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BRIEF REPORT

Brief Report: Are Autistic-Behaviors in Children Related to Prenatal Vitamin Use and Maternal Whole Blood Folate Concentrations?

Joseph M. Braun · Tanya Froehlich · Amy Kalkbrenner · Christine M. Pfeiffer · Zia Fazili · Kimberly Yolton · Bruce P. Lanphear

Abstract Prenatal multivitamin/folic acid supplement use may reduce the risk of autism spectrum disorders. We investigated whether 2nd trimester prenatal vitamin use and maternal whole blood folate (WBF) concentrations were associated with Social Responsiveness Scale (SRS) scores at 4–5 years of age in a prospective cohort of 209 mother–child pairs. After confounder adjustment, children born to women taking prenatal vitamins weekly/daily (n = 179) had lower odds of clinically elevated SRS scores (odds ratio 0.26; 95 % confidence interval 0.08, 0.89) than those who rarely/never took them (n = 30). WBF concentrations were not associated with SRS scores. The lack of association between WBF and autistic-behaviors may be due to the timing of biomarker measures relative to critical periods of brain development, confounding, or other modifying factors.

Keywords Autism spectrum disorders · Folate · Pregnancy · Prenatal vitamins

Abbreviations

ASDs	Autism spectrum disorders
CDC	Centers for Disease Control and Prevention
CI	95 % Confidence interval
OR	Odds ratio
SD	Standard deviation
SRS	Social Responsiveness Scale
WBF	Whole blood folate

Introduction

Despite intense public interest and an increasing prevalence of autism spectrum disorders (ASD), few modifiable risk factors have been identified. Four reports from two studies suggest that periconceptional vitamin use, dietary folic acid intake, or folic acid supplementation are associated with a decreased risk of ASD diagnosis, severe language delay, and emotional problems (Roth et al. 2011; Schmidt et al. 2011, 2012; Steenweg-de Graaff et al. 2012; Suren et al. 2013). In one study, the association between prenatal vitamin intake and ASD diagnosis was modified by maternal polymorphisms in folate transport and

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J. M. Braun (✉)
Department of Epidemiology, Brown University School of Public Health, Brown University, Providence, RI 02912, USA
e-mail: joseph_braun_1@brown.edu

T. Froehlich · K. Yolton
Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

A. Kalkbrenner
Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA

C. M. Pfeiffer · Z. Fazili
Division of Laboratory Sciences, Centers for Disease Control and Prevention, National Center for Environmental Health, Atlanta, GA 30341, USA

B. P. Lanphear
Faculty of Health and Sciences, Simon Fraser University, Burnaby V5A 1S6, Canada

B. P. Lanphear
Child and Family Research Institute, BC Children's and Women's Hospital, Vancouver V5Z 4H48, Canada

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bioavailability genes.(Schmidt et al. 2011) While these findings suggest a role for folate status in the development of ASD, the association between biomarkers of prenatal folate status and child ASD or autistic-behaviors has not been directly assessed. Thus, we investigated the relationship between *both* prenatal vitamin use *and* whole blood folate (WBF) concentrations during the 2nd trimester of pregnancy with child autistic-behaviors at 4–5 years of age in a prospective birth cohort.

Methods

From 2003 to 2006 we enrolled 398 pregnant women from the greater Cincinnati, Ohio area in the Health Outcomes and Measures of the Environment (HOME) Study. The HOME Study was designed to examine the association between low-level environmental chemical exposure and child growth and development. After delivery, women and their children returned to our study clinic annually for neurodevelopmental assessments until the child's 5th birthday. Eligibility criteria, questionnaire descriptions, biological specimen collection, and follow-up visits have been previously described (Braun et al. 2010a, b, 2011).

At study enrollment (mean: 16, range 11–21 weeks gestation), whole blood samples were collected from women via venipuncture and stored at ≤ -20 °C. In 2010, WBF concentrations were measured at the Centers for Disease Control and Prevention (CDC) using a sensitive and specific liquid chromatography tandem mass spectrometry method (Fazili and Pfeiffer 2004). During an in-home interview (mean: 21, range 14–39 weeks gestation), trained examiners asked women about the frequency of their current prenatal vitamin use. Potential confounding variables were collected during pregnancy. We used standardized questionnaires to collect information about maternal age, race, education, household income, marital status, health insurance, employment, frequency of fresh fruit/vegetable intake, and food security. We abstracted women's parity from medical chart reviews. Maternal weight and height were measured at enrollment using a scale and stadiometer, respectively. Depressive symptoms were measured with the Beck Depressive Inventory-II (Beck et al. 1996). We measured serum cotinine concentrations to assess tobacco exposures (Braun et al. 2010a, b).

