

ORIGINAL ARTICLE

Epidemiology of Allergic Disease

Maternal folic acid use during pregnancy, methylenetetrahydrofolate reductase gene polymorphism, and child's lung function and asthma

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Summary

Background: Folic acid supplement use during pregnancy might affect childhood respiratory health, potentially mediated by methylenetetrahydrofolate reductase polymorphism *C677T* (*MTHFR-C677T*) carriership.

Objectives: We examined the associations of maternal folic acid supplement use and folate, vitamin B₁₂ and homocysteine concentrations during pregnancy with childhood lung function and asthma.

Methods: This study was embedded in a population-based prospective cohort study among 5653 children. Folic acid supplement use was assessed by questionnaires. Folate, vitamin B₁₂ and homocysteine plasma concentrations were measured in early pregnancy and at birth. At age 10 years, forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), FEV₁/FVC, forced expiratory flow between 25% and 75% (FEF₂₅₋₇₅), at 75% of FVC (FEF₇₅), and asthma were examined.

Results: Maternal folic acid supplement use during pregnancy was associated with higher childhood FEV₁ and FVC and with a lower FEV₁/FVC, compared with no folic acid supplement use. Among mothers carrying *MTHFR-C677T* variants, preconceptional start of folic acid supplement use was associated with lower FEV₁/FVC (−0.17 [−0.32, −0.02]) and FEF₂₅₋₇₅ (−0.24 [−0.40, −0.07]). Among children carrying *MTHFR-C677T* wild-type, a higher vitamin B₁₂ level at birth was associated with a lower FEV₁ (−0.07 [−0.12, −0.01]) and FVC (−0.09 [−0.15, −0.04]). Folate and homocysteine concentrations were not consistently associated with lower childhood lung function or asthma.

Conclusions: Preconceptional start of maternal folic acid supplement use and higher vitamin B₁₂ concentrations at birth might adversely affect childhood lung function depending on *MTHFR-C677T* carriership. The clinical implications need to be evaluated.

KEYWORDS

asthma, epidemiology, genetics, pediatrics

1 | INTRODUCTION

Folate is vital for fetal development. Folic acid supplement use during pregnancy is recommended to prevent neural tube defects,¹ but high dosages have been associated with increased risks of asthma and allergic diseases in animal studies.² Human studies that assessed associations of maternal folic acid supplement use or related folate blood concentrations during pregnancy with childhood lung function and asthma show conflicting results.^{3–12} This could be due to different methods used to assess folic acid supplement use, including food frequency questionnaires, diet records and self-administered questionnaires, and differences in techniques and ages at which folate concentrations were measured. Besides folic acid use and related folate blood concentrations, vitamin B₁₂ and homocysteine are involved in the one-carbon metabolism, which is important for DNA-methylation.¹³ The one-carbon metabolism is influenced by polymorphisms in the methylenetetrahydrofolate reductase gene (*MTHFR*).^{14,15} The variant C677T is known to affect the activity of the *MTHFR* enzyme, leading to lower circulating folate and higher homocysteine concentrations.¹⁶ We previously observed that higher circulating maternal folate concentrations during pregnancy and not at birth were associated with increased risk of atopic dermatitis,¹⁰ but not with wheezing, higher respiratory resistance or fractional exhaled nitric oxide.⁴ The modifying effects of maternal or child's *MTHFR*-C677T variants on the associations of maternal folic acid supplement use during pregnancy with lung function and asthma are less clear.^{4,9,10} Furthermore, only a limited number of studies assessed the associations of folate, vitamin B12 and homocysteine blood concentrations during pregnancy with lung function and asthma.

Therefore, we aimed to assess in a population-based prospective cohort study the associations of folic acid supplement use and folate, vitamin B₁₂ and homocysteine concentrations in early pregnancy and at birth with lung function and asthma at age 10 years. Second, we assessed whether these associations were modified by *MTHFR*-C677T polymorphisms.¹⁷

2 | METHODS

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards situated in Rotterdam, the Netherlands.¹⁸ This study was approved by the Medical Ethical Committee of Erasmus MC, University Medical Center Rotterdam, the Netherlands (MEC_2012-165 NL40020.078.12). Written informed consent was obtained from parents or legal guardians. Twins, children with missing data on maternal folic acid supplement use and folate, vitamin B₁₂ and homocysteine concentrations in early pregnancy and at birth (*n* = 186), and with missing data on lung function and current asthma at age 10 years (*n* = 1554) were excluded, resulting in a total of 5653 children and their mothers for analyses (Fig. S1).

2.1 | Maternal folic acid supplement use

In the Netherlands, 400–500 µg folic acid solely or within multivitamin supplements are recommended during pregnancy, and mandatory food fortification with folic acid is not present. Mothers reported their folic acid or multivitamin use and when they started these supplements by questionnaires before 18 weeks of gestation (median 13.2 weeks; 90%-range 10.5–17.2 weeks). We categorized folic acid supplement use into “no use” (*n* = 855 [18.9%]) and “use” (*n* = 3670 [81.3%]), and further classified use of folic acid supplements into duration of use: (i) start ≥10 weeks of pregnancy (*n* = 575 [15.7%]), (ii) start <10 weeks of pregnancy (*n* = 1265 [34.5%]) and (iii) start preconceptional (*n* = 1830 [49.9%]). Self-reported folic acid use was validated by serum folate and homocysteine concentrations before 12 weeks of gestation in a small random subsample of this study (*n* = 276), which provided sensitivity and specificity scores of respectively 97% and 56%.¹⁹

2.2 | Folate, homocysteine and vitamin B₁₂ concentrations

Non-fasting maternal blood samples in early pregnancy (median 13.4 weeks of gestation, 95%-range 9.8–17.5) and cord blood samples were collected at birth (median 40.1 weeks of gestation, 95%-range 35.8–42.3). Folate, vitamin B₁₂ and homocysteine concentrations were analysed using an immunoelectrochemoluminescence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands).²⁰ The between-run coefficient of variation ranged from 1.5% to 8.9%. Detailed information is provided in the Appendix S1.

2.3 | Childhood lung function and asthma

Lung function measures at a median age of 9.7 years (range 8.5–12.0 years) comprised (forced expiratory volume in 1 second), FEV₁/FVC (forced vital capacity) and FEF₇₅ (Forced Expiratory Flow at 75% of FVC),²¹ which were converted into sex-, age-, height- and ethnicity-adjusted z-scores.²² Quality criteria required 3 reproducible curves and no use of inhalant bronchodilators or corticosteroids within 48 hours before spirometry, which was met for 4583 (87%) individuals. Additionally, 521 children were included with individual spirometry curves with >5% deviation, but with at least one blow according to ATS/ERS (American Thoracic Society and European Respiratory Society) criteria with adequate reach and duration of plateau. We observed no difference in lung function values between the group children with and without stringent criteria and no difference in size or direction of the effect estimates in our analyses when we included or excluded these children. Ever physician-diagnosed asthma, wheezing and use of bronchodilators or inhalant corticosteroids were reported by a parental questionnaire at age 10 years. Current asthma (no; yes) was defined as ever physician-diagnosed asthma, with either wheezing or the use of inhalant medication in the past 12 months.

2.4 | *MTHFR*-c677t

Maternal DNA was extracted from white blood cells obtained in early pregnancy. Genotyping of *MTHFR*-C677T was performed using TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA) and Abgene QPCR ROX mix (Abgene, Hamburg, Germany).¹⁸ Child's DNA was extracted from white cells in cord blood. Samples were genotyped using Illumina Infinium II HumanHap610-660 Quad Arrays following standard manufacturer's protocols. Genotype data were extracted from an imputed genome-wide association scan (1000G phase Iv3).¹⁸ The imputation quality of *MTHFR*-C677T was high ($r^2 = .99$).

2.5 | Covariates

We collected information about maternal age, parity (nulli-; multiparous), history of asthma, eczema or allergy (no; yes) and educational level (low: secondary school or lower; and high: higher than secondary school as highest finished education) from maternal questionnaires at enrollment. Maternal weight and height were measured at enrollment. We obtained information on maternal smoking (no; first trimester only; continued during pregnancy) and alcohol use (no; first trimester only, continued during pregnancy) by multiple questionnaires during pregnancy. Child's sex, gestational age at birth and birthweight were obtained from midwives and hospital records. The country of birth of parents was used to define the child's ethnicity.²² We measured inhalant allergic sensitization by skin prick test using the "scanned area method",²³ for which detailed information is provided in the Appendix S1.

2.6 | Statistical analyses

We used linear and logistic regression models to examine the associations of folic acid supplement use and folate, vitamin B₁₂ and homocysteine blood concentrations in early pregnancy and at birth with lung function and asthma. We created standard deviations scores (SDS) for folate, vitamin B₁₂ and homocysteine to enable comparison between effect estimates. For clinical interpretation, we categorized folate, vitamin B₁₂ and homocysteine concentrations into 3 groups: "low" (SDS < -1.00), "normal" (-1.00 ≤ SDS ≤ 1.00) and "high" (SDS > 1.00). These groups reflect the 16th and 84th percentile, respectively. The "normal" group was used as reference category. The modifying effects of inhalant allergic sensitization and *MTHFR*-C677T polymorphisms were tested by adding them as product terms with the exposures in the models. Models with *P*-values for interaction < .05 were stratified. The most common *MTHFR* genotype [CC] was used as reference genotype (wild-type). Because of low prevalences, the variant genotypes [CT] and [TT] were combined and considered as "variant" genotype. All models were adjusted for covariates based on literature, if the covariate was associated with the exposure and outcome, or if it changed the effect estimates of univariate associations with ≥ 10%. The models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational level, smoking or

alcohol use during pregnancy, child's gestational age at birth, birthweight and ethnicity. For all covariates, the percentage of missing values was < 20% (Table S1). Missing data on covariates were imputed using the Markov Chain Monte Carlo method to select the most likely value for a missing response. Ten imputed data sets were created and analysed together. No major differences in the magnitude or direction of the effect estimates were observed between imputed data and complete cases only. To further strengthen observed associations and to minimize reporting false-positive findings, we additionally performed canonical correlations analyses,²⁴ of which detailed information is provided in the Appendix S1. All measures of association are presented with their 95% Confidence Interval (95% CI). Statistical analyses were performed using SPSS 21.0 (IBM, Chicago, IL, USA).

