A. M., Guerrero Angel, E., & Ozonoff, S. (2019). Association of maternal prenatal vitamin use with risk for autism spectrum disorder recurrence in Young siblings. *JAMA Psychiatry*. https://doi.org/10.1001/jamapsychiatry.2018.3901

Schmidt, R. J., Tancredi, D. J., Ozonoff, S., Hansen, R. L., Hartiala, J., Allayee, H., Schmidt, L. C., Tassone, F., & Hertz-Picciotto, I. (2012). Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. *American Journal of Clinical Nutrition, 96*(1), 80–89. https://doi.org/10.3945/ajcn.110.004416

Schober, P., Bossers, S. M., & Schwarte, L. A. (2018). Statistical significance versus clinical importance of observed effect sizes: What do P values and confidence intervals really represent? *Anesthesia and Analgesia, 126*(3), 1068–1072. https://doi.org/10.1213/ANE.0000000000002798

Steenweg-de Graaff, J., Ghassabian, A., Jaddoe, V. W., Tiemeier, H., & Roza, S. J. (2015). Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study. *European Journal of Public Health, 25*(3), 431–433. https://doi.org/10.1093/eurpub/cku126

Suren, P., Roth, C., Bresnahan, M., Haugen, M., Hornig, M., Hirtz, D., Lie, K. K., Lipkin, W. I., Magnus, P., Reichborn-Kjennerud, T., Schjolberg, S., Davey Smith, G., Oyen, A. S., Susser, E., & Stoltenberg, C. (2013). Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA, 309*(6), 570–577. https://doi.org/10.1001/jama.2012.155925

Tamura, T., Goldenberg, R. L., Chapman, V. R., Johnston, K. E., Ramey, S. L., & Nelson, K. G. (2005). Folate status of mothers during pregnancy and mental and psychomotor development of their children at five years of age. *Pediatrics, 116*(3), 703–708. https://doi.org/10.1542/peds.2004-2189

Virk, J., Liew, Z., Olsen, J., Nohr, E. A., Catov, J. M., & Ritz, B. (2016). Preconceptional and prenatal supplementary folic acid and multivitamin intake and autism spectrum disorders. *Autism, 20*(6), 710–718. https://doi.org/10.1177/1362361315604076

Werler, M. M., Pober, B. R., Nelson, K., & Holmes, L. B. (1989). Reporting accuracy among mothers of malformed and nonmalformed infants. *American Journal of Epidemiology, 129*(2), 415–421. https://doi.org/10.1093/oxfordjournals.aje.a115145

Wilson, R. D., Genetics, C., Wilson, R. D., Audibert, F., Brock, J. A., Carroll, J., Cartier, L., Gagnon, A., Johnson, J. A., Langlois, S., Murphy-Kaulbeck, L., Okun, N., Pastuck, M., Special, C., Deb-Rinker, P., Dodds, L., Leon, J. A., Lowel, H. L., Luo, W., … Van den Hof, M. (2015). Pre-conception folic acid and multivitamin supplementation for the primary and secondary prevention of neural tube defects and other folic acid-sensitive congenital anomalies. *Journal of Obstetrics and Gynaecology Canada, 37*(6), 534–552.

Xu, G., Jing, J., Bowers, K., Liu, B., & Bao, W. (2014). Maternal diabetes and the risk of autism spectrum disorders in the offspring: A systematic review and meta-analysis. *Journal of Autism and Developmental Disorders, 44*(4), 766–775. https://doi.org/10.1007/s10803-013-1928-2

Ye, B. S., Leung, A. O. W., & Wong, M. H. (2017). The association of environmental toxicants and autism spectrum disorders in children. *Environmental Pollution, 227*, 234–242. https://doi.org/10.1016/j.envpol.2017.04.039