

High Circulating Folate and Vitamin B-12 Concentrations in Women During Pregnancy Are Associated with Increased Prevalence of Atopic Dermatitis in Their Offspring^{1–3}

Jessica C. Kiefte-de Jong,^{4,5} Sarah Timmermans,^{4,6} Vincent W. V. Jaddoe,^{4,5,7} Albert Hofman,⁷ Henning Tiemeier,⁸ Eric A. Steegers⁶ Johan C. de Jongste,⁹ and Henriette A. Moll^{5,*}

⁴Generation R Study Group, ⁵Department of Pediatrics, ⁶Department of Obstetrics and Gynecology, ⁷Department of Epidemiology, ⁸Department of Child and Adolescent Psychiatry, and ⁹Department of Pediatrics/Respiratory Medicine, Erasmus University Medical Centre, Rotterdam, The Netherlands

Abstract

Recent studies suggest that in utero exposure of methyl donors influences programming of the fetal immune system in favor of development of allergic disease. The aim of this study was to assess whether the *MTHFR* C677T polymorphism, folic acid supplementation, and circulating folate and vitamin B-12 concentrations during pregnancy were associated with wheezing, shortness of breath, and atopic dermatitis in offspring. The study was a population-based birth cohort from fetal life until 48 mo ($n = 8742$). The use of folic acid supplementation during pregnancy was assessed by questionnaire. Plasma folate and serum vitamin B-12 concentrations and the *MTHFR* C677T polymorphism were available from blood collected in early pregnancy. Atopic dermatitis, wheezing, and shortness of breath in the offspring were assessed by parental-derived questionnaires at 12, 24, 36, and 48 mo. Maternal folate >16.2 nmol/L and vitamin B-12 >178 pmol/L were positively associated with the development of atopic dermatitis [adjusted OR: 1.18 (95%CI: 1.05–1.33) and adjusted OR: 1.30 (95% CI: 1.06–1.60) for the highest quartiles of folate and vitamin B-12 concentrations, respectively] but not with wheezing and shortness of breath. Maternal *MTHFR* C677T polymorphism and folic acid supplementation were not associated with wheezing, shortness of breath, and atopic dermatitis. No interactions were found by age, family history of atopy, folic acid supplementation, *MTHFR* C677T polymorphism, or maternal smoking (P -interaction > 0.10). High folate and vitamin B-12 levels during pregnancy are associated with increased prevalence of atopic dermatitis in the offspring. Potential risks of high folate and vitamin B-12 concentrations on allergic outcomes should be evaluated when discussing mandatory fortification programs. J. Nutr. doi: 10.3945/jn.111.154948.

Introduction

In 1976 Smithells et al. (1) suggested an association between low maternal folate status and the occurrence of NTD⁴. In the early 1990s, this hypothesis was supported by two randomized trials showing that folic acid supplementation reduced the risk of

NTD (2,3). The firm scientific evidence of these studies has led to the general advice for women planning pregnancy to use a folic acid supplement from at least 1 mo before until 3 mo after conception in addition to consuming a healthy diet (4,5). Furthermore, fortification of food with folic acid to reduce the number of NTD has been introduced in the United States and Canada and is now increasingly being considered by other countries as well (4).

Despite long-term research, the precise mechanisms underlying the beneficial effects of folic acid remain unknown (5). Folate has many functions and along with vitamin B-12 it plays a critical role in the homocysteine pathway, which is important for protein, lipid, and DNA synthesis. In addition, folate provides, with vitamin B-12 as cofactor, methyl groups for the synthesis of methionine and its derivative, *S*-adenosyl-methionine. The latter is the most important methyl donor in the human body and is involved in epigenetic mechanisms (5). Initial evidence has shown that varying dietary inputs to the methionine/folate cycle

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³ Supplemental Tables 1–3 and Supplemental Figures 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

⁴ Abbreviations used: GEE, generalized estimating equation; k , Cohen's kappa; NTD, neural tube defect.

* To whom correspondence should be addressed. E-mail: h.a.moll@erasmusmc.nl.

during the periconceptional period can lead to widespread epigenetic alterations in the offspring influencing health-related phenotypes (6). Also, the *MTHFR* C677T polymorphism may play a role, because the TT genotype renders individuals more susceptible to epigenetic alterations in response to low folate status associated with this genotype (7).