3 | RESULTS

3.1 | Participant characteristics

An overview of the study population is given in Table 1. Prevalence of *MTHFR*-C677T variant genotype in mothers was 49.1%, and in children 50.6%. Mean (SD) FEV₁ was 2.02 (0.98) L/s, for FVC 2.34 (0.36) L, for FEV₁/FVC 0.87 (0.06), for FEF₂₅₋₇₅ (Forced Expiratory Flow between 25% and 75% of FVC) 2.71 (0.63) L/s and for FEF₇₅ 1.14 (0.34) L/s. Current asthma was reported for 269 children (5.7%). We observed differences in socio-economic characteristics between mothers who the different groups of folic acid supplement use during pregnancy. Children lost to follow-up had younger, lower educated mothers who smoked more and used less alcohol and were more often of non-European origin, compared to children included (Table S1).

3.2 | Folic acid supplement use and respiratory outcomes

Maternal folic acid supplement use during pregnancy was associated with higher FEV₁ and FVC (z-score difference [95% CI]: 0.14 [0.05, 0.22] and 0.17 [0.09, 0.25], respectively), but not with FEV₁/FVC, FEF₂₅₋₇₅, FEF₇₅ or current asthma, compared to mothers who did not use folic acid supplements (Table 2). Preconceptional start of folic acid supplement use was associated with a lower FEV₁/FVC (z-score difference [95% CI]: -0.11 [-0.21, -0.02]). A shorter duration of folic acid supplement use was associated with a higher FEF₂₅₋₇₅ (*P*-value for trend .04). When stratified for maternal *MTHFR*-C677T, folic acid supplement use was associated with a high FEV₁, FVC and FEF₂₅₋₇₅, compared with no folic acid supplement use, among mothers carrying wild-type (*P*-values for interaction: < .01, < .01 and .05, respectively) (Figure 1, Table S3). Preconceptional start of folic acid supplement use was associated with lower FEV₁/FVC and FEF₂₅₋₇₅ among mothers carrying variant alleles (z-score difference [95% CI]: -0.17 [-0.32, -0.02] and -0.24 [-0.40, -0.07], respectively). Effect estimates of preconceptional folic acid supplement use and higher FVC and lower FEV₁/FVC and FEF₂₅₋₇₅ were similar in size and direction among *MTHFR*-C677T genotypes in children (*P*-values for interaction < .05) (Table S4).

TABLE 1 Characteristics of children and their mothers

	Total group n = 5653	No use n = 855	Preconceptionally n = 575	First 10 wk n = 1265	Unclear/after 10 wk n = 1830	P-value
Maternal characteristics						
Age (y)	31.0 (4.9)	29.1 (5.8)	32.1 (3.9)	30.6 (4.7)	30.1 (5.2)	<.01
Body mass index at intake (kg/m ²) ^a	23.9 (18.9-35.4)	24.5 (18.7-38.6)	23.4 (18.9-35.1)	23.5 (18.7-34.6)	23.6 (18.5-35.2)	<.01
Nullipara (%)	57.0 (3215)	44.0	62.9	63.2	61.3	<.01
History of asthma or atopy, yes (%)	38.7 (2190)	33.5	39.9	41.5	44.4	.01
Higher education (%)	49.5 (2793)	22.6	64.0	53.4	41.3	<.01
Smoking during pregnancy (%)	24.1 (1363)	26.2	15.0	29.9	28.7	<.01
Alcohol consumption during pregnancy	56.0 (3200)	38.3	61.9	63.8	58.5	<.01
Folic acid supplement use (%) ^b		–	–	–	–	–
No use	15.1 (855)					
Start after first 10 wk of pregnancy	10.2 (575)					
Start within first 10 wk of pregnancy	22.4 (1265)					
Preconceptional start	32.4 (1830)					
Folate in early pregnancy (nmol l ⁻¹) ^{a,b}	17.5 (6.0-37.9)	8.7 (4.9-20.7)	23.0 (8.5-39.8)	17.7 (7.2-36.9)	17.3 (6.3-39.5)	<.01
Vitamin B ₁₂ in early pregnancy (pmol l ⁻¹) ^{a,b}	172 (77-406)	162 (66-399)	199 (82-421)	177 (77-428)	164 (78-404)	<.01
Homocysteine in early pregnancy (μmol l ⁻¹) ^{a,b}	9.1 (5.2-16.6)	7.4 (4.8-14.3)	6.7 (4.6-10.5)	6.9 (4.7-11.1)	7.1 (4.7-11.2)	<.01
MTHFR-C677T variant, [CT or TT] (%) ^b	49.1 (2127)	44.7	52.2	49.4	45.1	<.01
Child's characteristics						
Female sex (%)	50.6 (2860)	48.8	52.5	50.6	49.9	.10
Gestational age at birth (wk) ^a	40.1 (35.8-42.3)	40.0 (35.9-42.3)	40.3 (35.6-42.4)	40.1 (35.9, 42.4)	40.1 (36.0-42.3)	.01
Birth weight (g)	3440 (554)	3356 (553)	3510 (539)	3452 (551)	3401 (529)	<.01
European ethnicity (%)	68.7 (3674)	35.5	84.3	73.1	61.1	<.01
Folate at birth (nmol l ⁻¹) ^{a,b}	20.9 (10.7-38.4)	18.3 (9.8-35.0)	23.0 (11.4-39.7)	20.4 (10.6-38.4)	20.7 (10.3-38.2)	<.01
Vitamin B ₁₂ at birth (nmol l ⁻¹) ^{a,b}	303.0 (121-902)	294 (160-883)	306 (135-920)	304 (120-912)	310 (124-936)	<.01
Homocysteine at birth (μmol l ⁻¹) ^{a,b}	6.9 (4.6-12.0)	9.5 (5.5-17.5)	8.8 (5.1-16.1)	8.9 (5.1-16.0)	8.9 (5.0-15.9)	<.01
MTHFR-C677T variant, [CT or TT] (%) ^b	50.6 (1850)	43.6	54.0	51.1	49.1	<.01
Age at spirometry and asthma-assessment	9.8 (0.3)	9.9 (0.4)	9.8 (0.3)	9.8 (0.3)	9.8 (0.3)	<.01
FEV ₁ (L) ^b	2.02 (0.30)	1.97 (0.31)	2.02 (0.30)	2.02 (0.30)	2.00 (0.30)	.04
FVC (L) ^b	2.34 (0.36)	2.27 (0.38)	2.35 (0.35)	2.34 (0.36)	2.31 (0.37)	.53
FEV ₁ /FVC ^b	0.87 (0.06)	0.87 (0.06)	0.86 (0.06)	0.87 (0.06)	0.87 (0.06)	<.01
FEF ₂₅₋₇₅ (L/s) ^b	2.71 (0.63)	2.70 (0.68)	2.66 (0.63)	2.69 (0.65)	2.73 (0.63)	<.01
FEF ₇₅ (L/s) ^b	1.14 (0.34)	1.15 (0.37)	1.14 (0.34)	1.15 (0.34)	1.14 (0.35)	<.01
Current asthma, yes (%) ^b	5.7 (269)	9.2	7.4	9.7	7.4	.19

Values are means (SD), valid percentages (absolute numbers) or ^amedians (95% range). Total group: all included participants. FEV₁, forced expiratory volume in the 1st second; FEV₁, forced expiratory volume in the 1st second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of FVC; FEF₇₅, forced expiratory flow at 75% of exhalation.

^bNot imputed. P-values for differences between different strata of folic acid supplement use were calculated using ANOVA for normal distributed data, Kruskal-Wallis for non-normal distributed data and Pearson chi square to compare distributions within populations.

3.3 | Folate, vitamin B₁₂ and homocysteine concentrations and respiratory outcomes

In early pregnancy, higher maternal folate concentrations were associated with higher FEF₇₅ among mothers carrying wild-type only (z-

score [95% CI]: 0.06 [0.00, 0.11], per 1 SDS increase), but not with other lung function measures (Table 3). We observed a borderline significant association of higher maternal folate concentrations with childhood current asthma among mothers carrying wild-type only (OR [odds ratio] [95% CI]: 0.78 [0.61, 1.00]). Vitamin B₁₂

TABLE 2 Associations of folic acid supplementation use during pregnancy with childhood lung function and asthma

	FEV ₁ z-score (95% CI) n = 5099	FVC z-score (95% CI) n = 5104	FEV ₁ /FVC z-score (95% CI) n = 5099	FEF ₂₅₋₇₅ z-score (95% CI) n = 5102	FEF ₇₅ z-score (95% CI) n = 5103	Current asthma odds ratio (95% CI) n = 269/4707
Folic acid supplement use						
Never (n = 855)	Reference n = 794	Reference n = 795	Reference n = 794	Reference n = 794	Reference n = 795	Reference n = 43/597
Any use of folic acid (n = 3670)	0.14 (0.05, 0.22)** n = 3305	0.17 (0.09, 0.25)** n = 3309	−0.07 (−0.15, 0.01) n = 3305	0.05 (−0.05, 0.14) n = 3308	0.02 (−0.06, 0.10) n = 3308	0.99 (0.67, 1.46) n = 180/3213
Start > 10 wk (n = 575)	0.13 (0.02, 0.24)* n = 512	0.14 (0.04, 0.25)** n = 512	−0.05 (−0.16, 0.06) n = 512	0.12 (0.00, 0.25)* n = 512	0.04 (−0.07, 0.14) n = 512	0.85 (0.60, 1.43) n = 26/473
Start 0-10 wk (n = 1265)	0.17 (0.07, 0.26)** n = 1145	0.18 (0.09, 0.27)** n = 1148	−0.04 (−0.13, 0.05) n = 1148	0.05 (−0.05, 0.15) n = 1148	0.06 (−0.03, 0.15) n = 1148	1.33 (0.74, 1.73) n = 74/1105
Start preconceptional (n = 1830)	0.11 (0.02, 0.21)* n = 1648	0.18 (0.10, 0.27)** n = 1649	−0.11 (−0.21, −0.02)** n = 1648	0.02 (−0.09, 0.12) n = 1649	−0.02 (−0.11, 0.06) n = 1649	0.92 (0.50, 1.44) n = 80/1635
P-value for trend	.68	.39	.09	.04	.11	.56

Values are z-score mean differences or odds ratios (95% confidence intervals) with “never folic acid supplement use during pregnancy” as reference group. “n”, number of individuals assessed (lung function) and number of cases/number of individuals assessed (current asthma). Any use of folic acid supplements was categorized into start after the first 10 wk of pregnancy, start within the first 10 wk of pregnancy and preconceptional start. P-value for trend depicts line for increased duration of folic acid supplements use. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV₁, forced expiratory volume in the 1st second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of FVC; FEF₇₅, forced expiratory flow at 75% of exhalation. Models were adjusted for maternal age and body mass index at intake, parity, history of asthma or atopy, educational level, smoking or alcohol use during pregnancy, child's gestational age at birth, birthweight and ethnicity. *P < .05, **P < .01, ***P < .01 (presented in bold).

concentrations were not associated with lung function or current asthma. Higher homocysteine concentrations were associated with higher FVC (z-score difference [95% CI]: 0.04 [0.01, 0.07] per 1 SDS increase), but not among the different maternal *MTHFR*-C677T genotype groups (P-value for interaction >.05). When categorized, folate, vitamin B₁₂ and homocysteine concentrations were not associated with lung function or current asthma (Table S5).