Autistic-behaviors were measured one or two times by maternal-report with the Social Responsiveness Scale (SRS) at 4 and 5 years of age (Constantino 2005). The SRS is a valid and reliable instrument that measures children's social awareness, cognition, communication, motivation, and autistic mannerisms using 63 Likert-style questions. Outcomes included both continuous SRS Total T scores [mean: 50 and standard deviation (SD): 10 in the standardization sample] and whether a child scored $\geq$60. Scores $\geq$60 indicate reciprocal social behavior deficits associated with mild to moderate ASD symptoms and risk for ASD (Constantino 2005). Additional measures of autistic behaviors or diagnoses were not available.

We calculated mean child SRS scores, as well as prenatal WBF levels and vitamin use, according to categories of covariates; in cases where more than one SRS score per child was available, we used the first one so that the same child was not counted twice. We examined unadjusted and confounder-adjusted associations between maternal prenatal vitamin intake and WBF concentrations with SRS scores at 4 and 5 years of age using generalized linear mixed models (SAS v9.3 PROC MIXED and GLIMMIX) with a random intercept and unstructured correlation matrix (Fitzmaurice et al. 2004). These models allow us to examine the mean change in SRS scores or odds of elevated SRS scores at 4 and 5 years of age according to WBF levels or prenatal vitamin use, while accounting for repeated SRS measures.

Results

Among 389 singleton births in the cohort, 222 (57 %) mother–child pairs returned for a follow-up visit at 4 or 5 years of age. Among these, seven were missing covariates, another two were missing information on prenatal vitamin use, and six more were missing WBF values. Our analysis included 209 (54 %) mother–child pairs with all relevant variables who completed a total of 371 visits at 4 and 5 years of age. Of these, 16 completed a 4 year visit, 31 completed a 5 year visit, and 162 completed both visits. Average WBF concentrations (mean WBF: 485 vs. 480 nmol/L) and proportion of women using prenatal vitamins (proportion using: 86 vs. 82 %) was similar among women with and without complete follow-up.

Average WBF concentrations were lower among women who reported that they rarely/never took prenatal vitamins (mean WBF: 329, SD: 150 nmol/L, $n = 30$) compared to those who reported taking them weekly/daily (mean WBF: 516, SD: 238, $n = 179$). Mean SRS scores were similar at 4 (mean: 50.5, SD: 8.3) and 5 (mean: 50.3, SD: 10.3) years of age. Continuous SRS scores were reasonably well correlated at 4 and 5 years of age (intraclass correlation coefficient: 0.68); there was fair agreement for children having SRS scores $\geq$ 60 at 4 and 5 years of age (kappa: 0.46).

Traditional risk factors for poor maternal and child health (e.g., low socioeconomic status) were associated with lower WBF concentrations, decreased prenatal vitamin use, and higher SRS scores (Table 1). Maternal education, race, marital status, insurance, and household income were the strongest confounders in folate/vitamin-

Table 1 Proportion of women using prenatal vitamins during 2nd trimester, maternal WBF concentrations during 2nd trimester (nmol/L), and child SRS Total SRS T Scores at 4 or 5 years of age according to covariates (Cincinnati, OH 2003–2006)