Because folic acid may have a function on an epigenetic level, potential adverse effects of folic acid have also been debated (8). Hollingsworth et al. (9) found differential methylation in lung tissue between the offspring of mice fed either a high-methyl or low-methyl donor diet. They showed that an increased supply of folic acid and vitamin B-12 in mice was associated with an increased risk of allergic airway disease that was mediated through epigenetic mechanisms. Likewise, other studies have demonstrated that epigenetic mechanisms may influence gene expression regulating airway inflammation (10) and polarization of Th1/Th2 cells (11). Also, recent epidemiological studies on the association between folic acid supplementation during pregnancy and the increased risk of allergic disease support the hypothesis that epigenetics may be involved in the development of allergies (12,13).

In view of these findings and of the current recommendations and policy considerations concerning folic acid fortification in several countries, we aimed to assess whether folic acid supplementation, plasma folate, and serum vitamin B-12 concentrations during pregnancy were associated with the development of wheezing, shortness of breath, and atopic dermatitis in the offspring up to 48 mo of age. A second aim was to assess whether the maternal *MTHFR* C677T polymorphism was associated with wheezing, shortness of breath, and atopic dermatitis. The last aim was to assess whether any interaction with age, family history of atopy, folic acid supplementation, maternal smoking, and *MTHFR* C677T polymorphism was observed for the above associations.

Materials and Methods

Study design. This study was embedded in the Generation R study, a population-based prospective cohort study from fetal life until young adulthood and was previously described in detail (14). In total, 8742 mothers with a delivery date between April 2002 and January 2006 provided consent for prenatal or postnatal follow-up and were included in this study (Supplemental Fig. 1). Ethic approval for the study was obtained from the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam, The Netherlands. Written informed consent was obtained from all participants.

Assessment of periconceptional folic acid exposure. At enrollment, we assessed folic acid exposure during pregnancy by asking the following question by questionnaire: "Have you taken folic acid, either as a single supplement or as part of multivitamin supplement during the first trimester?" Self-reported maternal folic acid intake was classified as follows as previously described (15): 1) no exposure, defined as no folic acid use at all; 2) periconceptional exposure, defined as the start of a folic acid supplement prior to conception; and 3) start of folic acid supplement within first 10 wk of conception (i.e., from the moment that pregnancy was recognized but in any case before 10 wk of pregnancy). Approximately 15% of the mothers used folic acid as part of a multivitamin supplement. The doses of folic acid in these multivitamins were comparable with single supplements of folic acid (between 0.4 and 0.5 mg/d), which is the recommended dose of folic acid for women in the preconceptional phase in The Netherlands. No valid information on dietary intake of B vitamins from food was available.

Plasma folate and serum vitamin B-12 concentrations. Nonfasting blood plasma (EDTA) and serum samples were collected during the first trimester of pregnancy (13.5 ± 2.0 wk of gestation). After sampling, these were transported to the regional laboratory for storage at -80°C

(STAR-MDC, regional laboratory). Processing aimed to be finished within a maximum of 3 h after venous puncture. In 2008, all EDTA samples (Supplemental Fig. 1) were transported to the Department of Clinical Chemistry, Erasmus Medical Centre, Rotterdam. Subsequently, plasma folate and serum vitamin B-12 concentrations were analyzed using microparticle-enhanced immunoassay on the AxSYM and Architect system (Abbott Diagnostics). The between-run CV for plasma folate were 8.9% at 5.6 nmol/L, 2.5% at 16.6 nmol/L, and 1.5% at 33.6 nmol/L, with an analytic range of 1.8–45.3 nmol/L. This CV for serum vitamin B-12 was 3.6% at 142 pmol/L, 7.5% at 308 pmol/L, and 3.1% at 633 pmol/L, with an analytic range of 44–1476 pmol/L.

***MTHFR* C677T polymorphism.** Analyses of the maternal *MTHFR* C677T polymorphism (CC, CT, or TT genotype) were restricted to Western mothers ($n = 4955$) (Supplemental Fig. 1). Ethnicity was defined according to the classification of Statistics Netherlands, meaning that if both parents of the mother were born in The Netherlands, the ethnicity of the mother was defined as Dutch; if one of the parents was born in a country other than The Netherlands, that country applied; if parents of the mother were born in different countries other than The Netherlands, the country of the mother applied (16). In addition, Western ethnicity was defined as Dutch, European (excluding Turkey), or American (excluding Latin American). Genotype and allele frequencies in this Western population were in Hardy Weinberg equilibrium ($P = 0.68$). Mothers were genotyped for the *MTHFR* C677T polymorphism (rs1801133) using a TaqMan allelic discrimination assay (Applied Biosystems) and Abgene QPCR ROX mix. The genotyping reaction was amplified using the GeneAmp PCR system 9600 [95°C (15 min), then 40 cycles of 94°C (15 s) and 60°C (1 min)]. The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were determined using SDS software (version 2.3, Applied Biosystems).

