



Association Between Maternal 2nd Trimester Plasma Folate Levels and Infant Bronchiolitis

Shanda Vereen^{1,4,5} · Tebeb Gebretsadik^{2,5} · Nia Johnson⁶ · Terryl J. Hartman⁷ · Sreenivas P. Veeranki^{1,4,5} · Chandrika Piyathilake⁸ · Edward F. Mitchel³ · Mehmet Kocak⁹ · William O. Cooper^{1,4} · William D. Dupont^{2,5} · Frances Tylavsky⁹ · Kecia N. Carroll^{1,4,5,10} 

Published online: 19 July 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Objectives Viral bronchiolitis is the most common cause of infant hospitalization. Folic acid supplementation is important during the periconceptional period to prevent neural tube defects. An area of investigation is whether higher prenatal folate is a risk factor for childhood respiratory illnesses. We investigated the association between maternal 2nd trimester plasma folate levels and infant bronchiolitis. **Methods** We conducted a retrospective cohort analysis in a subset of mother-infant dyads ($n=676$) enrolled in the Conditions Affecting Neurocognitive Development and Learning in Early Childhood study and Tennessee Medicaid. Maternal folate status was determined using 2nd trimester (16–28 weeks) plasma samples. Bronchiolitis diagnosis in the first year of life was ascertained using *International Classification of Diagnosis-9* codes from Medicaid administrative data. We used multivariable logistic regression to assess the adjusted association of prenatal folate levels and infant bronchiolitis outcome. **Results** Half of the women in this lower-income and predominately African-American (84%) study population had high levels of folate (median 2nd trimester level 19.2 ng/mL) and 21% of infants had at least one bronchiolitis healthcare visit. A relationship initially positive then reversing between maternal plasma folate and infant bronchiolitis was observed that did not reach statistical significance ($p_{\text{overall}} = .112$, $p_{\text{nonlinear effect}} = .088$). Additional adjustment for dietary methyl donor intake did not significantly alter the association. **Conclusions for Practice** Results did not confirm a statistically significant association between maternal 2nd trimester plasma folate levels and infant bronchiolitis. Further work is needed to investigate the role of folate, particularly higher levels, in association with early childhood respiratory illnesses.

Keywords Folate · Bronchiolitis · Prenatal · Lower respiratory tract infection · Pregnancy

Shanda Vereen and Tebeb Gebretsadik have contributed equally to this work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10995-018-2610-2>) contains supplementary material, which is available to authorized users.

✉ Kecia N. Carroll
Kecia.carroll@vanderbilt.edu

- ¹ Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA
- ² Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN, USA
- ³ Department of Health Policy, Vanderbilt University Medical Center, Nashville, TN, USA
- ⁴ Divisions of General Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA
- ⁵ Center for Asthma Health Research, Vanderbilt University Medical Center, Nashville, TN, USA

- ⁶ Fisk University, Nashville, TN, USA
- ⁷ Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA
- ⁸ Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, USA
- ⁹ Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, USA
- ¹⁰ Vanderbilt University School of Medicine, 313 Oxford House, 1313 21st Avenue South, Nashville, TN 37232-4313, USA

Significance

What is already known? There are no effective vaccines to prevent viruses most commonly associated with bronchiolitis, a leading cause of infant hospitalization. Dietary in utero exposures, such as folic acid, may influence the developing immune system. Inconsistent associations have been reported between prenatal folate levels and early childhood respiratory illnesses.

What this study adds? Epidemiologic studies examining prenatal folate levels and infant bronchiolitis are few. In this cohort of mother–child dyads, prenatal plasma folate levels and infant bronchiolitis were captured objectively; we found weak evidence of a non-linear relationship between maternal folate levels and infant bronchiolitis.

Objectives

Bronchiolitis, a lower respiratory tract infection (LRTI), is the leading cause of hospitalizations among infants in the U.S. (Nair et al. 2010) and is associated with subsequent wheeze and asthma in childhood (Carroll et al. 2009). Currently, no effective vaccines prevent the most common viral causes of bronchiolitis (Graham 2011). Evidence suggests that in utero exposures, such as diet, may influence the developing fetal immune system (Martino and Prescott 2011); therefore, it is important to identify potentially modifiable risk factors for bronchiolitis.

An area of investigation is whether folate, a methyl donor in the process of DNA methylation, may contribute to altered gene expression, epigenetic changes and an allergic phenotype (Prescott and Clifton 2009). The U.S. Public Health Service (1992) and the Institute of Medicine (1998) recommend that women of childbearing age consume at least 400 mcg of folic acid daily to prevent neural tube defects (Centers for Disease Control and Prevention 2010). These recommendations, together with the subsequent implementation of the folic acid food fortification program in 1998 (Centers for Disease Control and Prevention 2010; Czeizel and Dudas 1992), may lead some pregnant women and their infants to be exposed to high amounts of folic acid. Evidence shows adequate folate levels prevent neural tube defects (Centers for Disease Control and Prevention 2010; Czeizel and Dudas 1992); however, a question of research interest is whether higher folic acid intake is associated with harmful effects.

Epidemiologic studies have reported inconsistent findings for the association between folate intake or supplementation and childhood respiratory health (Alfonso et al. 2018; Bekkers et al. 2012; Haberg et al. 2009; Magdelijns

et al. 2011; Martinussen et al. 2012; Whitrow et al. 2009). For example, some prospective cohort studies have found that higher prenatal folate levels were associated with increased asthma and atopic disease in children (Haberg et al. 2011; Kieft-de Jong et al. 2012), while others have found decreased risk of LRTI (Kim et al. 2015) and asthma (Magdelijns et al. 2011). Further research investigating these associations is necessary, especially among infants.