At birth, folate concentrations were not associated with lung function or current asthma (Table 4). When categorized, a low folate concentration at birth was associated with a higher FEV₁, FEV₁/FVC and FEF₇₅ (0.11 [0.01, 0.21], 0.14 [0.04, 0.23] and 0.10 [0.01, 0.19], respectively) (Table S6). Higher vitamin B₁₂ at birth was associated with lower FVC and higher FEV₁/FVC (z-score difference [95% CI]: −0.05 [−0.08, −0.01] and 0.04 [0.00, 0.07], respectively, per 1 SDS increase). Effect estimates were more prominent among children carrying wild-type showing that a higher vitamin B₁₂ concentration at birth was associated with a lower FEV₁ and FVC and higher FEV₁/FVC and FEF₇₅ (P-values for interaction: .15, .01, .06 and .14, respectively) (Table 4). When categorized, a high vitamin B₁₂ concentration was associated with a low FVC (−0.18 [−0.33, −0.03]) in children carrying wild-type. A low vitamin B₁₂ concentration was associated with a low FEF₂₅₋₇₅ (−0.23 [−0.40, −0.06]), compared with a normal vitamin B₁₂ concentration, only among children carrying variants (Table S6). Homocysteine concentrations at birth were not associated with lung function and current asthma. Canonical correlation analyses showed that folate, vitamin B₁₂ and homocysteine in early pregnancy and at birth explained 3.1% of the variance of the combined lung function measures (Tables S7). When stratified for *MTHFR*-C677T status, the explained variance was 5.7% (P-value .02)

among mothers carrying variants, and 5.4% (P-value .04) among children carrying wild-type. No explained variance was observed among mothers carrying wild-type and children carrying variants (P-values .71 and .35, respectively).

We did not observe any effect modification by child's sex. Furthermore, we did not observe interactions between inhalant allergic sensitization and folic acid supplement use, folate, vitamin B₁₂ or homocysteine for their association with lung function and current asthma.

4 | DISCUSSION

We observed that folic acid supplement use during pregnancy was associated with higher childhood FEV₁, FVC and FEF₂₅₋₇₅ among mothers carrying *MTHFR*-C677T wild-type and with lower FEV₁/FVC and FEF₂₅₋₇₅ among mothers carrying *MTHFR*-C677T variants, but not with childhood asthma. A higher vitamin B₁₂ concentration at birth was associated with a lower FEV₁ and FVC among children carrying *MTHFR*-C677T wild-type and no associations were observed when carrying *MTHFR*-C677T variants. Folate, vitamin B₁₂ and homocysteine concentrations in early pregnancy, or folate and homocysteine concentrations at birth, were not consistently associated with lower childhood lung function and asthma.

4.1 | Comparison with previous studies

Previous studies present conflicting epidemiologic evidence of the effects of maternal folic acid supplement use and folate

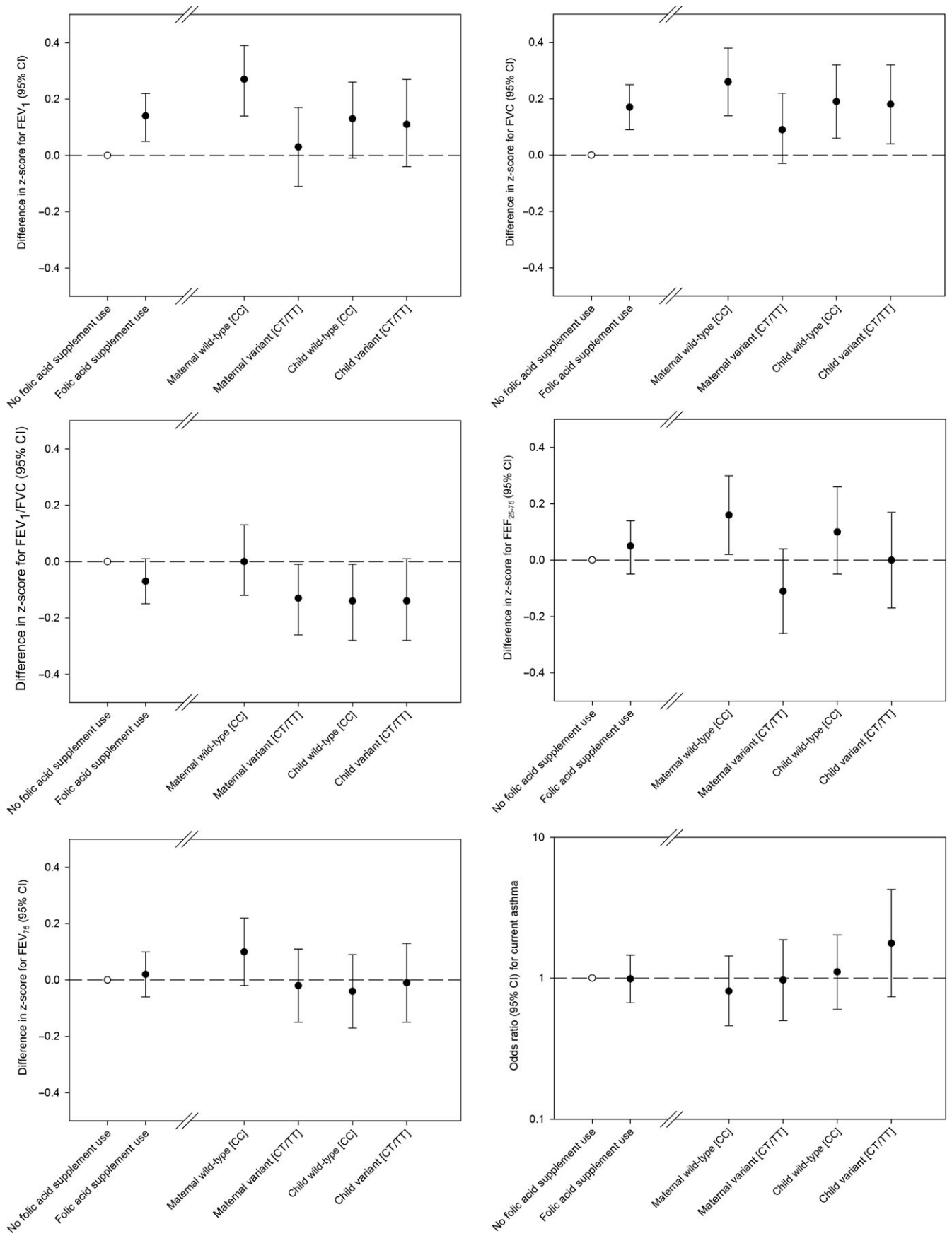


FIGURE 1 Associations of maternal folic acid supplement use during pregnancy with (A) FEV₁ (B) FVC (C) FEV₁/FVC (D) FEF₂₅₋₇₅ (E) FEF₇₅ and (F) current asthma, for the total population and stratified by maternal and child *MTHFR-C677T* genotype. Values are z-score mean differences or odds ratios (95% confidence intervals) with “never used folic acid supplements during pregnancy” as reference group. Associations for the entire population and stratification by maternal and child *MTHFR-C677T* genotype are shown. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV₁, forced expiratory volume in the 1st second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of FVC; FEF₇₅, forced expiratory flow at 75% of exhalation. Models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational level, smoking or alcohol use during pregnancy, child's gestational age at birth, birthweight and ethnicity. **P* < .05, ***P* < .01

concentrations during pregnancy on risk of childhood asthma.³⁻¹¹ Two studies among 32 077 and >100 000 children showed that folic acid supplement use or folic acid prescription during pregnancy was associated with an up to 1.3-fold increased risks of wheeze and respiratory tract infections up to 18 months of age,⁶ and of asthma at ages 4-6 years,¹¹ respectively. Similarly, in a population-based cohort study among 39 846 children, the higher quintile of maternal total folate intake was associated with an 1.2 increased relative risk of asthma at age 7 years, compared with the lowest quintile.¹² In contrast, a randomized controlled trial among 793 Nepalese children aged 8 years examined the effects of maternal micronutrient supplementation, including folic acid, and observed no differences in childhood FEV₁, FVC and FEV₁/FVC between the group with and without supplementation.²⁵ Results are difficult to generalize to western populations because the Nepalese study assessed multiple micronutrients in a population with depleted micronutrient sources. Folic acid supplement use during pregnancy was not associated with lung function, asthma or atopic outcomes among 1902 Dutch children aged 6-7 years.³ Higher intracellular folate concentrations in late pregnancy were associated with a lower risk of asthma in a dose-dependent manner. Results were limited by a small sample size with a low number of asthma cases and a young study population.^{26,27}

Studies on the associations of folate, vitamin B₁₂ and homocysteine concentrations during pregnancy with lung function and asthma are scarce and conflicting.^{3,8,10,28} Most studies were limited to an age of asthma diagnosis up to 4 years, which partly is biased by transient wheezing phenotypes. Only folate concentrations in late pregnancy seem to be consistently associated with an increased risk of wheezing and asthma in childhood.²⁶

A study among 5364 children aged 7-8 years⁹ observed no modifying effect of maternal *MTHFR-C677T* on the associations between folic acid supplement use at 18 and 32 weeks of gestation and risk of asthma and atopic disease. We also observed no associations between *MTHFR-C677T* genotypes and the risk of childhood asthma. We did observe that associations of folic acid supplement use in early pregnancy with childhood lung function were affected by maternal *MTHFR-C677T* variant carriership, and of vitamin B₁₂ concentrations at birth with FVC and FEF₂₅₋₇₅ by type of child's *MTHFR-C677T* carriership. Canonical correlation analyses taking simultaneous comparison of correlated variables into account supported the observed associations. This suggests a role of genetic predisposition affecting the associations of folic supplement use and vitamin B₁₂ blood concentrations with childhood lung function. The difference in findings with lung function and physician-diagnosed

asthma in the current study could be due to the low number of asthma cases, as an increased risk between high folic acid supplement use during pregnancy and childhood asthma was observed in large study populations.^{6,11,12}

4.2 | Interpretation of results

Folic acid supplement use in pregnancy was associated with higher childhood FEV₁ and FVC, but only among mothers carrying *MTHFR-C677T* wild-type. For these women, folic acid supplement use could be beneficial for their child's respiratory health in later life. Among mothers carrying *MTHFR-C677T* variants, we observed that folic acid supplement use was associated with lower childhood FEV₁/FVC and FEF₂₅₋₇₅. These results suggest that the folic acid metabolism during pregnancy could affect lung development in fetal life, thereby affecting the risk of respiratory diseases in later life.