Assessment of childhood atopic dermatitis, wheezing, and shortness of breath. Information regarding atopic dermatitis, wheezing, and shortness of breath was obtained using core questions from the validated International Study of Asthma and Allergies in Childhood (17) and doctor attendance for atopic dermatitis at the ages of 12, 24, 36, and 48 mo. Parents were asked the following questions from the International Study of Asthma and Allergies in Childhood questionnaire: "Has your child had problems with a wheezing chest during the last year?," "Has your child had problems with tightness of the chest or shortness of breath during the past year?," and "Has your child had an itchy rash that came and went during the past year?"

The observed agreement (k) for wheezing and shortness of breath between measurement by questionnaire and by physician interview was 75% ($k = 0.30$) and 81% ($k = 0.39$), respectively, as previously described (18). The observed agreement for atopic dermatitis measured by questionnaire and physician interview was 79% ($k = 0.44$).

Covariates. Several medical, behavioral, and sociodemographic characteristics obtained from a combination of pre- and postnatal questionnaires and community midwife and hospital registries as previously described in detail (14) were considered as potential confounders in the analyses of folate, vitamin B-12, and wheezing, shortness of breath, and atopic dermatitis. The selection of confounders was based on previous studies on determinants of folic acid supplementation (15) and asthma development (19). The following variables were considered as potential confounders: 1) maternal age at pregnancy; 2) maternal BMI at inclusion; 3) maternal educational level (low: no education, primary school, or <3 y of secondary school; mid: >3 y of secondary school; high: higher vocational training, bachelor's degree, or academic education) (20); 4) maternal ethnicity (Western: Dutch, European, or American vs. non-Western: Cape Verdian, Moroccan, Antillean, Surinam, Turkish, African, Asian, or Latin American) (16); 5) parity; 6) infant's sex; 7) infant's birth weight and gestational age at birth; 8) any maternal smoking during pregnancy; 9) any maternal alcohol consumption during pregnancy; 10) duration of breastfeeding; 11) any attendance of day care of the child in the first 24 mo of the infant's life (yes vs. no); and 12) parental atopic constitution, defined as any parental history of doctor-attended atopic dermatitis, asthma, hay fever, or allergy to house dust.

Statistical analysis. First, independent Student's *t* test and Chi-square tests were used to test differences in characteristics between mothers who used folic acid and women who did not use a folic acid supplement during pregnancy.

To assess how folic acid supplementation during pregnancy and plasma folate and serum vitamin B-12 concentrations were associated with wheezing, shortness of breath, and atopic dermatitis, logistic GEE analyses were performed. Briefly, GEE analysis assesses the association by correction for the within-subject dependence as a result of the repeated observations on wheezing, shortness of breath, and atopic dermatitis (21). Because the within-subject correlation coefficient for the outcome variables at the four time points were comparable ($r = 0.2$ – 0.4 for wheezing and shortness of breath and $r = 0.3$ – 0.5 for atopic dermatitis), an exchangeable working correlation structure was used for the GEE models. The primary independent variables in the GEE model were use of folic acid during pregnancy (0 = no, 1 = start within 10 wk of conception, and 2 = periconceptional start), plasma folate concentration (after stratification into quartiles with the first quartile as reference), serum vitamin B-12 concentration (after stratification into quartiles with the first quartile as reference) or *MTHFR* C677T polymorphism (CC genotype as reference). All crude models were adjusted for time (12, 24, 36, and 48 mo). Subsequently, we created multivariate models including adjustment for potential confounders as maternal age, maternal BMI, ethnicity, family history of atopic constitution, parity, maternal educational level, maternal smoking, maternal alcohol consumption, gender, parity, daycare attendance, breastfeeding duration, and birth weight SD score. Additionally, to assess whether the associations among folate and vitamin B-12 and wheezing, shortness of breath, and atopic dermatitis were different by age of the child or by the *MTHFR* C677T polymorphism, folic acid supplementation, maternal smoking, or parental atopic constitution, the statistical interaction was evaluated by

adding the product term of the covariate (e.g., folate concentrations) and stratum (folate $\times$ stratum) as an independent variable to the models.