Previously, we conducted analyses in mother–infant dyads enrolled in the Tennessee Medicaid program. We reported that infants of women who filled folic acid containing prescriptions in the 1st trimester only, or both during and after the 1st trimester, had increased relative odds of bronchiolitis and severe bronchiolitis (Veeranki et al. 2014). In the present study, we investigated the association between prenatal plasma folate levels and infant bronchiolitis diagnosis in a cohort of dyads enrolled in both the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) study and Tennessee Medicaid (TennCare).

Methods

Study Population

The CANDLE study, is a prospective prenatal cohort of mother–child dyads designed to represent the demographic profile of Shelby County, (Memphis, TN), which is a predominately low-income, African-American population (Völgyi et al. 2013). Women were recruited from community obstetric practices and an obstetric clinic during the 2nd trimester of pregnancy from December 2006 to June 2011 ($n = 1503$ women). CANDLE investigates the association of prenatal, genetic, and environmental exposures and early childhood development and health outcomes (Völgyi et al. 2013) using data and biospecimens collected during study visits at enrollment (2nd trimester), 3rd trimester, delivery, and early childhood. The current study included CANDLE mother–child dyads in which women reported having TennCare insurance at study enrollment (942, 63%) and the children had continuous TennCare coverage during infancy ($N = 835$). For children, we defined continuous enrollment as no more than 90 days of non-enrollment during the first year of life (Carroll et al. 2009).

In order to study infants without preexisting pulmonary, airway or cardiac disease, we excluded infants with *International Classification of Diseases* (ICD)-9 diagnoses indicating congenital heart disease, chronic lung disease or congenital upper airway anomalies (5%, $n = 41$) (Carroll et al. 2009), low birth weight infants (< 2500 g, 11%, $n = 73$), and infants born less than 37 weeks estimated gestational age (EGA) (5%, $n = 38$) (Veeranki et al. 2014). We excluded

seven dyads without a 2nd trimester plasma sample. After exclusions, our sample included 676 dyads. In multivariable regression analyses, we restricted to African-American or White as other races were too few to study ($n=7$). Written informed consent was given by the women before enrollment. This study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments. The study was approved by the Institutional Review Boards of Vanderbilt University, University of Tennessee Health Sciences Center, the Tennessee Department of Health, and by representatives of the Bureau of TennCare.

Infant Bronchiolitis Outcome

Bronchiolitis diagnosis during the first year of life was our primary outcome and was captured using ICD-9 codes as previously described (Carroll et al. 2009). We identified clinic visits, emergency department visits, 23 h observation stays (defined as non-inpatient hospital admission for less than 24 h) (Ross and Zalenski 2001), and hospitalizations using ICD-9 codes for bronchiolitis (466.1) and/or respiratory syncytial virus pneumonia (480.1) during infancy (Carroll et al. 2009; Veeranki et al. 2014). Consecutive 23 h observation stays were captured as hospitalizations. We classified infants as having at least one healthcare encounter for bronchiolitis during the first year of life versus none (Veeranki et al. 2014).

Maternal Plasma Folate

Plasma folate levels were measured using samples collected during the 2nd trimester. Trained research staff collected blood samples by venipuncture in a 10 mL purple top tube which was centrifuged for 10 min at 3000 rpm in order to separate the buffy coat and plasma. Samples were registered, processed, and stored in the CANDLE repository in the University of Tennessee Health Science Center, Department of Pathology at -70°C . Plasma folate level determinations were completed by the Molecular Epidemiology Laboratory in Birmingham, AL using a lactobacillus casei microbiological assay (Roy et al. 2018). The assay minimum detection limit was 3 ng/mL and the intra-assay and the inter-assay variability were 4.8–5.9 and 5.5–6.5% respectively.

Covariates

CANDLE research assistants collected socio-demographic information and dyad medical history at enrollment and subsequent study visits. We estimated infant bronchiolitis risk adjusting for confounders based on prior studies (Carroll et al. 2009; Haberg et al. 2011; Leermakers et al. 2013; Veeranki et al. 2014). We collected data on the following covariates via self-report: maternal race (African-Americans

vs. White), age at enrollment (years), educational attainment (less than high school, high school, greater than high school), smoking during pregnancy (yes, no), asthma history (yes, no), pre-pregnancy body mass index (BMI), weight gain during pregnancy (kg), and enrollment year. Birth weight (kg) and estimated gestational age (weeks) were collected from delivery records. Number of siblings relative to the index child and breastfeeding initiation (yes, no) was characterized using birth certificate data. Maternal pre-pregnancy BMI (weight [kg] per height [m^2]) and weight gain during early pregnancy (current weight [2nd trimester] minus pre-pregnancy weight) were calculated using self-reported height and weights.

Research assistants administered the validated semi-quantitative Block (2005) Food Frequency Questionnaire (FFQ) (<http://nutritionquest.com/company/our-research-questions/>) with standardized food visual aids for quantity references to the women during the 2nd trimester of pregnancy as previously described (Völgyi et al. 2013). Respondents reported usual dietary intake of 111 foods, beverages and dietary supplements over the previous 3 months. For dietary analyses, women with low (<500 kcal/day) or high (>5000 kcal/day) total energy intake were excluded, leaving 507 (76%) (Völgyi et al. 2013; Willett 1998). The following nutrients are thought to play a role in DNA methylation: total folate (μg), total choline (mg), betaine (mg), methionine (mg), total vitamin B12 (mg), total B6 (mg), and total energy intake. These nutrients were assessed as potential confounders in models with dyads having plausible dietary intakes.

Statistical Analyses

Descriptive statistics were calculated as median with interquartile range (median, IQR: 25th, 75th) for continuous variables, and frequency and percentages for categorical variables. In bivariate analyses, we compared differences in maternal and infant characteristics across quartiles of plasma folate levels using Chi-square and Kruskal–Wallis tests for categorical and continuous variables, respectively.