Variable	N	N using vitamins (%)	Mean folate (SD)	Mean SRS T Scores (SD)
Overall	209	179 (86)	489 (237)	51 (9.4)
Maternal age				
18–25 years	41	29 (70)	404 (192)	58 (13)
>25–35 years	136	118 (87)	492 (229)	50 (7.4)
>35 years	32	32 (100)	585 (284)	49 (8.5)
Maternal race				
White	135	127 (94)	536 (241)	49 (6.9)
Black	60	40 (67)	363 (172)	58 (12)
Other	14	12 (86)	580 (241)	47 (5.5)
Maternal education				
Bachelor's/Grad/Prof	114	108 (95)	531 (228)	48 (6.1)
Tech school/some college	55	49 (89)	482 (235)	53 (9.2)
HS	23	17 (74)	450 (270)	57 (13)
<12th grade	17	5 (29)	283 (125)	60 (11)
Marital status				
Married	147	139 (95)	542 (237)	48 (6.7)
Not married-living with someone	21	13 (62)	385 (197)	55 (9.5)
Not married-living alone	41	27 (66)	352 (177)	59 (12)
Household income				
>$80 K	56	53 (95)	549 (209)	47 (5.6)
$40–80 K	82	78 (95)	522 (249)	49 (6.5)
$20–40 K	28	24 (86)	471 (280)	53 (7.7)
<$20 K	43	24 (56)	360 (165)	60 (12)
Parity				
0	101	92 (91)	519 (227)	50 (8.8)
1–2	95	78 (82)	471 (254)	51 (9.7)
3+	13	9 (69)	391 (139)	57 (9.4)
Depressive symptoms				
Minimal	172	154 (89)	498 (244)	50 (8.1)
Mild	22	17 (77)	493 (207)	57 (9.8)
Moderate/severe	15	8 (53)	379 (164)	60 (14)
Serum cotinine concentrations (ng/mL)				
<LOD	67	64 (95)	544 (248)	48 (5.7)
LOD—3	131	109 (83)	477 (227)	53 (11)
>3	11	6 (54)	292 (156)	51 (5.8)
Employment status				
Unemployed	33	20 (61)	448 (247)	56 (14)
Employed	176	159 (90)	497 (235)	50 (8.0)
Insurance				
Private	158	149 (94)	537 (231)	49 (7.2)
Public/uninsured	51	30 (59)	342 (190)	59 (11)
Food security				
Not enough	199	172 (86)	499 (235)	51 (8.9)
Enough	10	7 (70)	303 (208)	62 (13)
Fresh fruit/vegetable intake frequency				
Monthly	24	18 (75)	415 (239)	54 (9.3)
Weekly	107	91 (85)	478 (221)	52 (9.7)
Daily or more	78	70 (90)	527 (252)	49 (8.5)

If a child had more than one SRS score, the 4 year value was used

SRS relationships. Additional adjustment for perinatal and nutritional variables attenuated our estimates and improved their precision.

In unadjusted analyses, children born to women who used prenatal vitamins weekly/daily had lower SRS scores (β −9.7; CI −14, −5.5) and odds of SRS scores ≥60 [odds ratio (OR) 0.14; CI 0.06, 0.32) compared to those who rarely or never used them (Table 2). After adjustment for confounders, we observed a *trend* suggesting lower SRS scores among children born to women taking prenatal vitamins (β −2.7; CI −6.5, 1.1) as the 95 % CI included the null value. Prenatal vitamin use continued to be associated with reduced odds of having SRS scores ≥60 after confounder adjustment (OR 0.26, CI 0.08, 0.89).

Compared to self-reported vitamin intake, WBF concentrations exhibited weaker associations with SRS scores (β per SD increase: −1.5; CI −2.6, −0.5) and SRS scores ≥60 (OR 0.69; CI 0.48, 0.99) before confounder adjustment. These associations were attenuated towards the null after confounder adjustment (β per SD increase 0.6; CI −0.3, 1.5 and OR 1.42; CI 0.81, 2.49) (Table 2).

Our results were similar when we also adjusted for year of child birth, maternal BMI, and paternal age (results not shown). In addition, our results were not appreciably different when we ran separate confounder-adjusted models for prenatal vitamins and WBF, instead of jointly adjusting for both.

Discussion

Low periconceptional folate levels, either due to deficient intake or genetic variants conferring inefficient folate metabolism, are known to increase the risk of neural tube defects (De-Regil et al. 2010; Zhang et al. 2013). This has spurred interest in determining whether periconceptional folic acid intake is also associated with offspring neurodevelopment, including ASDs (Anjos et al. 2013).