Nonresponse analysis showed that mothers who had no postnatal data on the child's health had lower mean folate (14.7 ± 8.3 nmol/L) and vitamin B-12 (183 ± 92 pmol/L) concentrations during pregnancy relative to mothers who filled out the questionnaires concerning the child's health (19 ± 9 nmol/L and 191 ± 93 pmol/L, respectively; $P < 0.01$) and used folic acid supplements less frequently during pregnancy (54 vs. 81%; $P < 0.01$). To reduce potential bias associated with attrition, a multiple imputation procedure for all variables used in this study was performed ($n = 5$ imputations) (Supplemental Fig. 1; Supplemental Tables 1 and 2). The multiple imputation was based on the correlation between each variable with missing values with other participant characteristics as previously described (22). GEE analyses were then separately performed in each of the five datasets to obtain the desired effect sizes and SEE. OR were pooled by taking the mean of the effect sizes of the five imputed datasets. The pooled SEE was then calculated by using Rubin's rules (23). The pooled regression results of the five imputed datasets were reported in this paper as OR and 95% CI or as mean $\pm$ SD values. $P < 0.05$ was considered significant. Statistical analyses were carried out by using SPSS 17.0 for Windows.

Results

Overall, 69% of the mothers used folic acid during early pregnancy. A history of asthma or other allergies was present in about one-half of the parents and was more often present in mothers who used folic acid during pregnancy (Table 1). The folate concentration during pregnancy was 17.6 ± 9.1 nmol/L

TABLE 1 Mother and child characteristics according to folic acid supplementation during pregnancy ($n = 8742$)

	Folic acid exposure during pregnancy		<i>P</i> -value
	No (<i>n</i> = 2738)	Yes (<i>n</i> = 6004)	
Children's characteristics			
Gender, % <i>male</i>	52	50	0.05
Birth weight, <i>g</i>	3338 ± 565	3457 ± 556	<0.01
Gestational age, <i>wk</i>	39.7 ± 2.0	39.9 ± 1.8	<0.01
Any daycare in first 24 mo of life, %	92	91	0.37
Parental atopic constitution, %	46	53	<0.01
Breastfeeding, %			0.09
None	17	12	
Partial until 4 mo	57	60	
Exclusive until 4 mo	26	28	
Mother's characteristics			
Western ethnicity, %	27	70	<0.01
Maternal age, <i>y</i>	28 ± 6	31 ± 5	<0.01
Educational level, %			<0.01
Low	27	6	
Mid	55	43	
High	17	51	
Nullipara, %	44	65	<0.01
BMI, <i>kg/m</i> ²	26 ± 5	24 ± 4	<0.01
Plasma folate, <i>nmol/L</i>	9.6 ± 4.3	20.8 ± 8.6	<0.01
Serum vitamin B-12, <i>pmol/L</i>	177 ± 91	195 ± 96	<0.01
<i>MTHFR</i> C677T polymorphism, ¹ %			0.96
CC	45	46	
CT	44	43	
TT	11	10	
Maternal smoking during pregnancy, %	34	26	<0.01
Maternal alcohol use during pregnancy, %	27	45	<0.01

¹ Restricted to Western mothers only ($n = 4955$).

and that of vitamin B-12 was 188 ± 93 pmol/L. Mothers who took folic acid during pregnancy had significantly higher concentrations of plasma folate and serum vitamin B-12 and had a lower BMI relative to those without folic acid supplementation during pregnancy (Table 1). Folic acid use during pregnancy was more often seen in mothers who were older, had a high educational background, were of Western ethnicity, and who had no multiple parities (Table 1). Children of mothers who used folic acid during pregnancy had a slightly higher birthweight than children of mothers who did not use folic acid supplementation (Table 1). Folate levels were lower in Western mothers who had the TT genotype (18.1 ± 8.2 nmol/L) than in mothers with the CC (19.6 ± 8.8 ; $P = 0.01$) or CT (19.2 ± 8.8 ; $P = 0.02$) genotype.