We evaluated the association between prenatal folate levels and infant bronchiolitis using two approaches. Multivariable logistic regression analyses were conducted to assess the association of 2nd trimester plasma folate levels with infant bronchiolitis, while adjusting for relevant covariates: maternal age, race, education, smoking, asthma, BMI (kg/m^2), pregnancy weight gain (kg), year of study enrollment and infant gestational age, birth weight (kg), breastfeeding initiation, and sibling number. We compared the odds of bronchiolitis in infants categorized by maternal plasma folate levels in quartiles (reference: lowest quartile). The results from logistic regression suggested a non-linear relationship between folate levels and the log-odds of bronchiolitis. The effect of plasma folate levels on bronchiolitis related

healthcare visits was then modeled without any assumption on the underlying relationship using flexible terms (splines). Thus, the logistic regression analysis was carried out using restricted cubic splines with four knots for maternal plasma folate to assess a potential non-linear relationship with the log-odds of bronchiolitis (Dupont 2009). Breastfeeding data was missing in 25 subjects (~4%). Thus, in sensitivity analysis we applied multiple imputations using predictive mean matching and carried multivariable regression analysis that avoid case wise deletions of observations when breast feeding or other covariates were missing (Harrell 2015).

For dyads with plausible 2nd trimester dietary data, dietary folate equivalents (DFEs) were calculated from reported dietary folate, folic acid fortification, and supplement intakes (Online Resource 2). Spearman's rank correlation coefficients (ρ) was used to assess the bivariate relationships of plasma folate and dietary and supplement intake of folate, vitamin B6, vitamin B12, choline (diet only), and betaine (diet only). We also considered intakes of vitamin B6, vitamin B12, choline, and betaine as potential confounders and included them in separate models after adjusting for total energy intake (Willett 1998).

Maternal asthma and infant sex were hypothesized a priori to be potential effect modifiers (Carroll et al. 2009; Sharma and Litonjua 2014; Veeranki et al. 2014). Hence, we allowed the association of 2nd trimester folate to be modified by either infant sex or maternal asthma via an interaction term (cross-product) in separate models. The global test of significance for the interaction term was used to assess statistical significance. A two-sided 5% significance level was used for all statistical inferences. SAS version 9.1 (SAS Inc., Cary, NC, US) was used for data management. All analyses were performed using R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria, <https://www.r-project.org>).

Results

Of the 1503 women enrolled in CANDLE, 962 had TennCare insurance at enrollment. After excluding dyads with infants without continuous TennCare enrollment and infants with pre-existing conditions, 676 eligible dyads had plasma folate measured in the 2nd trimester (Table 1). The median maternal age at delivery was 23 years [IQR: 20, 27]. Among participating women, 84% were African-American and 18% had less than a high school education. Among infants, 52% were male; median birth weight and EGA were 3.2 kilograms [IQR: 3.0, 3.5] and 39 weeks [38, 40], respectively.

The distribution of prenatal folate levels ranged from 3.8 to 85.0 ng/mL (median: 19.2, IQR: 12.9, 26.8) (Fig. 1). Some statistically significant differences were found among quartiles of plasma folate, including differences

by education and race. Women with higher folate levels tended to be more highly educated ($p = .004$). African-American women had a median energy-adjusted dietary folate intake of 1745 DFE (IQR: 1507, 1913) and White women had a median dietary folate intake of 1796 DFE (IQR: 1653, 1976, $p < .01$; data not shown). African-American women had lower plasma folate levels (median: 18 ng/mL, IQR: 13, 26) compared to White women (median: 24 ng/mL, IQR: 18, 32, $p < .01$). Folate levels stratified by race are displayed in Online Resource 1. Overall, 21% of infants in the study had at least one healthcare visit for bronchiolitis and the proportion did not differ by quartiles of 2nd trimester folate levels ($p = .30$).

In multivariable logistic regression analyses, we did not observe a significant association between bronchiolitis diagnosis and plasma folate, but the results suggested a possible non-monotonic trend in the relationship (Table 2). Using a spline approach, the adjusted relationship of maternal folate levels with predicted probability of any bronchiolitis visit is shown in Fig. 1. At low levels, 5–12 ng/mL, there appears to be a positive association between maternal folate levels and bronchiolitis risk. This relationship appears to reverse for folate levels between 13 and 27 ng/mL. The overall association of maternal plasma folate and infant bronchiolitis was not statistically significant ($p = .112$) and the test of non-linear effects approached but did not reach statistical significance ($p = .088$) (Fig. 2). In sensitivity analysis that applied multiple imputations, results were consistent with the model that used complete cases. The overall association of maternal plasma folate and infant bronchiolitis was not statistically significant ($p = .106$) and the test of non-linear effects was of marginal significance ($p = .055$).

We considered dietary measures of folate, vitamin B6, vitamin B12, choline, methionine, and betaine as they related to maternal plasma folate levels and as potential confounders (Fig. 3). Plasma folate was positively correlated with total dietary folate equivalents ($\rho = 0.39$), B6 ($\rho = 0.38$), B12 ($\rho = 0.27$) (all p -values $< .001$) and betaine ($\rho = 0.09$, $p = .05$, Fig. 3). We did not detect a bivariate relationship between plasma folate and choline ($\rho = 0.08$, $p = .083$) and methionine ($\rho = 0.04$, $p = 0.41$). The addition of folate, vitamin B6, vitamin B12, choline, methionine, and betaine to our models did not appreciably change our results and interpretation (data not shown). In the analysis of prenatal DFE and infant bronchiolitis, we did not detect a significant association (Online Resource 2). Lastly, we did not observe significant effect modification by maternal history of asthma (p -interaction = .84) or infant sex (p -interaction = .78) for the relationship of plasma folate levels and the diagnosis of infant bronchiolitis.