Methylenetetrahydrofolate reductase gene produces an enzyme that converts dietary folic acid to folate.²⁹ Homozygous C677T [TT] individuals have ~30% of the expected *MTHFR* enzyme activity, and heterozygotes [CT] have ~65% activity, compared to wild-type [CC].¹⁶ Therefore, less conversion of folic acid occurs in *MTHFR-C677T* variant carriership. Folate, vitamin B₁₂ and homocysteine are cofactors and co-substrates for biological methylation and nucleic acid synthesis and also function as regulatory molecules. They act as mobile cofactors in several key enzymatic reactions and are essential components in the one-carbon metabolism, which is responsible for the synthesis of purines and pyrimidines, numerous amino acids and S-adenosylmethionine (SAM). SAM is a key methyl donor in DNA-methylation. High concentrations of folic acid could inhibit the formation of folate and lead to a decrease in methionine synthesis and DNA hypomethylation.³⁰⁻³² Similarly, vitamin B₁₂ deficiency results in an excessive incorporation of uracil into DNA and DNA hypomethylation.³³ Experimental studies support the role of folate in cell division, nucleotide synthesis and DNA-methylation during fetal development.²⁶ Elevated folic acid exposure during pregnancy has been linked to cellular modifications associated with asthma and allergic diseases.³⁴ In animal models, enriched maternal diet with among others folic acid and vitamin B₁₂ during pregnancy was associated with epigenetic changes and phenotypic differences in offspring, including immune functioning and insulin resistance, compared with offspring of mothers fed a low methyl donor diet.^{34,35} Human studies are scarce. We previously observed that folate concentrations in early pregnancy were associated with differential DNA-methylation of 443 CpGs linked to 320 genes at birth.³⁶ These findings suggest that

TABLE 3 Associations of folate, vitamin B₁₂ and homocysteine concentrations in early pregnancy with childhood lung function and asthma

	FEV ₁ z-score (95% CI) n = 5099	FVC z-score (95% CI) n = 5104	FEV ₁ /FVC z-score (95% CI) n = 5099	FEF ₂₅₋₇₅ z-score (95% CI) n = 5102	FEF ₇₅ z-score (95% CI) n = 5103	Current asthma odds ratio (95% CI) n = 269/4707
Folate concentration early pregnancy (SDS)						
Total population (n = 3719)	0.01 (−0.02, 0.05)	0.02 (−0.02, 0.05)	−0.01 (−0.04, 0.03)	−0.01 (−0.05, 0.03)	0.00 (−0.03, 0.04)	0.93 (0.79, 1.09)
Maternal wild-type [CC] (n = 1666)	0.04 (−0.01, 0.10)	0.02 (−0.03, 0.08)	0.04 (−0.02, 0.09)	0.01 (−0.05, 0.08)	0.06 (0.00, 0.11)*	0.78 (0.61, 1.00)
Maternal variant [CT/TT] (n = 1656)	−0.01 (−0.07, 0.04)	−0.01 (−0.06, 0.04)	−0.01 (−0.06, 0.05)	−0.05 (−0.11, 0.01)	−0.01 (−0.06, 0.04)	1.01 (0.78, 1.31)
Vitamin B ₁₂ concentration early pregnancy (SDS)						
Total population (n = 3559)	−0.00 (−0.04, 0.03)	−0.00 (−0.03, 0.03)	0.01 (−0.03, 0.05)	0.00 (−0.04, 0.04)	0.01 (−0.02, 0.04)	0.95 (0.81, 1.11)
Maternal wild-type [CC] (n = 1599)	−0.01 (−0.06, 0.05)	0.00 (−0.05, 0.05)	0.01 (−0.04, 0.06)	0.02 (−0.03, 0.08)	0.00 (−0.05, 0.05)	1.00 (0.79, 1.27)
Maternal variant [CT/TT] (n = 1578)	−0.01 (−0.06, 0.04)	0.00 (−0.05, 0.05)	−0.03 (−0.08, 0.02)	−0.01 (−0.07, 0.05)	−0.00 (−0.05, 0.05)	0.89 (0.69, 1.15)
Homocysteine concentration early pregnancy (SDS)						
Total population (n = 3683)	0.03 (−0.01, 0.06)	0.04 (0.01, 0.07)*	−0.02 (−0.06, 0.01)	−0.02 (−0.06, 0.02)	0.00 (−0.03, 0.03)	1.05 (0.90, 1.22)
Maternal wild-type [CC] (n = 1654)	0.00 (−0.06, 0.06)	0.02 (−0.04, 0.07)	−0.03 (−0.09, 0.03)	−0.01 (−0.08, 0.05)	−0.01 (−0.06, 0.04)	1.18 (0.95, 1.47)
Maternal variant [CT/TT] (n = 1635)	0.01 (−0.04, 0.06)	0.03 (−0.02, 0.08)	−0.02 (−0.07, 0.03)	−0.02 (−0.08, 0.04)	−0.01 (−0.05, 0.04)	0.93 (0.71, 1.22)

Values are z-score mean differences or odds ratios (95% confidence intervals) with “never folic acid supplement use during pregnancy” as reference group. “n”: number of individuals assessed (lung function at age 10 y) and number of cases/number of individuals assessed (current asthma at age 10 y). Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV₁, forced expiratory volume in the 1st second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of FVC; FEF₇₅, forced expiratory flow at 75% of exhalation; SDS, standard deviations scores. Models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational level, smoking or alcohol use during pregnancy, child’s gestational age at birth, birthweight and ethnicity. Models were stratified for maternal or child *MTHFR-C677T* carriership when interaction (*P*-value <.05) was present. No significant interactions were observed between folate, vitamin B₁₂ and homocysteine concentrations at birth with child *MTHFR-C677T* carriership. **P* < .05, ***P* < .01 (presented in bold).

insufficient or excess intake of methyl donors leads to altered folate, vitamin B₁₂ and homocysteine concentrations, which could affect DNA-methylation. Whether this mechanism affects lung development, lung function and risk of asthma remains to be studied.

We did not observe associations between folate, vitamin B₁₂ and homocysteine concentrations in early pregnancy, or folate and homocysteine concentrations at birth with childhood lung function and asthma. Plasma folate, vitamin B₁₂ and homocysteine levels do not reflect self-reported dietary supplementation use in early pregnancy. The concentrations of these metabolites are sensitive to dietary changes and reflect short-term balance of folate. Therefore, the folate, vitamin B₁₂ and homocysteine concentrations do not reflect concentrations throughout the entire pregnancy, which could explain the lack of associations between serum levels and childhood lung function and asthma.

4.3 | Strengths and limitations

We used a prospective population-based design with a large number of participants being studied from early life with detailed and

repeatedly measured exposures and multiple respiratory outcomes. An association of folic acid supplement use with lung function is supported by our stratified analyses on *MTHFR-C677T*. This genetic variant has an independent distribution in the population. Therefore, it is unlikely that the differences in associations between folic acid supplement use among mothers with *MTHFR-C677T* variants and lung function were biased by residual socio-economic and lifestyle-related factors. Still, some limitations do apply. The major source of selection bias in cohort studies usually arises from loss to follow-up,³⁷ which was 25.3% in our study. We observed differences in characteristics between included participants and those lost to follow-up and can therefore not fully exclude selection bias. Furthermore, we observed differences in socio-economic characteristics between mothers who the different groups of folic acid supplement use during pregnancy. We adjusted for these differences in the statistical analyses, but cannot exclude residual confounding by socio-economic factors. Plasma folate does not reflect self-reported folic acid supplementation use in early pregnancy.³⁸ Plasma folate concentrations are sensitive to dietary changes and reflect short-term balance of folate. We did not have information on the frequency

TABLE 4 Associations of folate, vitamin B₁₂ and homocysteine concentrations at birth with childhood lung function and asthma

	FEV ₁ z-score (95% CI) n = 5099	FVC z-score (95% CI) n = 5104	FEV ₁ /FVC z-score (95% CI) n = 5099	FEF ₂₅₋₇₅ z-score (95% CI) n = 5102	FEF ₇₅ z-score (95% CI) n = 5103	Current asthma odds ratio (95% CI) n = 269/ 4707
Folate concentration birth (SDS)						
Total population (n = 3095)	−0.02 (−0.06, 0.02)	−0.00 (−0.04, 0.04)	−0.03 (−0.06, 0.01)	−0.03 (−0.07, 0.02)	−0.01 (−0.05, 0.02)	1.05 (0.88, 1.24)
Child wild-type [CC] (n = 1430)	−0.03 (−0.09, 0.02)	−0.03 (−0.08, 0.03)	−0.00 (−0.06, 0.05)	−0.05 (−0.11, 0.01)	0.01 (−0.04, 0.07)	1.12 (0.98, 1.43)
Child variant [CT/TT] (n = 1464)	−0.00 (−0.06, 0.05)	0.03 (−0.02, 0.08)	−0.04 (−0.10, 0.01)	0.00 (−0.06, 0.06)	−0.03 (−0.08, 0.02)	0.97 (0.74, 1.26)
Vitamin B ₁₂ concentration birth (SDS)						
Total population (n = 3135)	−0.03 (−0.07, 0.00)	−0.05 (−0.08, −0.01)*	0.04 (0.00, 0.07)*	0.02 (−0.02, 0.06)	0.02 (−0.01, 0.06)	0.99 (0.84, 1.18)
Child wild-type [CC] (n = 1437)	−0.07 (−0.12, −0.01)*	−0.09 (−0.15, −0.04)*	0.07 (0.01, 0.12)*	−0.04 (−0.10, 0.03)	0.05 (0.00, 0.10)*	1.15 (0.91, 1.46)
Child variant [CT/TT] (n = 1489)	−0.02 (−0.08, 0.03)	−0.02 (−0.07, 0.03)	0.01 (−0.05, 0.06)	0.04 (−0.02, 0.10)	−0.00 (−0.05, 0.05)	0.81 (0.61, 1.07)
Homocysteine concentration birth (SDS)						
Total population (n = 3003)	0.02 (−0.01, 0.06)	0.01 (−0.02, 0.05)	0.01 (−0.02, 0.05)	0.02 (−0.03, 0.06)	0.01 (−0.02, 0.05)	1.04 (0.88, 1.24)
Child wild-type [CC] (n = 1389)	0.04 (−0.02, 0.09)	0.04 (−0.02, 0.09)	−0.02 (−0.07, 0.04)	0.04 (−0.03, 0.10)	−0.01 (−0.06, 0.05)	0.89 (0.68, 1.17)
Child variant [CT/TT] (n = 1420)	0.02 (−0.03, 0.07)	−0.00 (−0.05, 0.05)	0.04 (−0.01, 0.09)	0.01 (−0.05, 0.07)	0.04 (−0.01, 0.08)	1.13 (0.89, 1.42)