Table 2 Unadjusted and adjusted change in child SRS scores and ORs of elevated SRS scores (≥60) at 4–5 years of age with maternal prenatal vitamin use or increasing WBF concentrations during pregnancy (Cincinnati, OH; 2003-2006)

	Vitamin use: weekly/daily versus never/rarely (95 % CI)		WBF concentration: per SD increase (95 % CI)[a]	
	Change in continuous SRS scores (β)[b]	SRS Score ≥60 (OR)[b,c]	Change in continuous SRS scores (β)[b]	SRS score ≥60 (OR)[b,c]
Unadjusted	−9.7 (−14.0, −5.5)	0.14 (0.06, 0.32)	−1.5 (−2.6, −0.5)	0.69 (0.48, 0.99)
Adjusted[d]	−2.7 (−6.5, 1.1)	0.26 (0.08, 0.89)	0.6 (−0.3, 1.5)	1.42 (0.81, 2.49)

[a] SD of WBF is 239 nmol/L

[b] Continuous models are presented as the change in SRS scores with prenatal vitamin use or increasing WBF concentrations. Lower SRS scores indicate less autistic-behaviors. Dichotomous scores are presented as OR for elevated SRS score (≥60) with prenatal vitamin use or increasing WBF concentrations

[c] 21 (11.8 %) and 34 (17.6 %) children had SRS scores ≥60 at 4 and 5 years of age, respectively

[d] Fully adjusted model includes WBF and prenatal vitamin use, as well as maternal age (continuous years), race (non-Hispanic Whites, non-Hispanic Blacks, and other), education (<12, 12, some college, college completed, and graduate school), household income (continuous, dollars per year), marital status (married, unmarried/living with someone, and unmarried/living alone), employment during pregnancy (any and none), insurance status (private insurance and public/uninsured), depressive symptoms (continuous), serum cotinine concentrations (continuous $\log_{10}$-transformed concentrations), food security (always enough and often/sometimes not enough), and fresh fruit/vegetable intake (≥daily, weekly, and ≤monthly)

SD standard deviation, *CI* 95 % confidence interval, *WBF* whole blood folate

In this cohort, children born to women taking prenatal vitamins during the 2nd trimester of pregnancy were less likely to have parent-reported SRS scores ≥60 at 4 and 5 years of age. Prior studies have found that children born to women taking prenatal vitamins or folic acid supplements preconceptionally or during the first 2 months of pregnancy had a 40 % reduction in the risk of ASD (Schmidt et al. 2011; Schmidt 2013; Suren et al. 2013). In contrast, we did not observe the hypothesized protective relationship between 2nd trimester WBF concentrations and SRS scores, despite prenatal vitamin use being positively associated with WBF concentrations.

Similar to our findings, results from a large, prospective cohort study found that child emotional behavior problems at 18 months of age was *not* related to variations in maternal serum folate measured between 10 and 17 weeks gestation when levels were indicative of sufficient folate intake.(Steenweg-de Graaff et al. 2012) While this study did not find a protective relationship between maternal serum folate levels and emotional problems, the authors observed that self-reported prenatal folic acid supplementation was associated with reduced risk of emotional problems in children.

If folate availability during *discrete* developmental windows is important in predicting ASD risk or autistic behaviors, then differences in the timing of prenatal vitamin questions and folate biomarker level measurements during pregnancy may explain the observed discrepancy between our results and those of other studies. We only assessed prenatal vitamin use during the last 6 months of pregnancy and not during the periconceptional period. Prior studies observed a protective association between prenatal vitamin use and ASD diagnosis only when use occurred preconceptionally or during the first 2 months of gestation, but not later (Schmidt et al. 2011; Suren et al. 2013). Neither our folate biomarker nor self-reported prenatal vitamin use question was temporally aligned with this period of brain development that might be most dependent on folate.

There is evidence that vitamin intake and folate status may vary over short time periods within pregnancy. A prior study from Norway found that self-reported periconceptional folic acid supplement use before 3 months of gestation was not associated with serum folate levels at 4–5 months of gestation, whereas folic acid supplement use closer to the 4–5 month time period was associated with higher average serum folate concentrations (Roth et al. 2013). This indicates that repeated measures of *both* serum folate and supplement use are needed to identify unique windows of vulnerability to reduced folate availability since serum levels may only reflect recent folic acid intake.