Table 1 Demographic characteristics of mother–child dyads enrolled in the Tennessee Medicaid Program and in the CANDLE study by quartile of maternal 2nd trimester plasma folate levels

Characteristic	Quartile 1 (N= 169) (3.76–12.9 ng/ml)	Quartile 2 (N= 169) (12.94–19.2 ng/ml)	Quartile 3 (N= 169) (19.23–26.8 ng/ml)	Quartile 4 (N= 169) (26.8–85.0 ng/ml)	All (N= 676)
Maternal race, n (%)^a					
Black	152 (90)	150 (89)	140 (83)	127 (76)	569 (84)
White	15 (9)	18 (11)	28 (17)	38 (23)	99 (15)
Other	2 (1)	1 (1)	1 (1)	3 (2)	7 (1)
Missing					1
Maternal education, n (%)^a					
Less than high school	39 (23)	40 (24)	19 (11)	26 (15)	124 (18)
High school	103 (61)	101 (60)	110 (65)	95 (57)	409 (61)
Greater than high school	27 (16)	28 (17)	40 (24)	47 (28)	142 (21)
Missing					1
Maternal asthma, n (%)					
Yes	18 (11)	16 (9)	17 (10)	21 (12)	72 (11)
No	149 (89)	153 (91)	150 (90)	148 (88)	600 (89)
Missing					4
Maternal prenatal smoking, n (%)					
Yes	23 (14)	22 (13)	16 (9)	25 (15)	86 (13)
No	146 (86)	147 (87)	153 (91)	144 (85)	590 (87)
Maternal prenatal vitamin use, n (%)^a					
Yes	141 (87)	153 (94)	161 (98)	161 (97)	616 (94)
No	21 (13)	10 (6)	3 (2)	5 (3)	39 (6)
Missing					21
Maternal age (years) ^{a,b}	24 [20, 26]	22 [19, 26]	24 [21, 28]	23 [20, 28]	23 [20, 27]
Maternal pre-pregnancy BMI ^b	27 [22, 35]	26 [22, 29]	27 [23, 33]	26 [22, 32]	26 [22, 32]
Missing					3
Maternal betaine intake (mg) ^{b,c}	225 [191, 267]	236 [209, 276]	237 [216, 282]	236 [214, 271]	235[207,275]
Maternal methionine intake (mg) ^{b,c}	2.3 [2.1, 2.6]	2.3 [2.2, 2.6]	2.3 [2.2, 2.5]	2.3 [2.2, 2.6]	2.3 [2.1, 2.6]
Maternal total choline intake (mg) ^{a,b,c}	426 [380, 480]	430 [380, 497]	444 [402, 517]	433 [395, 498]	433[388,499]
Maternal total B6 intake (mg) ^{a,b,c}	4.1 [3.2, 4.5]	4.4 [4.0, 5.0]	4.7 [4.4, 5.2]	4.8 [4.4, 5.1]	4.6 [4.1, 5.0]
Maternal total B12 intake (mg) ^{a,b,c}	9 [7, 11]	10 [8, 11]	10 [9, 12]	10 [9, 11]	10 [9, 11]
Maternal total folate (DFE) (μg) ^{a,b,c,d}	1489 [1060,1810]	1676 [1397,1813]	1833 [1700,1990]	1827 [1669,2019]	1757 [1551,1930]
Infant birth weight (kg) ^{a,b}	3.2 [3.0, 3.5]	3.2 [3.0, 3.4]	3.2 [3.0, 3.5]	3.3 [3.1, 3.6]	3.2 [3.0, 3.5]
Infant estimated gestational age (weeks) ^b	39 [38, 40]	39 [39, 40]	39 [38, 40]	39 [39, 40]	39 [38, 40]
Infant sex n (%)					
Female	76 (45)	94 (56)	73 (43)	83 (49)	326 (48)
Male	93 (55)	75 (44)	96 (57)	86 (51)	350 (52)
Breastfeeding initiation n (%)					
Yes	86 (52)	92(57)	97(60)	104(64)	379(58)
No	79(48)	70(43)	64(40)	59(36)	272(42)
Missing					25
Infant bronchiolitis n (%)					
Yes	41 (24)	40 (24)	31 (18)	29 (17)	141 (21)
No	128 (76)	129 (76)	138 (82)	140 (83)	535 (79)

^ap<.05 for difference between quartiles^bValues are given as median and [IQR], Total N with plausible dietary intake data = 507^cValues calculated from 2nd trimester food frequency questionnaire data and are adjusted for total energy intake^dDFE Dietary folate equivalents; total folate includes dietary folate, dietary folic acid, and supplemental folic acid intakes

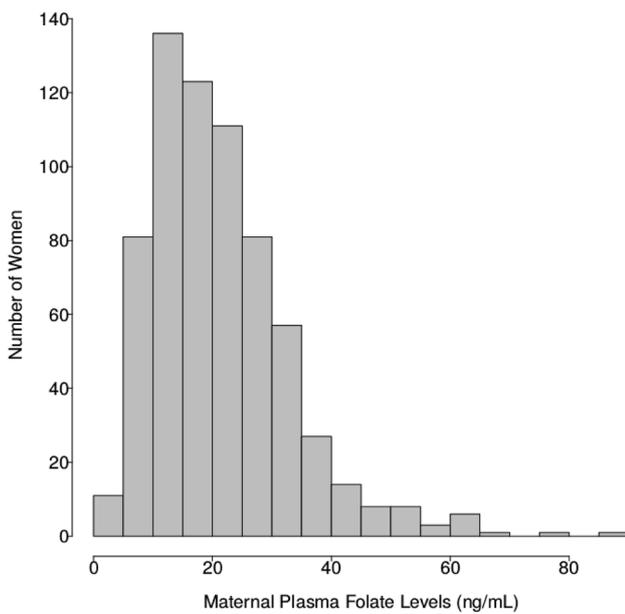


Fig. 1 Histogram of 2nd trimester plasma folate levels (ng/mL) in CANDLE-TennCare cohort