Values are z-score mean differences or odds ratios (95% confidence intervals) with “never folic acid supplement use during pregnancy” as reference group. “n”, number of individuals assessed (lung function at age 10 y) and number of cases/number of individuals assessed (current asthma at age 10 y). Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV₁, forced expiratory volume in the 1st second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of FVC; FEF₇₅, forced expiratory flow at 75% of exhalation; SDS, standard deviations scores. Models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational level, smoking or alcohol use during pregnancy, child’s gestational age at birth, birthweight and ethnicity. Models were stratified for maternal or child *MTHFR-C677T* carriership when interaction (*P*-value < .05) was present. No significant interactions were observed between folate, vitamin B₁₂ and homocysteine concentrations at birth with maternal *MTHFR-C677T* carriership. **P* < .05, ***P* < .01 (presented in bold).

and dosage of folic acid supplement use throughout pregnancy. Therefore, the associations of folate concentrations with lung function and asthma do not reflect the mean daily dosage of folic acid supplement use or folate concentrations throughout pregnancy. Last, analyses stratified on genotype were limited by numbers of asthma cases, which might have led to under- or overestimation of the true effect sizes.

In conclusion, folic acid supplement use during pregnancy was associated with higher childhood lung function among mothers carrying *MTHFR-C677T* wild-type and with lower childhood lung function among mothers carrying *MTHFR-C677T* variants, but not with childhood asthma. Further understanding of the interactions between folic acid exposure, blood methyl donor concentrations and genetic vulnerability is needed to provide insight into mechanisms for the development of chronic obstructive respiratory diseases in later life. Given the importance of folic acid during pregnancy to

prevent congenital abnormalities, potential adverse consequences of supplement use need to be carefully evaluated before modifications in current recommendations are considered.

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AUTHOR CONTRIBUTION

HD and LD had full access to all of the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis, and contributed to the study concept and design, data analysis and interpretation, drafting, and revision of the manuscript. IR, JJ, and VJ contributed to the study concept, data interpretation, and writing of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Maternal folic acid use during pregnancy, *MTHFR* polymorphism, and child's lung function and asthma.

SUPPLEMENTARY TEXT

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METHODS

Folate, homocysteine and vitamin B₁₂ concentrations Samples were transported to a dedicated laboratory facility (STAR-MDC) (1). In 2008, plasma samples (folate and homocysteine) and serum samples (vitamin B₁₂) were analysed at the Department of Clinical Chemistry at the Erasmus MC, University Medical Center Rotterdam (2). After thawing, folate, vitamin B₁₂ and homocysteine concentrations were analysed using an immunoelectrochemoluminescence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands) (2). The between-run coefficient of variation for plasma folate was 8.9% at 5.6 nmol/l, 2.5% at 16.6 nmol/l and 1.5% at 33.6 nmol/l, with an analytical range of 1.8-45.3 nmol/l. The between-run coefficient of variation for serum vitamin B₁₂ was 3.6% at 142 pmol/l, 7.5% at 308 pmol/l and 3.1% at 633 pmol/l, with an analytical range of 44-1476 pmol/l (2). The between-run coefficient of variation for plasma homocysteine was 3.1% at 7.2 µmol/l, 3.1% at 12.9 µmol/l and 2.1% at 26.1 µmol/l, with an analytic range of 1-50 µmol/l. Folate, vitamin B₁₂ and homocysteine concentrations were available for 80.8%, 76.5% and 79.9% mothers and 71.2%, 71.2% and 68.4% children (1).

Inhalant allergic sensitization Allergic sensitization was measured by skin prick tests on the volar surface of the left forearm with single-use sterile 1 mm-tip metal lancets (ALK-Abelló A/S, Hørsholm, Denmark). Histamine dihydrochloride (10 mg/mL) and a saline solution (NaCl 0.9%) served as 2 positive controls and 1 negative control, respectively. Inhalant allergens tested were *Dermatophagoides pteronyssinus*, 5-grass mixture (*Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*), birch (*Betula verrucosa*), cat (*Felis catus*) and dog (*Canis familiaris*) (ALK-Abelló B.V., Almere, The Netherlands). Skin responses were recorded 15 minutes after applying allergens to the skin by measuring the area of the wheal (in mm²) using the Precise Automated Area Measurement of Skin Test (PAAMOST) software and considered positive if the area of the wheal was ≥40% of the histamine response (i.e., histamine equivalent prick (HEP) index area ≥0.40) (3). A skin prick test was not performed if children could not omit antihistamine

intake or corticosteroid ointment ≤ 72 h and ≤ 48 h prior to the test, respectively, used oral prednisone ≥ 10 mg daily or had eczema on the volar surface of the left forearm (n=51).

Canonical correlation analysis Canonical correlation analysis represents the highest level of the general linear modeling and can be interpreted as a method similar to the Pearson correlation coefficient (4). Canonical correlation analysis allows for simultaneous comparison among correlated variables. We tested whether 6 variables (folic acid, vitamin B₁₂ and homocysteine during pregnancy and at birth) could predict 5 lung function variables. In that case, 30 multiple regressions are needed to examine the associations between each exposure and outcome separately. This will result in an increased experiment-wise (EW) Type I error rate. At a test-wise (TW) error rate of 0.05, the experiment-wise error rate could be estimated as $\alpha_{EW} = 1 - (1 - \alpha_{TW})^k = 1 - (1 - .05)^{30} = 0.79$, which is substantial. Also, we cannot identify which of the statistically significant results are true or false. Multivariate techniques such as canonical correlation analysis enables simultaneous assessment of the relationships between the 6 exposure variables and the 5 lung function variables. Because only one test is performed, the risk of committing a Type I error is minimized (4). Therefore, we conducted a canonical correlation analysis using folate, vitamin B₁₂ and homocysteine levels in early pregnancy and at birth as predictors of FEV₁, FVC, FEV₁/FVC, FEF₂₅₋₇₅ and FEF₇₅ to evaluate the multivariate shared relationship between the two variable sets. In the full study population (n = 5,653), the analysis yielded 5 functions with squared canonical correlations of 0.02452, 0.00483, 0.00176, 0.00040 and 0.00006 for each successive function. Collectively, the full model across all functions was statistically significant (Wilks's $\lambda = .96861$, p=.002). Because Wilks's λ represents the unexplained variance, $1 - \lambda$ shows the full model effect size as an r^2 value. Thus, for the set of 5 canonical functions, the r^2 type effect size was 0.03139, which indicates that the full model explained about 3.1% of the variance shared between the variable sets. Only function 1 was significant in all models.

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Supplementary Table 1. Characteristics of children and their mothers (n = 5,653)

	Original Data	Data after multiple imputation
Maternal characteristics		
Age (years)	31.0 (4.9)	31.0 (4.9)
<i>Missing</i>	-	-
Body mass index at intake (kg/m ²) [#]	23.7 (18.8 – 35.6)	23.9 (18.9 – 35.4)
<i>Missing</i>	9.8 (554)	-
Parity (%)		
Nullipara	57.4 (3,141)	57.0 (3,215)
Multipara	42.6 (2,331)	43.0 (2,438)
<i>Missing</i>	3.2 (181)	-
History of asthma or atopy (%)		
No	60.4 (2,772)	61.3 (3,463)
Yes	39.6 (1,818)	38.7 (2,190)
<i>Missing</i>	18.8 (1,063)	-
Education (%)		
Low (no, primary, secondary education)	49.0 (2,557)	50.5 (2,860)
High (higher education)	51.0 (2,661)	49.5 (2,793)
<i>Missing</i>	7.7 (435)	-
Smoking during pregnancy (%)		
No smoking throughout pregnancy	76.6 (3,802)	75.9 (4,288)
Smoking during first trimester	8.7 (432)	8.6 (485)
Smoking throughout pregnancy	14.7 (729)	15.6 (880)
<i>Missing</i>	12.2 (690)	-
Alcohol consumption during pregnancy (%)		
No alcohol throughout pregnancy	42.7 (1,943)	43.4 (2,453)
Alcohol during first trimester	14.1 (642)	14.2 (803)
Alcohol throughout pregnancy	43.2 (1,966)	42.4 (2,397)
<i>Missing</i>	19.5 (1,102)	-
Folic acid supplement use (%)		NI
No use	15.1 (855)	
Start after first 10 weeks of pregnancy	10.2 (575)	
Start within first 10 weeks of pregnancy	22.4 (1,265)	
Preconceptional start	32.4 (1,830)	

<i>Missing</i>	19.9 (1,128)	
Folate in early pregnancy (nmol/l) [#]	17.5 (6.0 – 37.9)	NI
<i>Missing</i>	34.2 (1,933)	
Vitamin B ₁₂ in early pregnancy (pmol/l) [#]	172.0 (77.0 – 406.0)	NI
<i>Missing</i>	37.1 (2,097)	
Homocysteine in early pregnancy (μmol/l) [#]	9.1 (5.2 – 16.6)	NI
<i>Missing</i>	34.6 (1,956)	
MTHFR-C677T (%)		NI
CC	51.0 (2,214)	
CT	40.0 (1,738)	
TT	9.0 (391)	
<i>Missing</i>	23.2 (1,310)	
Child's characteristics		
Sex (%)		
Female	50.6 (2,860)	50.6 (2,860)
Male	49.4 (2,793)	49.4 (2,793)
<i>Missing</i>	-	-
Gestational age at birth (weeks) [#]	40.1 (35.8 – 42.3)	40.1 (35.8 – 42.3)
<i>Missing</i>	0.5 (33)	
Birth weight (grams)	3440 (554)	3440 (554)
<i>Missing</i>	0.1 (7)	-
Ethnicity (%)		
European	68.7 (3,674)	68.9 (3,895)
Non-European	31.3 (1,674)	31.1 (1,758)
<i>Missing</i>	5.4 (305)	
Folate at birth (nmol/l) [#]	20.9 (10.7 – 38.4)	NI
<i>Missing</i>	45.2 (2,555)	
Vitamin B ₁₂ at birth (nmol/l) [#]	303.0 (121.0 – 902.2)	NI
<i>Missing</i>	44.6 (2,521)	
Homocysteine at birth (μmol/l) [#]	6.9 (4.6 – 12.0)	NI
<i>Missing</i>	46.9 (2,651)	
MTHFR-C677T (%)		NI
CC	49.3 (1,773)	
CT	41.7 (1,500)	

TT	8.9 (320)	
<i>Missing</i>	35.1 (1,660)	
FEV ₁ (L/s)	2.02 (0.30)	NI
FVC (L)	2.34 (0.36)	NI
FEV ₁ /FVC	0.87 (0.06)	NI
FEF ₂₅₋₇₅ (L/s)	2.71 (0.63)	NI
FEF ₇₅ (L/s)	1.14 (0.34)	NI
Current asthma (%)		NI
No	94.3 (4,438)	
Yes	5.7 (269)	
<i>Missing</i>	16.7 (946)	

Values are means (SD), valid percentages (absolute numbers) or #medians (95% range).