The highest prevalence of wheezing and shortness of breath during childhood was at the age of 12 mo (39 and 34%, respectively) and this decreased to a prevalence of 22 and 18% at 24 mo ($P < 0.01$). Wheezing prevalence remained stable at 15% at the ages of 36 and 48 mo ($P = 0.91$) as did the prevalence of shortness of breath (12–13% at 36 and 48 mo) ($P = 0.21$). The prevalence of atopic dermatitis remained relatively stable during the first 24 mo of life (28 and 30%) ($P = 0.27$), slightly decreased to 20% at the age of 36 mo ($P = 0.01$), and remained stable afterwards at 25% ($P = 0.18$). Both periconceptional folic acid supplementation and folic acid supplementation within the first 10 wk of conception were not significantly associated with an increased prevalence of wheezing, shortness of breath, or atopic dermatitis during childhood after adjustment for confounders (Table 2). No overall association was found between folate and vitamin B-12 concentration during pregnancy and symptoms of wheezing and shortness of breath in childhood (Table 3 and Table 4). However, maternal plasma folate concentrations during pregnancy of at least 16.2 nmol/L were significantly associated with an overall increased prevalence of atopic dermatitis in children up to 48 mo (Table 3). Also, maternal serum vitamin B-12 concentrations during pregnancy of at least 178 pmol/L were significantly associated with an increased prevalence of atopic dermatitis in the offspring in the first 48 mo of life (Table 4). Both associations were not explained by potential confounders (Tables 3 and 4; Supplemental Table 3).

Time-specific effects of folate and vitamin B-12 concentration on wheezing, shortness of breath, and atopic dermatitis revealed that the OR of maternal folate and vitamin B-12 concentration

on wheezing, shortness of breath, and atopic dermatitis did not differ between the different age groups (Supplemental Figs. 2–4; P -interaction > 0.10).

The association between folate and vitamin B-12 concentrations and wheezing, shortness of breath, and atopic dermatitis did not significantly differ within strata of parental atopic constitution, maternal smoking, folic acid supplementation, and *MTHFR* C677T polymorphism (P -interaction > 0.10) (data not shown).

No overall significant association was found between the *MTHFR* C677T polymorphism and the development of wheezing, shortness of breath, or atopic dermatitis in the offspring of Western mothers (Table 5), which was not different according to the age of the child (P -interaction > 0.10).

Discussion

This study demonstrated that high folate and vitamin B-12 concentrations in the first trimester of pregnancy, but not maternal *MTHFR* C677T polymorphism and folic acid supplementation, were significantly associated with the development of atopic dermatitis, but not with asthma-like symptoms in early childhood.

Results from previous studies on folate and asthma or allergic outcomes are conflicting. Haberg et al. (12) and Whitrow et al. (13) showed an increased risk of early asthma-like symptoms and asthma in the offspring of mothers who took folic acid supplements in the first and third trimesters of pregnancy, respectively. Additionally, Haberg et al. (24) confirmed that folate concentrations in the second trimester of pregnancy were associated with an increased risk of asthma in the offspring. In contrast, Martinussen et al. (25) showed no association between folic acid supplementation in the first trimester of pregnancy and asthma in the offspring. Results from the KOALA birth cohort study (26), however, showed no association with folic acid supplementation during pregnancy, but high intracellular folate levels in late pregnancy tended to be associated with a lower risk of asthma in the offspring at the age of 6–7 y. Also, in the Avon Longitudinal Study of Parents and Children (ALSPAC) study, no evidence for an association between folic acid supplementation and asthma development was found in the second and third trimesters of pregnancy (27). Recently, the Prevention and Incidence of Asthma

TABLE 2 Association between folic acid supplementation during pregnancy and wheezing, shortness of breath, and atopic dermatitis in the offspring ($n = 8742$)

Symptoms	Maternal folic acid use		
	No use (Reference)	Start within 10 wk of conception OR (95%CI)	Periconceptional start OR (95%CI)
Wheezing			
Crude ¹	1.00	0.94 (0.81–1.07)	0.83 (0.66–1.05)
Multivariate ²	1.00	1.02 (0.90–1.16)	0.99 (0.89–1.09)
Shortness of breath			
Crude ¹	1.00	1.16 (0.86–1.55)	1.02 (0.84–1.24)
Multivariate ²	1.00	1.16 (0.85–1.57)	1.04 (0.84–1.29)
Atopic dermatitis			
Crude ¹	1.00	1.16 (0.91–1.48)	1.16 (0.98–1.37)
Multivariate ²	1.00	1.15 (0.90–1.47)	1.17 (0.97–1.40)

¹ Overall crude OR (95% CI) adjusted for time/age.