Discussion

In our cohort of dyads enrolled in the CANDLE study and Tennessee Medicaid, 21% of infants had at least one bronchiolitis-related healthcare visit. Rates were consistent with findings in our large, population-based retrospective cohort study (Veeranki et al. 2014). In addition, this low-income population had relatively high levels of plasma folate with 50% of the levels above 19.2 ng/mL. Yet, African-American women had lower dietary folate intake and plasma folate levels than White women in our cohort. We did not find an overall significant association between 2nd trimester plasma folate levels and bronchiolitis during the first year of life. Weak evidence of a non-linear relationship between maternal folate levels and infant bronchiolitis was found; however, these results should be

interpreted cautiously given the distribution of folate levels in our population. At lower levels there appeared to be a positive relationship between maternal plasma folate and infant bronchiolitis, the observed relationship was reversed for mid-range levels; fewer women had higher folate levels > 40 ng/mL.

Our study linked a longitudinal birth cohort with prenatal plasma folate data with administrative database files, enabling the objective assessment of provider-diagnosed bronchiolitis and minimizing participant recall bias, while also accounting for critical timing of the measurement of blood folate levels. The results of this study should be considered in the context of the limited number of studies investigating this association.

Women in this study had a wide range of plasma folate levels (3.76–85.02 ng/mL or 8.6–193.2 nmol/L). With a median plasma folate level of 19.8 ng/mL (45 nmol/L), the women in our study had higher prenatal folate levels than reported median levels from previous cohort studies (Dunstan et al. 2012 [37.2 nmol/L or 16.4 ng/mL]; Haberg et al. 2011 [9.1 nmol/L or 4 ng/mL]; Kim et al. 2015 [9.5–11.2 ng/mL or 21.5–25.4 nmol/L]). The high folate levels we observed may be due to high dietary folic acid intake and reported folic acid supplementation (94%) in the 2nd trimester; which was also higher than reported supplementation in other studies (~55–89%) (Branum et al. 2013; Dunstan et al. 2012; Kiefte-de Jong et al. 2012; Kim et al. 2015). In addition to the recommendation that women of child bearing age consume at least 400 mcg of folic acid daily (Centers for Disease Control and Prevention 2010), the US has a mandatory food fortification program for grains and cereals (Bentley et al. 1999), unlike other countries where previous studies have been conducted (Haberg et al. 2011; Kiefte-de Jong et al. 2012; Kim et al. 2015; Magdelijns et al. 2011). Based on lower limit cutoffs for folate (range < 3–4.5 ng/mL), < 5% of the women in our study had low levels during the 2nd trimester of pregnancy (McDowell et al. 2008; Selhub et al. 2008). Approximately 84% of the women in our study were African-American. Previous studies have observed lower folate concentrations in African-American women compared

Table 2 Bronchiolitis diagnosis and maternal plasma folate in 2nd trimester of pregnancy (quartiles) among term, otherwise healthy infants enrolled in CANDLE and TennCare

2nd trimester plasma folate levels in ng/ml (quartiles)	Bronchiolitis ^a	
	Unadjusted OR (95% CI)	Adjusted OR ^b (95% CI)
Q1: [3.76, 12.9, ng/mL]	Referent	Referent
Q2: [12.94, 19.2, ng/mL]	1.03 (0.62, 1.71)	1.17 (0.68, 2.00)
Q3: [19.23, 26.8, ng/mL]	0.75 (0.44, 1.27)	0.74 (0.42, 1.31)
Q4: [26.8, 85.0, ng/mL]	0.66 (0.38, 1.14)	0.73 (0.40, 1.31)

^aAt least one healthcare visit for bronchiolitis during the first year of life (Binary logistic regression model)

^bOdds Ratio adjusted for infant sex, gestational age (in weeks), infant birth weight (kg), breastfeeding initiation, number of siblings, maternal race (African America, White race), maternal age at delivery, maternal smoking during pregnancy, maternal asthma, BMI (kg/m²), weight gain (kg), and year of study enrollment

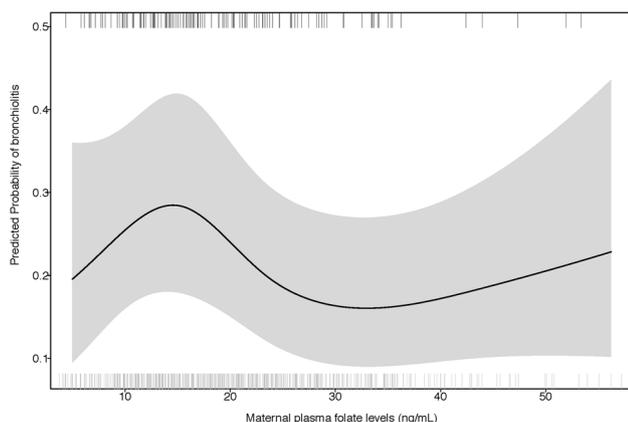


Fig. 2 The adjusted relationship of 2nd trimester plasma folate levels and infant bronchiolitis. Multivariable logistic regression adjusted for the following covariates: maternal age, race, education, smoking, asthma, BMI (kg/m^2), pregnancy weight gain (kg), year of study enrollment and infant gestational age, birth weight (kg), breastfeeding initiation, and number of siblings. Rug plots at the top and bottom of this figure indicate folate levels of the women whose infants who did develop bronchiolitis (top) and did not develop bronchiolitis (bottom), respectively

to White women of childbearing age (McDowell et al. 2008). Similarly, in our cohort of pregnant women, plasma folate and dietary measures of folate were significantly lower in African-American women compared to White women. Differences in both dietary intake and actual use of prenatal supplements may have contributed to this finding.