FEV₁: forced expiratory volume in the 1st second, FEV₁: forced expiratory volume in the 1st second, FVC: forced vital capacity, FEF₂₅₋₇₅: forced expiratory flow between 25% and 75% of FVC, FEF₇₅: forced expiratory flow at 75% of exhalation. NI: not imputed.

Supplementary Table 2. Characteristics of included (population for analysis) and excluded (lost to follow-up) participants.

	Included (n = 5,653)	Lost to follow-up (n = 1,554)	P-value
Maternal characteristics			
Age (years)	31.0 (4.9)	30.9 (4.9)	
Body mass index at intake (kg/m ²) [#]	23.9 (18.9 – 35.3)	24.4 (19.1 – 35.3)	0.18
Nulli-parity	57.4 (3,141)	52.3 (811)	<0.01
History of asthma or atopy, yes (%)	39.3 (2,180)	40.3 (626)	0.10
Lower education (%)	50.5 (2,860)	50.6 (786)	<0.01
Smoking during pregnancy (%)			
No smoking throughout pregnancy	75.9 (4,288)	70.4 (1,094)	0.46
Smoking during first trimester	8.6 (485)	8.4 (131)	
Smoking throughout pregnancy	15.6 (880)	21.2 (329)	
Alcohol consumption during pregnancy			
No alcohol throughout pregnancy	43.4 (2,453)	55.2 (858)	<0.01
Alcohol during first trimester	14.2 (803)	12.7 (197)	
Alcohol throughout pregnancy	42.4 (2,397)	32.1 (499)	
Folic acid supplement use	84.6 (3,616)	79.0 (1,228)	<0.01
Folate in early pregnancy (nmol/l) [#]	17.4 (6.0 – 37.9)	14.8 (5.3, 37.4)	<0.01
Vitamin B ₁₂ in early pregnancy (pmol/l) [#]	172 (77 – 406)	165 (68 – 411)	0.06
Homocysteine in early pregnancy (μmol/l)	7.2 (2.0)	7.3 (2.3)	0.08
Child's characteristics			
Female sex (%)	50.6 (2,860)	49.2 (765)	0.30
Gestational age at birth (weeks) [#]	40.1 (35.8 – 42.3)	39.7 (32.5 – 42.1)	0.05
Birth weight (grams)	3440 (554)	3213 (690)	<0.01
European ethnicity (%)	68.9 (3,895)	67.8 (1,054)	0.62
Folate at birth (nmol/l) [#]	20.9 (10.7 – 38.4)	20.0 (10.0, 38.5)	<0.01
Vitamin B ₁₂ at birth (nmol/l) [#]	313 (117 – 929)	294 (118 – 919)	0.19
Homocysteine at birth (μmol/l)	9.7 (3.2)	9.7 (3.1)	0.51

Values are means (SD), valid percentages (absolute numbers) or #medians (95% range).

Differences between the included population and loss to follow-up were assessed with T-tests, Mann-Whitney U tests and chi-square tests.

Supplementary Table 3. Associations of folic acid supplementation use during pregnancy with childhood lung function and asthma, stratified for maternal rs1801133.

		FEV₁ Z-score (95% CI) n = 5,099	FVC Z-score (95% CI) n = 5,104	FEV₁/FVC Z-score (95% CI) n = 5,099	FEF₂₅₋₇₅ Z-score (95% CI) n = 5,102	FEF₇₅ Z-score (95% CI) n = 5,103	Current asthma Odds Ratio (95% CI) n = 269 / 4,707
Maternal genotype	Folic acid use						
Wildtype [CC]	No use n = 388	<i>Reference</i> <i>n = 362</i>	<i>Reference</i> <i>n = 363</i>	<i>Reference</i> <i>n = 363</i>	<i>Reference</i> <i>n = 363</i>	<i>Reference</i> <i>n = 363</i>	<i>Reference</i> <i>n = 24 / 257</i>
	Any folic acid supplementation n = 1,533	0.27 (0.14, 0.39)** n = 1,389	0.26 (0.14, 0.38)** n = 1,391	0.00 (-0.12, 0.13) n = 1,389	0.16 (0.02, 0.30)* n = 1,391	0.10 (-0.02, 0.22) n = 1,391	0.81 (0.46, 1.44) n = 82 / 1,386
	Start > 10 weeks n = 268	0.24 (0.08, 0.41)** n = 240	0.23 (0.07, 0.37)** n = 240	0.03 (-0.13, 0.19) n = 240	0.13 (-0.05, 0.10) n = 240	0.14 (-0.01, 0.29) n = 240	0.71 (0.33, 1.49) n = 13 / 224
	Start ≤ 10 weeks n = 537	0.29 (0.15, 0.43)** n = 491	0.25 (0.11, 0.38)** n = 491	0.05 (-0.09, 0.19) n = 491	0.14 (-0.02, 0.30) n = 491	0.17 (0.04, 0.31)* n = 491	0.93 (0.49, 1.75) n = 29 / 461
	Start preconceptional n = 749	0.26 (0.12, 0.40)** n = 660	0.30 (0.16, 0.43)** n = 660	-0.07 (-0.21, 0.07) n = 660	0.19 (0.03, 0.35)* n = 660	0.02 (-0.11, 0.15) n = 660	0.78 (0.40, 1.52) n = 29 / 668
	<i>P-value for trend</i>	<i>0.99</i>	<i>0.26</i>	<i>0.07</i>	<i>0.47</i>	<i>0.38</i>	<i>0.66</i>
Variant [CT/TT]	No use n = 313	<i>Reference</i> <i>n = 290</i>	<i>Reference</i> <i>n = 290</i>	<i>Reference</i> <i>n = 290</i>	<i>Reference</i> <i>n = 290</i>	<i>Reference</i> <i>n = 290</i>	<i>Reference</i> <i>n = 14 / 228</i>
	Any folic acid supplementation n = 1,541	0.03 (-0.11, 0.17) n = 1,420	0.09 (-0.03, 0.22) n = 1,421	-0.13 (-0.26, -0.00)* n = 1,419	-0.11 (-0.26, 0.04) n = 1,421	-0.02 (-0.15, 0.11) n = 1,421	0.97 (0.50, 1.88) n = 77 / 1,424
	Start > 10 weeks n = 225	0.04 (-0.14, 0.22) n = 198	0.08 (-0.09, 0.25) n = 198	-0.010 (-0.28, 0.08) n = 198	0.10 (-0.10, 0.30) n = 198	0.01 (-0.16, 0.18) n = 198	1.05 (0.45, 2.45) n = 11 / 182
	Start ≤ 10 weeks	0.08	0.12	-0.09	-0.09	0.02	1.07

n = 530	(-0.07, 0.23)	(-0.02, 0.26)	(-0.24, 0.06)	(-0.26, 0.08)	(-0.12, 0.16)	(0.52, 2.19)
	n = 477	n = 477	n = 477	n = 477	n = 477	n = 28 / 471
Start	-0.02	0.08	-0.17	-0.24	-0.04	0.85
preconceptional	(-0.17, 0.13)	(-0.06, 0.22)	(-0.32, -0.02)*	(-0.40, -0.07)*	(-0.18, 0.10)	(0.41, 1.76)
n = 811	n = 746	n = 746	n = 746	n = 746	n = 746	n = 35 / 725
<i>P-value for trend</i>	<i>0.24</i>	<i>0.96</i>	<i>0.16</i>	<0.01	<i>0.31</i>	<i>0.74</i>

Values are z-score differences or odds ratios (95% confidence intervals) with “never uses folic acid supplements during pregnancy” as

reference group. Any use of folic acid supplements was categorized into start after the first 10 weeks of pregnancy, start within the first 10 weeks of pregnancy and preconceptional start. P-value for trend depicts line for increased duration of folic acid supplements use. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV₁: forced expiratory volume in the 1st second, FVC: forced vital capacity, FEF₂₅₋₇₅: forced expiratory flow between 25% and 75% of FVC, FEF₇₅: forced expiratory flow at 75% of exhalation. Models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational concentration, smoking or alcohol use during pregnancy, child's gestational age at birth, birthweight and ethnicity. *p < 0.05, ** p < 0.01.

Supplementary table 4. Associations of folic acid supplementation use during pregnancy with childhood lung function and asthma, stratified for child's rs1801133.