Greater misclassification of WBF concentrations compared to prenatal vitamin intake may not explain the null associations for WBF levels and protective associations for self-reported prenatal vitamin use since the WBF measurement error would have to be greater than self-reported prenatal vitamin use measurement error. This is unlikely given that folate biomarkers and repeated food frequency questionnaire assessments of folate intake exhibit comparable correlations (~0.5–0.7) over periods of weeks to years (Erkkola et al. 2001; Tamura et al. 2005; Lacher 2010; Shiraishi et al. 2012; Leenders et al. 2013).

Biological, genetic, or environmental factors may have obscured the association between WBF concentrations and SRS scores if they are acting as modifiers. For instance, the

association between prenatal vitamin use and ASD risk may be modified by the presence of folate receptor antibodies (Rothenberg et al. 2004) or maternal polymorphisms in folate transport and bioavailability genes (Schmidt et al. 2011). Alternatively, the association between prenatal vitamin use and SRS scores may be due other micronutrients present in prenatal vitamins and necessary for normal brain development, such as iron (Thomas et al. 2009). However, a prior study by Suren and colleagues found that folic acid supplements, and not other supplements, were associated with decreased risk of ASD (Suren et al. 2013).

Different sociodemographic and racial compositions across studies could influence the direction and magnitude of confounding and differences in results. While previous studies did not observe large differences in unadjusted and adjusted results, our results were substantially attenuated after confounder adjustment. This could be due to different confounding patterns for the SRS versus clinical diagnosis, as has been suggested by studies examining predictors of the SRS (Hus et al. 2013).

Loss to follow-up is a concern in longitudinal studies and selection bias could influence our results. However, it is reassuring that WBF levels and the proportion of women using prenatal vitamins were similar in families who did and did not complete follow-up. Additional selection factors at enrollment may be another source of bias.

Continuous measures of autistic-behavior, like the SRS, have desirable properties for prospective cohort studies like this one, but they are not equivalent to clinical diagnoses (Bellinger 2004; Jones and Lord 2013). Impairments measured by the SRS may also be correlates of, or due to other behavioral disorders, including ADHD or internalizing disorders (Reiersen et al. 2007; Hus et al. 2013). The SRS and clinical instruments may not measure identical neurobehavioral phenotypes, resulting in different estimates of the prevalence of clinically significant behaviors and possibly causing differences between our observations and those from previous reports. Because our study had a small number of children with SRS scores indicative of clinically significant impairments in reciprocal social behaviors, large studies using thorough clinical evaluations are necessary to determine if exposures associated with subtle changes in the population mean can impact the tails of continuous autistic-behavior distributions and change the risk of clinical disease.

Given that few modifiable risk factors for ASD have been identified, the potential protective association between prenatal vitamin use and ASD risk may have important public health implications. To confirm the precise timing and specificity of the protective association between folic-acid supplementation and ASD observed in this and prior studies, future studies should examine multiple nutritional biomarkers preconceptionally and throughout pregnancy, as well as genetic, biological, or environmental modifiers of folate status and ASD risk.

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Conflict of interest Dr. Lanphear has served as an expert witness and as a consultant to the California Attorney General's Office, but he has not personally received any compensation for these services. Dr. Lanphear has also served as a consultant on a US Environmental Protection Agency research study which he does receive compensation. Dr. Braun was financially compensated for conducting a reanalysis of the international pooled study of lead exposure for the plaintiffs in a public nuisance case.

References

Anjos, T., Altmae, S., et al. (2013). Nutrition and neurodevelopment in children: Focus on NUTRIMENTHE project. *European Journal of Nutrition, 52*(8), 1825–1842.

Beck, A. T., Steer, R. A., et al. (1996). *Beck depression inventory—2nd edition (BDI-II)*. San Antonio: The Psychological Corporation.

Bellinger, D. C. (2004). Assessing environmental neurotoxicant exposures and child neurobehavior: confounded by confounding? *Epidemiology, 15*(4), 383–384.

Braun, J. M., Daniels, J. L., et al. (2010a). Prenatal environmental tobacco smoke exposure and early childhood body mass index. *Paediatric and Perinatal Epidemiology, 24*(6), 524–534.