Pregnancy is a critical period for development of the fetal immune system, as emerging evidence indicates that the fetal T Helper 1/T Helper 2 cytokine balance, thought to play a role in the pathogenesis of allergic asthma, may be susceptible to epigenetic effects during this time (Adcock et al. 2007). Folate is a methyl donor in DNA methylation (Dhur et al. 1991), a process that is essential to the gene expression of transcription factors that regulate immune T cell development (Adcock et al. 2007; White et al. 2006, 2002). Although adequate folate intake during the periconceptional period is important for the prevention of neural tube defects (Czeizel and Dudas 1992), it is important to study potential effects on the developing immune system, especially effects that may result in respiratory disease (Sharma and Litonjua 2014).

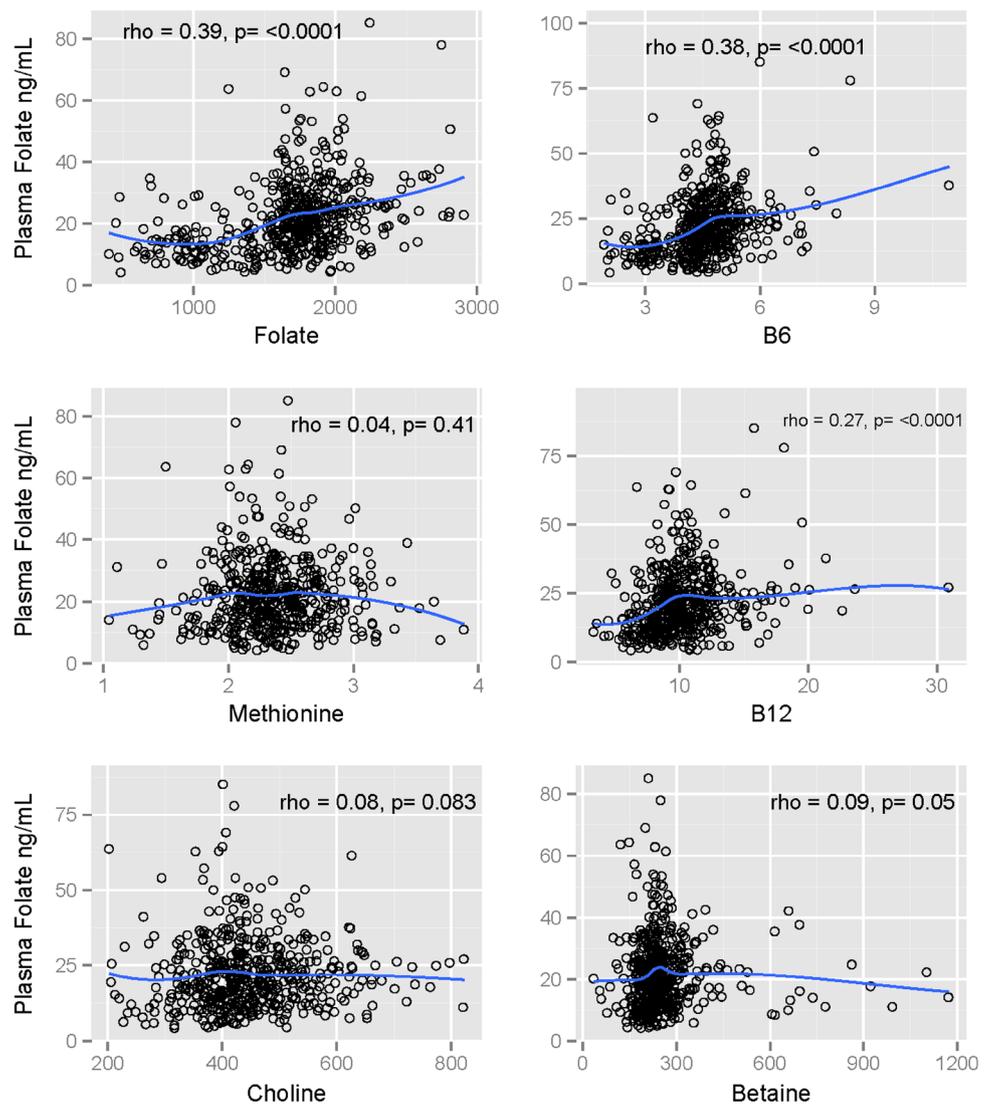
Our study contributes to the limited number of epidemiologic studies with the capability to examine prenatal folate levels and bronchiolitis during infancy. In an epidemiologic investigation examining self-reported prenatal folic acid supplementation, Haberg et al. reported that folic acid supplementation in the 1st trimester was associated with increased relative odds of LRTI and wheezing (Haberg et al. 2009). Although the authors did not investigate LRTI outcomes, Bekkers et al. reported an

increased risk for wheezing at 1 year of age for children of women who reported folic acid supplementation (Bekkers et al. 2012). In a study of timing of prenatal folic acid supplementation initiation and child respiratory outcomes, Alfonso et al. (2018) reported no overall association with timing of folic acid supplementation and wheeze or LRTI, but children born to women with a history of atopy who initiated folic acid after the 1st trimester had an increased risk of wheeze (Alfonso et al. 2018). Folic acid exposure in this study was reported postnatally and was not confirmed by blood folate samples. Kim et al. found that higher folate levels during pregnancy were associated with a 50% decreased relative odds of LRTI in the first 6 months of life (Kim et al. 2015). Although folate levels were measured during mid and late pregnancy, the study was conducted in a country without a folic acid supplementation program and the median plasma folate levels and prevalence of folic acid supplementation were lower than women in our study (Kim et al. 2015). A study by Kieft-de Jong et al. did not observe a significant association between higher maternal folate levels in early pregnancy and childhood wheeze (Kieft-de Jong et al. 2012). Blood samples in these studies were taken at different time points in pregnancy, suggesting that temporality may be an important consideration. Further research to determine whether the developing fetal immune system is more vulnerable to epigenetic mechanisms associated with folate exposure during a particular period of pregnancy is warranted.