² Adjusted OR (95% CI) controlled for time, maternal ethnicity, parental atopic constitution, parity, maternal BMI, maternal age, breastfeeding duration, daycare attendance, maternal educational level, maternal smoking and alcohol consumption, and fetal gender and birthweight SD score derived from generalized estimation equations.

TABLE 3 Associations between maternal folate concentrations during pregnancy and wheezing, shortness of breath, and atopic dermatitis in the offspring ($n = 8742$)

Symptoms	Maternal folate concentration			
	≤10.30 nmol/L (Reference)	10.31–16.20 nmol/L OR (95%CI)	16.21–23.20 nmol/L OR (95%CI)	≥23.21 nmol/L OR (95%CI)
Wheezing				
Crude ¹	1.00	0.93 (0.82–1.06)	0.88 (0.70–1.09)	0.86 (0.67–1.11)
Multivariate ²	1.00	1.00 (0.90–1.11)	0.98 (0.85–1.12)	1.02 (0.89–1.18)
Shortness of breath				
Crude ¹	1.00	1.01 (0.84–1.22)	0.97 (0.76–1.23)	0.96 (0.74–1.23)
Multivariate ²	1.00	1.03 (0.87–1.22)	0.98 (0.78–1.23)	0.98 (0.79–1.22)
Atopic dermatitis				
Crude ¹	1.00	1.13 (1.00–1.26) ^a	1.20 (1.05–1.36) ^a	1.21 (1.07–1.38) ^a
Multivariate ²	1.00	1.10 (0.98–1.25)	1.16 (1.03–1.32) ^a	1.18 (1.05–1.33) ^a

¹ Overall crude OR (95% CI) adjusted for time/age.² Adjusted OR (95% CI) controlled for time/age maternal ethnicity, parental atopic constitution, parity, maternal BMI, maternal age, breastfeeding duration, daycare attendance, maternal educational level, maternal smoking and alcohol consumption, and fetal gender and birth weight SD score derived from generalized estimating equations.^a $P < 0.05$ for comparison with reference category.

and Mite Allergy (PIAMA) study demonstrated a weak association between folic acid supplementation in the third trimester and asthma-like symptoms at the age of 1 y but not at other ages (28). To our knowledge, only a few studies reported data on (atopic) dermatitis. A recent prospective cohort study reported no association between maternal folate consumption in the second trimester, assessed by food questionnaire, and asthma-like symptoms and atopic dermatitis at the age of 16–24 mo (29). Also, the PIAMA study showed no association with eczema (28). Magdalijs et al. (26) showed an increased risk of eczema in the offspring of mothers using folic acid supplements for the entire pregnancy, but this association remained nonsignificant after adjustment of confounders. Although differences in measurement methods and the timing and amount of folate exposure make it difficult to compare our results with previous studies, assessment of folic acid supplementation by questionnaire can be prone to information bias and adherence bias, ensuring that association studies on this topic are challenging and vary in results. Also, we found no significant association with folic acid supplementation but we did

find an association with folate and vitamin B-12 concentrations, advocating that assessing maternal folate and vitamin B-12 concentrations during pregnancy in future studies can be a promising approach to shed light on the associations previously observed.

Pregnancy is a critical period for the programming of the fetal immune system insofar as emerging evidence shows that regulation of neonatal Th1/Th2 balance is under epigenetic control (10). Epigenetics refers to the heritable modifications of phenotypes and gene expression that are not coded in the DNA sequences (10). Folate together with vitamin B-12 plays a crucial role in providing methyl donors for methylation of DNA (5). Methylation of DNA substitutes can lead to the activation or inactivation of certain genes that control cell growth or proliferation and determine when and where a gene is expressed, e.g., during neonatal immune development (10,11). In mice it was demonstrated that in utero exposure to methyl donors such as folic acid and vitamin B-12 is associated with altered lymphocyte development. This skewed the fetal immune system

TABLE 4 Association between maternal vitamin B-12 concentrations during pregnancy and wheezing, shortness of breath, and atopic dermatitis in the offspring ($n = 8742$)