		FEV₁ Z-score (95% CI) n = 5,099	FVC Z-score (95% CI) n = 5,104	FEV₁/FVC Z-score (95% CI) n = 5,099	FEF₂₅₋₇₅ Z-score (95% CI) n = 5,102	FEF₇₅ Z-score (95% CI) n = 5,103	Current asthma Odds Ratio (95% CI) n = 269 / 4,707
Childs genotype Wildtype [CC]	Folic acid use						
	No use n = 318	<i>Reference</i> <i>n = 295</i>	<i>Reference</i> <i>n = 295</i>	<i>Reference</i> <i>n = 295</i>	<i>Reference</i> <i>n = 295</i>	<i>Reference</i> <i>n = 295</i>	<i>Reference</i> <i>n = 18 / 228</i>
	Any folic acid supplementation n = 1,250	0.13 (-0.01, 0.26) n = 1,020	0.19 (0.06, 0.32)* n = 1,020	-0.14 (-0.28, -0.00)* n = 1,020	0.10 (-0.05, 0.26) n = 1,020	-0.04 (-0.17, 0.09) n = 1,020	1.11 (0.60, 2.03) n = 68 / 1,101
	Start > 10 weeks n = 202	0.08 (-0.11, 0.26) n = 178	0.15 (-0.03, 0.33) n = 178	-0.14 (-0.33, 0.04) n = 178	0.08 (-0.13, 0.29) n = 178	-0.03 (-0.20, 0.14) n = 178	0.98 (0.43, 1.99) n = 11 / 170
	Start ≤ 10 weeks n = 449	0.16 (0.01, 0.32)* n = 408	0.21 (0.06, 0.36)** n = 408	-0.12 (-0.28, 0.03) n = 408	0.08 (-0.10, 0.25) n = 408	0.00 (-0.14, 0.14) n = 408	1.27 (0.66, 2.46) n = 31 / 386
	Start preconceptional n = 599	0.11 (-0.04, 0.27) n = 539	0.19 (0.04, 0.34)** n = 539	-0.16 (-0.32, -0.01)* n = 539	0.15 (-0.03, 0.33) n = 539	-0.09 (-0.24, 0.05) n = 539	0.98 (0.48, 1.99) n = 26 / 545
	<i>P-value for trend</i>	<i>0.94</i>	<i>0.77</i>	<i>0.76</i>	<i>0.35</i>	<i>0.25</i>	<i>0.80</i>
Variant [CT/TT]	Folic acid use						
	No use n = 244	<i>Reference</i> <i>n = 222</i>	<i>Reference</i> <i>n = 222</i>	<i>Reference</i> <i>n = 222</i>	<i>Reference</i> <i>n = 222</i>	<i>Reference</i> <i>n = 222</i>	<i>Reference</i> <i>n = 8 / 174</i>
	Any folic acid supplementation n = 1,398	0.11 (-0.04, 0.27) n = 1,256	0.18 (0.04, 0.32)* n = 1,256	-0.14 (-0.28, 0.01) n = 1,256	0.00 (-0.17, 0.17) n = 1,256	-0.01 (-0.15, 0.13) n = 1,256	1.77 (0.74, 4.28) n = 64 / 1,235
	Start > 10 weeks n = 196	0.18 (-0.01, 0.37) n = 177	0.20 (0.02, 0.38)* n = 177	-0.07 (-0.26, 0.12) n = 177	0.19 (-0.03, 0.41) n = 177	0.04 (-0.15, 0.22) n = 177	1.70 (0.57, 4.09) n = 8 / 158
	Start ≤ 10 weeks n = 469	0.11 (-0.06, 0.27) n = 426	0.16 (0.01, 0.32)* n = 426	-0.13 (-0.29, 0.04) n = 426	-0.01 (-0.20, 0.17) n = 426	-0.01 (-0.16, 0.15) n = 426	1.94 (0.77, 4.88) n = 25 / 418

Start	0.09	0.19	-0.18	-0.07	-0.04	1.63
preconceptional	(-0.08, 0.26)	(0.04, 0.34)*	(-0.34, -0.01)*	(-0.26, 0.11)	(-0.20, 0.12)	(0.64, 4.17)
n = 733	n = 653	n = 653	n = 653	n = 653	n = 653	n = 31 / 659
<i>P-value for trend</i>	<i>0.51</i>	<i>0.41</i>	<i>0.22</i>	0.01	<i>0.43</i>	<i>0.80</i>

Values are z-score differences or odds ratios (95% confidence intervals) with “never uses folic acid supplements during pregnancy” as

reference group. Any use of folic acid supplements was categorized into start after the first 10 weeks of pregnancy, start within the first 10 weeks of pregnancy and preconceptional start. P-value for trend depicts line for increased duration of folic acid supplements use. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores. FEV₁: forced expiratory volume in the 1st second, FVC: forced vital capacity, FEF₂₅₋₇₅: forced expiratory flow between 25% and 75% of FVC, FEF₇₅: forced expiratory flow at 75% of exhalation. Models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational concentration, smoking or alcohol use during pregnancy, child's gestational age at birth, birthweight and ethnicity. *p < 0.05, ** p < 0.01.

Supplementary Table 5. Associations of folate, vitamin B₁₂, and homocysteine concentrations in early pregnancy with spirometry measures and childhood asthma, additionally stratified by maternal genotype.

Genotype		FEV₁ Z-score (95% CI) n = 5,099	FVC Z-score (95% CI) n = 5,104	FEV₁/FVC Z-score (95% CI) n = 5,099	FEF₂₅₋₇₅ Z-score (95% CI) n = 5,102	FEF₇₅ Z-score (95% CI) n = 5,103	Current asthma Odds Ratio (95% CI) n = 269 / 4,707
Folate concentration early pregnancy (SDS)							
Total population	Low (n = 702)	-0.04 (-0.14, 0.05)	-0.06 (-0.15, 0.03)	0.03 (-0.06, 0.13)	-0.02 (-0.12, 0.09)	0.01 (-0.08, 0.09)	1.35 (0.91, 2.01)
	Normal (n = 2,324)	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>
	High (n = 693)	-0.01 (-0.10, 0.07)	-0.01 (-0.10, 0.07)	-0.01 (-0.09, 0.08)	-0.03 (-0.13, 0.07)	-0.01 (-0.10, 0.07)	1.11 (0.74, 1.66)
Maternal wildtype [CC]	Low (n = 314)	-0.07 (-0.21, 0.07)	-0.06 (-0.19, 0.08)	-0.02 (-0.16, 0.12)	0.00 (-0.15, 0.16)	-0.06 (-0.19, 0.07)	1.80 (0.97, 3.21)
	Normal (n = 1,579)	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>
	High (n = 315)	-0.02 (-0.15, 0.12)	-0.03 (-0.16, 0.10)	0.03 (-0.10, 0.16)	-0.05 (-0.19, 0.10)	0.03 (-0.10, 0.15)	1.05 (0.55, 1.99)
Maternal variant [CT/TT]	Low (n = 298)	0.02 (-0.12, 0.16)	0.01 (-0.12, 0.15)	0.01 (-0.13, 0.15)	-0.04 (-0.19, 0.12)	0.06 (-0.07, 0.19)	1.17 (0.60, 2.28)
	Normal (n = 1,607)	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>
	High (n = 303)	0.05 (-0.09, 0.18)	0.04 (-0.09, 0.16)	-0.01 (-0.14, 0.13)	-0.05 (-0.20, 0.10)	0.02 (-0.10, 0.15)	1.05 (0.58, 1.92)
Vitamin B₁₂ concentration early pregnancy (SDS)							
Total population	Low (n = 524)	0.03 (-0.06, 0.13)	-0.01 (-0.10, 0.09)	0.05 (-0.05, 0.14)	-0.05 (-0.16, 0.06)	0.05 (-0.04, 0.14)	0.98 (0.63, 1.53)

	Normal (n = 2,485)	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>
	High (n = 550)	0.00 (-0.09, 0.10)	-0.04 (-0.13, 0.05)	0.07 (-0.03, 0.16)	0.01 (-0.09, 0.12)	0.08 (-0.01, 0.17)	0.82 (0.51, 1.31)
Homocysteine concentration early pregnancy (SDS)							
Total population	Low (n = 275)	-0.01 (-0.13, 0.12)	-0.18 (-0.20, 0.04)	0.11 (-0.02, 0.24)	-0.05 (-0.19, 0.09)	0.11 (-0.01, 0.23)	0.80 (0.41, 1.55)
	Normal (n = 3,047)	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>
	High (n = 361)	0.02 (-0.09, 0.13)	0.04 (-0.07, 0.14)	-0.03 (-0.14, 0.08)	-0.01 (-0.14, 0.11)	0.02 (-0.08, 0.13)	1.07 (0.64, 1.77)
Maternal wildtype [CC]	Low (n = 131)	0.06 (-0.13, 0.24)	-0.01 (-0.19, 0.17)	0.16 (-0.03, 0.34)	-0.07 (-0.28, 0.15)	0.15 (-0.02, 0.33)	0.86 (0.33, 2.28)
	Normal (n = 1,392)	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>
	High (n = 131)	-0.01 (-0.19, 0.17)	0.02 (-0.16, 0.19)	-0.07 (-0.25, 0.11)	-0.04 (-0.25, 0.17)	0.03 (-0.14, 0.23)	1.20 (0.55, 2.62)
Maternal variant [CT/TT]	Low (n = 112)	-0.02 (-0.22, 0.17)	-0.11 (-0.30, 0.07)	0.10 (-0.10, 0.30)	0.00 (-0.22, 0.22)	0.10 (-0.08, 0.29)	0.56 (0.17, 1.85)
	Normal (n = 1,347)	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>	<i>Reference</i>
	High (n = 176)	-0.08 (-0.24, 0.08)	-0.06 (-0.21, 0.09)	-0.02 (-0.17, 0.15)	-0.01 (-0.19, 0.17)	-0.02 (-0.17, 0.13)	0.94 (0.42, 2.14)

Values are z-score differences or odds ratios (95% confidence intervals) with “never uses folic acid supplements during pregnancy” as

reference group. Any use of folic acid supplements was categorized into start after the first 10 weeks of pregnancy, start within the first 10

weeks of pregnancy and preconceptional start. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores.

FEV₁: forced expiratory volume in the 1st second, FVC: forced vital capacity, FEF₂₅₋₇₅: forced expiratory flow between 25% and 75% of FVC,

FEF₇₅: forced expiratory flow at 75% of exhalation. Models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational concentration, smoking or alcohol use during pregnancy, child's gestational age at birth, birthweight and ethnicity. Models were stratified for maternal or child *MTHFR-C677T* carriership when interaction (p-value < 0.05) was present. *p < 0.05, ** p < 0.01.

Supplementary Table 6. Associations of folate, vitamin B₁₂, and homocysteine concentrations at birth with spirometry measures and childhood asthma, stratified by child genotype.