This study has potential limitations to consider. We measured 2nd trimester plasma folate concentration as an objective biomarker; however, red blood cell folate concentration may provide a better indication of longer term folate status (Gibson 2005). Our cohort reflects a predominately African-American, demographically diverse population that is generally understudied; however, our results may be less generalizable to other populations. Data for potential covariates such as pre-pregnancy and 2nd trimester weight, dietary intake, and prenatal vitamin supplementation were obtained by participant self-report, which may lead to misclassification. The outcome of bronchiolitis in the first year of life, the period with the highest incidence, may be subject to under or over detection. These measures were objectively captured using well-characterized health plan administrative data, reducing the likelihood of systematic bias. As with all epidemiologic studies, unmeasured confounding or effect modifying variables may have influenced our results.

In summary, our results suggest a possible non-monotonic trend in the relationship between prenatal folate levels and infant bronchiolitis; nevertheless, our results did not confirm an overall association. Based on the findings from this study, we are not suggesting changes in recommendations by providers regarding folic acid supplementation. Further research

Fig. 3 Scatter plots with non-parametric curves illustrating the relationship of plasma folate with dietary markers (dietary food intake and supplements combined, dietary intake for choline and betaine). Dietary markers have been corrected for energy consumption (daily caloric intake). Blue lines represent the locally weighted scatter plot smoothing (LOESS) and Spearman rank correlation coefficient (ρ) and corresponding p value reported



on prenatal folate levels, especially higher levels, and early childhood respiratory illnesses is warranted to fully delineate potential clinical implications of this work.

Acknowledgements We acknowledge the vital contributions of the CANDLE study research staff and the families that are enrolled in the Conditions Affecting Neurocognitive Development and Learning in Early Childhood study. We thank the Tennessee Bureau of TennCare (Department of Finance and Administration) and the Tennessee Department of Health (Office of Policy, Planning, and Assessment) for providing data needed for this study. This work was supported by the National Institutes of Health, National Heart, Blood, and Lung Institute (Grant R01 HL109977-KNC), the Urban Child Institute (FT) and the National Institutes of Health, National Center for Research Resources (Vanderbilt CTSA Grant UL1 RR024975).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Adcock, I. M., Tsaprouni, L., Bhavsar, P., & Ito, K. (2007). Epigenetic regulation of airway inflammation. *Current Opinions in Immunology*, 19(6), 694–700.
- Alfonso, V. H., Bandoli, G., von Ehrenstein, O., & Ritz, B. (2018). Early folic acid supplement initiation and risk of adverse early childhood respiratory health: A population-based Study. *Maternal and Child Health Journal*, 22(1), 111–119:11.
- Bekkers, M. B., Elstgeest, L. E., Scholtens, S., Haveman-Nies, A., de Jongste, J. C., Kerkhof, M., ... Wijga, A. H. (2012). Maternal use of folic acid supplements during pregnancy, and childhood respiratory health and atopy. *European Respiratory Journal*, 39(6), 1468–1474.
- Bentley, J. R., Ferrini, R. L., & Hill, L. L. (1999). American College of Preventive Medicine public policy statement. Folic acid fortification of grain products in the U.S. to prevent neural tube defects. *American Journal of Preventive Medicine*, 16(3), 264–267.
- Branum, A. M., Bailey, R., & Singer, B. J. (2013). Dietary supplement use and folate status during pregnancy in the United States. *Journal of Nutrition*, 143(4), 486–492.