Symptoms	Maternal vitamin B-12 concentration			
	≤130.00 pmol/L (Reference)	130.01–178.00 pmol/L OR (95%CI)	178.01–227.00 pmol/L OR (95%CI)	≥227.01 pmol/L OR (95%CI)
Wheezing				
Crude ¹	1.00	0.93 (0.82–1.06)	0.94 (0.85–1.06)	0.91 (0.81–1.03)
Multivariate ²	1.00	0.95 (0.84–1.07)	0.96 (0.87–1.06)	0.95 (0.85–1.08)
Shortness of breath				
Crude ¹	1.00	1.01 (0.89–1.14)	1.05 (0.92–1.21)	1.06 (0.90–1.24)
Multivariate ²	1.00	1.01 (0.89–1.14)	1.05 (0.91–1.22)	1.07 (0.98–1.27)
Atopic dermatitis				
Crude ¹	1.00	1.16 (1.01–1.34) ^a	1.17 (1.01–1.35) ^a	1.34 (1.07–1.67) ^a
Multivariate ²	1.00	1.14 (1.00–1.31)	1.15 (1.00–1.32) ^a	1.30 (1.06–1.60) ^a

¹ Overall crude OR (95% CI) adjusted for time/age.² Overall adjusted OR (95% CI) controlled for time, maternal ethnicity, parental atopic constitution, parity, maternal BMI, maternal age, breastfeeding duration, daycare attendance, maternal educational level, maternal smoking and alcohol consumption, and fetal gender and birthweight SD score derived from generalized estimating equations.^a $P < 0.05$ for comparison with reference category.

TABLE 5 Associations between maternal *MTHFR* C677T polymorphism and wheezing, shortness of breath, and atopic dermatitis in the offspring (*n* = 4955)

Symptoms	<i>MTHFR</i> C677T polymorphism		
	CC (Reference)	CT OR ¹ (95%CI)	TT OR ¹ (95%CI)
Wheezing	1.00	1.03 (0.90–1.18)	0.96 (0.75–1.23)
Shortness of breath	1.00	0.99 (0.82–1.20)	0.95 (0.77–1.16)
Atopic dermatitis	1.00	0.94 (0.82–1.07)	1.00 (0.82–1.21)

¹ Overall crude OR (95% CI) adjusted for time/age.

toward a Th2 profile, which favored the development of allergic disease (9). Also, neonates at high risk for allergy are born with altered DNA methylation in their dendritic cells that process antigens and may change the Th1/Th2 balance (30). Other studies have found that during early immune development, increased DNA methylation plays a role when naive T-cells differentiate into Th2 cells (31,32). It has been hypothesized that a decreased methylation of DNA in particular may support the switch of T-cells into a Th1 phenotype (33). When assuming that folate and vitamin B-12 enhance gene-specific methylation of DNA, the latter hypothesis suggests that increasing folate concentrations during pregnancy might lead to adverse effects in utero besides the protective effect on NTD (2,3). Nevertheless, Magedelijns et al. (26) showed that high intracellular folic acid concentrations in late pregnancy in particular was associated with a lower risk of asthma, whereas Haberg et al. (24) showed that high folate concentrations in the second trimester of pregnancy are associated with a higher risk of asthma in the offspring. Thus, the timing of folate exposure during pregnancy that increases the risk of allergic disease might be important and needs further elucidation. Other studies with respect to postnatal folate intake during childhood or adulthood show a protective effect of folate on allergic disease instead of an increased risk as seen in studies on folate intake during pregnancy (34,35). Increased folate levels may be associated with a healthy lifestyle that may protect against allergic disease; thus, the inverse association between folate and allergic disease can be easily influenced by residual confounding (36). Nevertheless, the difference in effect of pre- and postnatal folate intake on allergic disease could support the hypothesis that infants are especially susceptible to an adverse immune effect of methyl donors in utero. Unfortunately, we did not have data on the B vitamin status of the children to ascertain whether effects of B vitamins in our study were restricted to exposure during pregnancy.