Genotype		FEV ₁ Z-score (95% CI) n = 5,099	FVC Z-score (95% CI) n = 5,104	FEV ₁ /FVC Z-score (95% CI) n = 5,099	FEF ₂₅₋₇₅ Z-score (95% CI) n = 5,102	FEF ₇₅ Z-score (95% CI) n = 5,103	Current asthma Odds Ratio (95% CI) n = 269 / 4,707
Total Population	Folate concentration birth (SDS)						
	Low (n = 548)	0.11 (0.01, 0.21)*	0.02 (-0.07, 0.12)	0.14 (0.04, 0.23)**	0.07 (-0.04, 0.18)	0.10 (0.01, 0.19)*	0.75 (0.46, 1.22)
	Normal (n = 1,981)	Reference	Reference	Reference	Reference	Reference	Reference
	High (n = 566)	0.02 (-0.07, 0.12)	0.01 (-0.09, 0.10)	0.04 (-0.05, 0.14)	0.02 (-0.08, 0.13)	0.04 (-0.05, 0.13)	1.11 (0.72, 1.70)
Total Population	Vitamin B₁₂ concentration birth (SDS)						
	Low (n = 475)	0.03 (-0.08, 0.13)	0.08 (-0.02, 0.18)	-0.07 (-0.18, 0.03)	-0.11 (-0.22, 0.01)	-0.05 (-0.14, 0.05)	0.94 (0.58, 1.53)
	Normal (n = 2,174)	Reference	Reference	Reference	Reference	Reference	Reference
	High (n = 486)	-0.09 (-0.19, 0.02)	-0.09 (-0.19, 0.01)	0.04 (-0.06, 0.15)	-0.05 (-0.16, 0.07)	0.05 (-0.05, 0.14)	1.04 (0.65, 1.66)
Child wildtype [CC]	Low (n = 218)	0.09 (-0.07, 0.24)	0.14 (-0.01, 0.29)	-0.10 (-0.25, 0.05)	0.05 (-0.12, 0.22)	-0.11 (-0.25, 0.03)	0.70 (0.33, 1.48)
	Normal (n = 995)	Reference	Reference	Reference	Reference	Reference	Reference
	High (n = 224)	-0.14 (-0.29, 0.02)	-0.18 (-0.33, -0.03)*	0.11 (-0.04, 0.26)	-0.02 (-0.19, 0.15)	0.06 (-0.08, 0.19)	1.43 (0.78, 2.63)
Child variant [CT/TT]	Low (n = 225)	-0.02 (-0.17, 0.13)	0.02 (-0.12, 0.16)	-0.05 (-0.20, 0.10)	-0.23 (-0.40, -0.06)**	-0.00 (-0.14, 0.14)	1.21 (0.60, 2.47)
	Normal (n = 1,036)	Reference	Reference	Reference	Reference	Reference	Reference
	High (n = 228)	-0.08 (-0.23, 0.07)	0.03 (-0.16, 0.14)	-0.05 (-0.20, 0.10)	-0.14 (-0.31, 0.03)	0.01 (-0.13, 0.15)	0.63 (0.26, 1.51)

		Homocysteine concentration birth (SDS)					
Total population	Low (n = 304)	0.04 (-0.09, 0.16)	-0.01 (-0.13, 0.11)	0.08 (-0.04, 0.20)	-0.04 (-0.18, 0.09)	0.08 (-0.03, 0.19)	0.72 (0.37, 1.41)
	Normal (n = 2,315)	Reference	Reference	Reference	Reference	Reference	Reference
	High (n = 384)	0.11 (-0.14, 0.22)	0.08 (-0.03, 0.19)	-0.03 (-0.08, 0.14)	0.08 (-0.04, 0.21)	0.05 (-0.05, 0.15)	0.97 (0.58, 1.63)

Values are z-score differences or odds ratios (95% confidence intervals) with “never uses folic acid supplements during pregnancy” as

reference group. Any use of folic acid supplements was categorized into start after the first 10 weeks of pregnancy, start within the first 10 weeks of pregnancy and preconceptional start. Lung function variables were converted into sex-, height-, age- and ethnicity-adjusted z-scores.

FEV₁: forced expiratory volume in the 1st second, FVC: forced vital capacity, FEF₂₅₋₇₅: forced expiratory flow between 25% and 75% of FVC, FEF₇₅: forced expiratory flow at 75% of exhalation. Models were adjusted for maternal age and BMI at intake, parity, history of asthma or atopy, educational concentration, smoking or alcohol use during pregnancy, child's gestational age at birth, birthweight and ethnicity. Models were stratified for maternal or child *MTHFR-C677T* carriership when interaction (p-value < 0.05) was present. *p < 0.05, ** p < 0.01.

Supplementary Table 7a. Canonical correlation analyses of folate, vitamin B12 and homocysteine levels in early pregnancy and at birth with lung function in childhood in the full cohort (n = 5,554).

	Coef	R _s	R ² _s
Folic acid in early pregnancy	0.81521	-0.68265	46.6011
Vitamin B ₁₂ in early pregnancy	0.19843	-0.10116	1.023335
Homocysteine in early pregnancy	0.31683	0.12982	1.685323
Folic acid at birth	-0.09881	0.13971	1.951888
Vitamin B ₁₂ at birth	-0.73317	0.58409	34.11553
Homocysteine at birth	-0.17574	-0.04560	0.207936
zFEV ₁	5.25966	-0.06397	0.409216
zFVC	-4.20692	0.11063	1.2239
zFEV ₁ /FVC	-1.75401	-0.43037	18.52183
zFEF ₂₅₋₇₅	-0.79941	-0.39034	15.23653
zFEF ₇₅	-1.5503	-0.47407	22.47424

Coef = standardized canonical function coefficient; R_s = structure coefficient; R²_s = squared structure coefficient. The analysis yielded 5 functions with squared canonical correlations of 0.02452, 0.00483, 0.00176, 0.00040 and 0.00006 for each successive function. Collectively, the full model across all functions was statistically significant using the Wilks's $\lambda = .96861$ criterion, $F(30, 7138.00) = 1.904783$, $p = .002$. The r^2 type effect size was 0.03139, which indicates that the full model explained about 3.1%, of the variance shared between the variable sets. For emphasis, structure coefficients <-0.45 or >0.45 are presented bold, indicating a "fair" factor loading, following Comrey et al. (Comrey A.L., Lee H.B.; A First encounter in factor analysis; Psychology Press; 2nd edition (1992)).

Supplementary Table 7b. Canonical correlation analyses of folate, vitamin B₁₂ and homocysteine levels in early pregnancy and at birth with lung function in childhood, stratified by maternal *MTHFR*-genotype.

	Maternal wildtype [CC]			Maternal variant [CT/TT]		
	Coef	R _s	R ² _s	Coef	R _s	R ² _s
Folic acid in early pregnancy	-0.87894	-0.76565	58.62199	-0.698	-0.62437	38.98379
Vitamin B ₁₂ in early pregnancy	-0.0917	0.14038	1.970654	-0.40151	-0.05235	0.274052
Homocysteine in early pregnancy	-0.06106	0.01838	0.033782	-0.23213	-0.01964	0.038573
Folic acid at birth	0.24131	-0.06156	0.378963	-0.08728	-0.24184	5.848659
Vitamin B ₁₂ at birth	0.60445	0.58503	34.22601	0.90281	0.56253	31.644
Homocysteine at birth	-0.02777	-0.08183	0.669615	0.17493	0.05519	0.304594
zFEV ₁	-5.53056	-0.4227	17.86753	-4.94953	0.24971	6.235508
zFVC	4.05997	-0.3622	13.12178	4.14401	0.07658	0.58645
zFEV ₁ /FVC	1.31866	0.01057	0.011172	1.74442	0.40981	16.79442
zFEF ₂₅₋₇₅	0.59887	0.05505	0.30305	0.96257	0.64068	41.04709
zFEF ₇₅	1.65360	0.05200	0.27040	1.33863	0.43852	19.22998

Coef = standardized canonical function coefficient; R_s = structure coefficient; R²_s = squared structure coefficient. For maternal wildtype genotype [CC], the full model across all functions was not statistically significant using the Wilks's $\lambda = 0.95641$ criterion, $F(30, 3122.00) = 1.16591$,

p=0.245. For maternal variant genotype [CT/TT], the full model across all functions was statistically significant using the Wilks's $\lambda = 0.94309$ criterion, $F(30, 3218.00) = 1.58296$, $p=0.023$. The r^2 type effect size was 0.05691, which indicates that the full model explained about 5.7% of the variance shared between the variable sets. For emphasis, structure coefficients <-0.45 or >0.45 are presented bold, indicating a "fair" factor loading, following Comrey et al. (Comrey A.L., Lee H.B.; A First encounter in factor analysis; Psychology Press; 2nd edition (1992)).

Supplementary Table 7c. Canonical correlation analyses of folate, vitamin B₁₂ and homocysteine levels in early pregnancy and at birth with lung function in childhood, stratified by child *MTHFR*-genotype.

	Child wildtype [CC]			Child [CT/TT]		
	Coef	R _s	R ² _s	Coef	R _s	R ² _s
Folic acid in early pregnancy	-0.80174	-0.68318	46.67349	-0.5916	-0.37101	13.76484
Vitamin B ₁₂ in early pregnancy	0.01434	0.32690	10.68636	-0.31106	0.0171	0.029241
Homocysteine in early pregnancy	-0.11453	-0.02947	0.086848	-0.4014	-0.20952	4.389863
Folic acid at birth	0.15967	-0.02351	0.055272	0.25349	0.06207	0.385268
Vitamin B ₁₂ at birth	0.65312	0.67500	45.5625	0.90781	0.63458	40.26918
Homocysteine at birth	-0.04658	-0.15288	2.337229	0.47162	0.23306	5.431696
zFEV ₁	-4.78868	-0.22450	5.040025	-1.74626	0.75850	57.53223
zFVC	3.43064	-0.39679	15.74423	2.29663	0.54205	29.38182
zFEV ₁ /FVC	1.07971	0.43914	19.28439	1.45735	0.35409	12.53797
zFEF ₂₅₋₇₅	0.49417	-0.00925	0.008556	0.66005	0.82766	68.50211
zFEF ₇₅	1.82369	0.44780	20.05248	0.03688	0.46946	22.03927

Coef = standardized canonical function coefficient; R_s = structure coefficient; R²_s = squared structure coefficient. For children with child wildtype genotype, the full model across all functions was statistically significant using the Wilks's $\lambda = 0.94639$ criterion, $F(30, 3250.00) = 1.50257$,

$p=0.039$. The r^2 type effect size was 0.05361, which indicates that the full model explained about 5.4% of the variance shared between the variable sets. For children with variant genotype [CT/TT], the full model across all functions was not statistically significant using the Wilks's $\lambda = 0.96266$ criterion, $F(30, 3382.00) = 1.07760$, $p=0.353$. For emphasis, structure coefficients <-0.45 or >0.45 are presented bold, indicating a “fair” factor loading, following Comrey et al. (Comrey A.L., Lee H.B.; A First encounter in factor analysis; Psychology Press; 2nd edition (1992)).

Supplementary Figure 1. Flowchart of inclusion of participants.