- Carroll, K. N., Wu, P., Gebretsadik, T., Griffin, M. R., Dupont, W. D., Mitchel, E. F., & Hartert, T. V. (2009). The severity-dependent relationship of infant bronchiolitis on the risk and morbidity of early childhood asthma. *Journal of Allergy and Clinical Immunology*, *123*(5), 1055.e1–1061.e1
- Centers for Disease Control & Prevention. (2010). Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *Morbidity and Mortality Weekly Report* 1992, *41*, 1–7.
- Czeizel, A. E., & Dudas, I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *New England Journal of Medicine*, *327*(26), 1832–1835.
- Dhur, A., Galan, P., & Hercberg, S. (1991). Folate status and the immune system. *Progress in Food & Nutrition Science*, *15*(1–2), 43–60.
- Dunstan, J. A., West, C., McCarthy, S., Metcalfe, J., Meldrum, S., Oddy, W. H., ... Prescott, S. L. (2012). The relationship between maternal folate status in pregnancy, cord blood folate levels, and allergic outcomes in early childhood. *Allergy*, *67*(1), 50–57.
- Dupont, W. D. (2009). *Statistical modeling for biomedical researchers: A simple introduction to the analysis of complex data*. Cambridge: Cambridge University Press.
- Gibson, R. S. (2005). *Principles of nutritional assessment*. Oxford: Oxford University Press.
- Graham, B. S. (2011). Biological challenges and technological opportunities for respiratory syncytial virus vaccine development. *Immunological Reviews*, *239*(1), 149–166.
- Haberg, S. E., London, S. J., Nafstad, P., Nilsen, R. M., Ueland, P. M., Vollset, S. E., & Nystad, W. (2011). Maternal folate levels in pregnancy and asthma in children at age 3 years. *Journal of Allergy and Clinical Immunology*, *127*(1), e262–e264
- Haberg, S. E., London, S. J., Stigum, H., Nafstad, P., & Nystad, W. (2009). Folic acid supplements in pregnancy and early childhood respiratory health. *Archives of Disease in Childhood*, *94*(3), 180–184.
- Harrell, F. E. (2015). *Regression modeling strategies with applications to linear models, logistic and ordinal regression, and survival analysis* (2nd edn.). New York: Springer.
- Kiefte-de Jong, J. C., Timmermans, S., Jaddoe, V. W., Hofman, A., Tiemeier, H., & Steegers, E. A., ... Moll, H. A. (2012). High circulating folate and vitamin B-12 concentrations in women during pregnancy are associated with increased prevalence of atopic dermatitis in their offspring. *Journal of Nutrition*, *142*(4), 731–738.
- Kim, J. H., Jeong, K. S., Ha, E. H., Park, H., Ha, M., Hong, Y. C., ... Chang, N. (2015). Relationship between prenatal and postnatal exposures to folate and risks of allergic and respiratory diseases in early childhood. *Pediatric Pulmonology*, *50*(2), 155–163.
- Leermakers, E. T., Sonnenschein-van der Voort, A. M., Gaillard, R., Hofman, A., de Jongste, J. C., Jaddoe, V. W., & Duijts, L. (2013). Maternal weight, gestational weight gain and preschool wheezing: The Generation R Study. *European Respiratory Journal*, *42*(5), 1234–1243.
- Magdelijns, F. J., Mommers, M., Penders, J., Smits, L., & Thijs, C. (2011). Folic acid use in pregnancy and the development of atopy, asthma, and lung function in childhood. *Pediatrics*, *128*(1), e135–e144.
- Martino, D., & Prescott, S. (2011). Epigenetics and prenatal influences on asthma and allergic airways disease. *Chest*, *139*(3), 640–647.
- Martinussen, M. P., Risnes, K. R., Jacobsen, G. W., & Bracken, M. B. (2012). Folic acid supplementation in early pregnancy and asthma in children aged 6 years. *American Journal of Obstetrics & Gynecology*, *206*(1), e-72.e7
- McDowell, M. A., Lacher, D. A., Pfeiffer, C. M., Mulinare, J., Picciano, M. F., Rader, J. I., ... Johnson, C. L. (2008). Blood folate levels: The latest NHANES results. *NCHS Data Brief*, *6*, 1–8.
- Nair, H., Nokes, D. J., Gessner, B. D., Dherani, M., Madhi, S. A., Singleton, R. J., ... Campbell, H. (2010). Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: A systematic review and meta-analysis. *Lancet*, *375*(9725), 1545–1555.
- Prescott, S. L., & Clifton, V. (2009). Asthma and pregnancy: Emerging evidence of epigenetic interactions in utero. *Current Opinion in Allergy and Clinical Immunology*, *9*(5), 417–426.
- Ross, M. A., & Zalenski, R. J. (2001). Observation services: Past, present, and future. *The American Journal of Medicine*, *110*(4), 324–325.
- Roy, A., Kocak, M., Hartman, T. J., Vereen, S., Adgent, M., Piyathilake, C., ... Carroll, K. N. (2018). Association of prenatal folate status with early childhood wheeze and atopic dermatitis. *Pediatric Allergy and Immunology*, *29*(2), 144–150.
- Selhub, J., Jacques, P. F., Dallal, G., Choumenkovitch, S., & Rogers, G. (2008). The use of blood concentrations of vitamins and their respective functional indicators to define folate and vitamin B12 status. *Food and Nutrition Bulletin*, *29*(2 Suppl), S67–S73.
- Sharma, S., & Litonjua, A. (2014). Asthma, allergy, and responses to methyl donor supplements and nutrients. *The Journal of Allergy and Clinical Immunology*, *133*(5), 1246–1254.
- Veeranki, S. P., Gebretsadik, T., Dorris, S. L., Mitchel, E. F., Hartert, T. V., Cooper, W. O., ... Carroll, K. N. (2014). Association of folic acid supplementation during pregnancy and infant bronchiolitis. *American Journal of Epidemiology*, *179*(8), 938–946.
- Völgyi, E., Carroll, K. N., Hare, M. E., Ringwald-Smith, K., Piyathilake, C., Yoo, W., & Tyllavsky, F. A. (2013). Dietary patterns in pregnancy and effects on nutrient intake in the mid-south: The Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) Study. *Nutrients*, *5*(5), 1511–1530.
- White, G. P., Hollams, E. M., Yerkovich, S. T., Bosco, A., Holt, B. J., Bassami, M. R., ... Holt, P. G. (2006). CpG methylation patterns in the IFN-gamma promoter in naive T cells: variations during Th1 and Th2 differentiation and between atopics and non-atopics. *Pediatric Allergy and Immunology*, *17*(8), 557–564.
- White, G. P., Watt, P. M., Holt, B. J., & Holt, P. G. (2002). Differential patterns of methylation of the IFN-gamma promoter at CpG and non-CpG sites underlie differences in IFN-gamma gene expression between human neonatal and adult CD45RO- T cells. *Journal of Immunology*, *168*(6), 2820–2827.
- Whitrow, M. J., Moore, V. M., Rumbold, A. R., & Davies, M. J. (2009). Effect of supplemental folic acid in pregnancy on childhood asthma: A prospective birth cohort study. *American Journal of Epidemiology*, *170*(12), 1486–1493.
- Willett, W. (1998). *Nutritional epidemiology* (2nd edn.). New York: Oxford University Press.