A strength of this study is the prospective study design and the fact that the population was not selected according to their allergy risk. In the United States, food fortification with folic acid is mandatory (37), whereas in The Netherlands, food is fortified with folic acid only on a voluntary basis. This makes it easier to assess the effect of folate during pregnancy in The Netherlands, because certain heterogeneity of the exposure is necessary (38). Also, we used actual concentrations of folate and vitamin B-12 during pregnancy, which is less susceptible to information and attrition bias than assessment of folic acid supplementation during pregnancy, which may explain the fact that we found a significant association only with folate and vitamin B-12 concentration and not with folic acid supplementation during pregnancy. Another strength of this study was that we explored several potential interactions, including with *MTHFR* C677T polymorphism and family history of atopic disorders. Limitations of this study include the relatively short

follow-up, which precludes an asthma diagnosis. Earlier studies on maternal folate and allergy focused on asthma as the outcome (12,13,29). We did not have data on asthma diagnosis, and wheezing and shortness of breath during childhood are not optimal predictors of childhood asthma (39). Hence, our results do not allow for conclusions regarding folate and childhood asthma. Nevertheless, because ~50% of the children with atopic dermatitis develop asthma later in life (40), follow-up studies on maternal folate and vitamin B-12 and asthma in the offspring is worthwhile. This epidemiological study does not permit conclusions on causality regarding maternal folate, vitamin B-12, and atopic dermatitis in childhood. In addition, residual confounding is a common phenomenon in epidemiological studies that may have influenced our results. Because intake of folate may also be associated with a healthy lifestyle, health consciousness might be a residual confounder in our study. If health-conscious mothers are more alert to health problems in their child, this residual confounding may have lead to overestimation of the association between folate and atopic dermatitis. Most data were obtained by parental-derived questionnaires and no additional information from medical records or physical examinations was available. Therefore, some children may be misclassified concerning the outcome of atopic dermatitis or wheezing or shortness of breath. However, only if this misclassification was also related to maternal folate or vitamin B-12 status would this misclassification have influenced our results.

We used principles of Mendelian randomization by using the *MTHFR* C677T polymorphism as a genetic determinant of folate status. Although this has been considered to provide the highest epidemiological evidence because it assumes that genes are not confounded by other factors, we did not find any evidence that the maternal *MTHFR* C677T polymorphism had a role in the development of wheezing, shortness of breath, and atopic dermatitis. These results are in accordance with Thuesen et al. (41) and the ALSPAC study (27). People homozygous for the minor T allele have reduced folate concentrations (42). Because we found an association between high folate concentration and atopic dermatitis, we expected to at least find an inverse association between TT genotype and atopic dermatitis. However, the assumptions of Mendelian randomization when the *MTHFR* C677T polymorphism is used has recently been challenged (43). Also, the deficiency state associated with the TT genotype might influence dietary behavior of these mothers, which may cancel out the association between the *MTHFR* C677T polymorphism and atopic dermatitis. Unfortunately, we had no data available on maternal dietary intake of folate to further elucidate the latter suggestion. In addition, the difference in folate concentrations between mothers with the TT genotypes relative to the CC genotype was ~2 nmol/L and these levels were still in the third tertile of folate concentrations of the study group (16.2–23.2 nmol/L).

In the majority of the longitudinal cohort studies, missing data may lead to decreased external validity of the study, because attrition frequently leads to a population with fewer women with low education and an unhealthy lifestyle. Indeed, we found that mothers who had no data on the child's health had lower folate and vitamin B-12 concentrations during pregnancy. To reduce attrition bias, we performed the final analyses after a multiple imputation procedure, which is considered to be a very appropriate method to deal with missing data, because it requires the fewest assumptions and reduces potential bias when missing data are not completely at random (22). As a result of this procedure, the 95% CI in our

study results reflected the uncertainty associated with missing data.

We had data only on folate concentrations measured in plasma, which fluctuate with recent changes in folate intake or temporary alteration in folate metabolism, whereas folate levels measured in RBC provide a more accurate indication of folate status in the preceding 3 mo (44). Therefore, conclusions on the relationship between maternal folate status very early in pregnancy and allergic outcomes should be made with caution.

In conclusion, this study showed that high maternal plasma folate and serum vitamin B-12 concentrations are associated with the development of atopic dermatitis but not with wheezing and shortness of breath in childhood. Further studies are needed to confirm this association with the outcomes of IgE sensitization and asthma diagnosis at older ages as well as additional measurements on gene-specific DNA methylation. With regard to policy decisions concerning mass interventions such as mandated folic acid fortification, potential adverse effects on allergic disease in childhood should be evaluated.

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