Braun, J. M., Daniels, J. L., et al. (2010b). A prospective cohort study of biomarkers of prenatal tobacco smoke exposure: the correlation between serum and meconium and their association with infant birth weight. *Environ Health, 9*, 53.

Braun, J. M., Kalkbrenner, A. E., et al. (2011). Impact of early-life bisphenol a exposure on behavior and executive function in children. *Pediatrics, 128*(5), 873–882.

Constantino, J. (2005). *Social Responsiveness Scale*. Los Angeles, CA: Western Psychological Services.

De-Regil, L. M., Fernandez-Gaxiola, A. C., et al. (2010). Effects and safety of periconceptional folate supplementation for preventing birth defects. *Cochrane Database Systematic Review, 10*, CD007950.

Erkkola, M., Karppinen, M., et al. (2001). Validity and reproducibility of a food frequency questionnaire for pregnant Finnish women. *American Journal of Epidemiology, 154*(5), 466–476.

Fazili, Z., & Pfeiffer, C. M. (2004). Measurement of folates in serum and conventionally prepared whole blood lysates: Application of an automated 96-well plate isotope-dilution tandem mass spectrometry method. *Clinical Chemistry, 50*(12), 2378–2381.

Fitzmaurice, G. M., Laird, N. M., et al. (2004). *Applied longitudinal data analysis*. Hoboken: Wiley.

Hus, V., Bishop, S., et al. (2013). Factors influencing scores on the Social Responsiveness Scale. *Journal of Child Psychology and Psychiatry, 54*(2), 216–224.

Jones, R. M., & Lord, C. (2013). Diagnosing autism in neurobiological research studies. *Behavioural Brain Research, 251*, 113–124.

Lacher, D., J. Hughes, et al. (2010). Biological variation of laboratory analytes based on the 1999–2002 National Health and Nutrition Examination Survey. *National health statistics reports*. Hyattsville, MD, National Center for Health Statistic 21.

Leenders, M., Ros, M. M., et al. (2013). Reliability of selected antioxidants and compounds involved in one-carbon metabolism in two Dutch cohorts. *Nutrition and Cancer, 65*(1), 17–24.

Reiersen, A. M., Constantino, J. N., et al. (2007). Autistic traits in a population-based ADHD twin sample. *Journal of Child Psychology and Psychiatry, 48*(5), 464–472.

Roth, C., Magnus, P., et al. (2011). Folic acid supplements in pregnancy and severe language delay in children. *JAMA, 306*(14), 1566–1573.

Roth, C., Bjorke-Monsen, A. L., et al. (2013). Use of folic acid supplements in early pregnancy in relation to maternal plasma levels in week 18 of pregnancy. *Molecular Nutrition & Food Research, 57*(4), 653–660.

Rothenberg, S. P., da Costa, M. P., et al. (2004). Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect. *New England Journal of Medicine, 350*(2), 134–142.

Schmidt, R. J. (2013). Maternal folic acid supplements associated with reduced autism risk in the child. *Evidence-Based Medicine, 18*, e53.

Schmidt, R. J., Hansen, R. L., et al. (2011). Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. *Epidemiology, 22*(4), 476–485.

Schmidt, R. J., Tancredi, D. J., et al. (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.

Shiraishi, M., Haruna, M., et al. (2012). Validity and reproducibility of folate and vitamin B(12) intakes estimated from a self-administered diet history questionnaire in Japanese pregnant women. *Nutrition Journal, 11*, 15.

Steenweg-de Graaff, J., Roza, S. J., et al. (2012). Maternal folate status in early pregnancy and child emotional and behavioral problems: The Generation R Study. *American Journal of Clinical Nutrition, 95*(6), 1413–1421.

Suren, P., Roth, C., et al. (2013). Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA, 309*(6), 570–577.

Tamura, T., Goldenberg, R. L., et al. (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.

Thomas, D. G., Grant, S. L., et al. (2009). The role of iron in neurocognitive development. *Developmental Neuropsychology, 34*(2), 196–222.

Zhang, T., Lou, J., et al. (2013). Genetic variants in the folate pathway and the risk of neural tube defects: a meta-analysis of the published literature. *PLoS ONE, 8*(4), e59570.