



Is Folate Status a Risk Factor for Asthma or Other Allergic Diseases?

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Purpose: It is controversial whether folate status is a risk factor for the development of asthma or other allergic diseases. This study was conducted to investigate whether indirect or direct exposure to folate and impaired folate metabolism, reflected as methylene-tetrahydrofolate reductase (*MTHFR*) C677T polymorphism, would contribute to the development of asthma and other allergic diseases. **Methods:** Electronic databases were searched to identify all studies assessing the association between folate status and asthma or other allergic diseases. Two reviewers independently assessed the eligibility of studies and extracted data. The relative risk (RR) or odds ratio (OR) with 95% confidence intervals (CI) was calculated and pooled. **Results:** Twenty-six studies (16 cohort, 7 case-control, and 3 cross-sectional studies) were identified. Maternal folic acid supplementation was not associated with the development of asthma, atopic dermatitis (AD), eczema, and sensitization in the offspring, whereas exposure during early pregnancy was related to wheeze occurrence in the offspring (RR=1.06, 95% CI=[1.02-1.09]). The TT genotype of *MTHFR* C677T polymorphism was at high risk of asthma (OR=1.41, 95% CI=[1.07-1.86]). **Conclusions:** It is indicated that maternal folic acid supplementation during early pregnancy may increase the risk of wheeze in early childhood and that the TT genotype of *MTHFR* C677T polymorphism impairing folic acid metabolism would be at high risk of asthma development. These results might provide additional information for recommendations regarding forced folate consumption or folic acid supplements during pregnancy based on its well-established benefits for the prevention of congenital malformations. However, currently available evidence is of low quality which is needed to further elucidate.

Key Words: Folic acid; asthma; risk; review, systematic; meta-analysis

INTRODUCTION

The prevalence of asthma has dramatically increased during the past decades as in other allergic diseases, but underlying mechanisms that account for this remarkable trend remain unclear.^{1,2} This allergy epidemic has been reported in relation to changes in environmental factors, which has generated intense interest in changes of lifestyle such as dietary patterns. One particular dietary component that has recently received much attention is folate, which functions as an essential cofactor for one-carbon transfers, primarily methyl groups, and relates to a complex network of biological processes that are vital to growth.³ Thus, it could directly influence the propensity for epigenetic modifications and might tilt the immunophenotypic balance in favor of allergic disease.

Since maternal folic acid (a synthetic form of folate) supplementation before and during pregnancy has been widely recommended to reach a protective effect on the occurrence of

neural tube defects^{4,5} as well as other congenital malformations⁶ for many years, it has been questioned whether folic acid supplementation might be associated with the development of adverse health outcomes in the offspring. Previous findings in mice suggested that maternal dietary supplementation with methyl donors (including folic acid) leads to allergic disease in the offspring,⁷ and similar conditions were observed in humans.^{8,9} These results raised concern about possible adverse effects of folic acid supplementation during pregnancy on respiratory and allergic outcomes in the offspring. However, several

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studies have not detected any relationship.¹⁰⁻¹³ In addition, studies conducted to detect the role of impaired folate metabolism, reflected by polymorphism of the methylene-tetrahydrofolate reductase (*MTHFR*) C677T in the development of asthma or other atopic diseases, have reported inconsistent results.^{14,15}

Although several reviews do not support an exact effect of maternal folate status on asthma risk, the timing, course, and dose effect of folate status, and the association between *MTHFR* C677T polymorphisms and asthma risk are still of increasing interest.¹⁶⁻¹⁹ There is no adequate evidence to exclude such effects, either.^{16,18,19} Therefore, we performed this comprehensive systematic review and meta-analysis of existing evidence to answer the following questions: (1) whether indirect or direct exposure to folate is associated with the development of asthma and other allergic diseases, including atopic dermatitis (AD), eczema, and sensitization; (2) whether there is a dose-dependent relationship between folate status and allergic diseases; (3) whether this relationship could be interpreted by exposure timing; and (4) whether the manifestations could be objectively measured.

MATERIALS AND METHODS

Searching and selection

Comprehensive literature search was performed using databases MEDLINE (PubMed), Cochrane Central, Web of Science, and EMBASE until August 2014. The following terms “folic” or “folate” in combination with “asthma,” “wheeze,” “allergy,” “allergic,” “AD,” “eczema,” “rhinitis,” or “hay fever” was used as search terms to identify potential studies. The search strategies were adjusted on the basis of the characteristics of each database. Reference lists of all identified potential studies were manually screened to obtain other relevant articles. No language or initial time restriction was performed.

All trials conducted on humans involving the association of folate status and asthma or other allergic diseases were included except only abstracts or no available data. Replicative publications on the same population with the same outcomes (for example, Thuesen 2010²⁰ and Husemoen 2006¹⁴) were evaluated, one of which with the largest sample size was included when performed meta-analysis.

Data extraction and management

Data was independently extracted by 2 reviewers (T.W. and H.P.Z.). For each study, the following details were extracted: study characteristics (country, study design, and sample size), folate status (reported as maternal use, personal use, objective measure, or gene polymorphism), the duration of follow-up, factors adjusted for the analysis, definition, and estimates of outcomes. To avoid confusing, variations in the definitions used for each outcome in original studies were unified. In this

study, atopy was defined as any positive skin prick test (SPT), and sensitization was defined as a positive test for specific immunoglobulin E (sIgE) to at least one of the allergens tested. The Newcastle-Ottawa quality assessment scale was used to evaluate the quality of eligible studies.²¹ A study with score ≥ 6 was considered to be of sufficient quality for our meta-analysis. In cases of disagreement, consensus was achieved following discussion with a third adjudicator (G.W.).

Statistical analysis

We followed the guidelines recommended by the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group.²² For dichotomous outcomes, the relative risk (RR) or odds ratio (OR) with 95% confidence intervals (CI) was calculated. When original data had been adjusted for potential confounding factors, adjusted estimates were pooled using the generic inverse variance method. The associations between maternal folate/folic acid status at different gestation periods (pre-pregnancy, early pregnancy, including the first trimester, whole pregnancy or other periods) and asthma/wheeze or other allergic outcomes were investigated. Heterogeneity was examined using the chi-square test with $P < 0.10$ indicating significant heterogeneity, and a I-square (I^2) statistic greater than 50% indicated moderate or high heterogeneity. The fixed-effects model was used for the meta-analysis if there was no significant heterogeneity ($I^2 < 50\%$); otherwise, the random-effects model. The Z test was used to determine the significance of the pooled estimates. Funnel plots and the Egger test were used to investigate study size effects indicating possible publication bias when outcomes were reported in at least 10 studies. Statistical analysis was conducted with Stata 11.0 (Stata Corp. LP, College Station, TX, USA), and a 2-sided P value less than 0.05 was regarded as significant.

The quality of the evidence related to the estimation of the association between maternal folic acid supplementation and asthma/wheeze or other allergic diseases in childhood or between *MTHFR* C677T polymorphisms and asthma risk followed the suggestions of the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Working Group by using the GradePro software (version 3.6).²³

RESULTS

Trials included, study characteristics and quality of reporting

The primary search strategy initially yielded a total of 1,814 citations from MEDLINE (PubMed) ($n = 141$), Cochrane Central ($n = 29$), Web of Science ($n = 297$), and EMBASE ($n = 1,347$). After removing duplicates and scanning the titles and abstracts, 23 articles were left over. After full-text reading and reference tracing, 26 eligible studies^{8-13,15,20,24-41} remained (Fig. 1).

The characteristics of the studies included are summarized in Tables S1-3 as supplementary data. Among these studies, 16

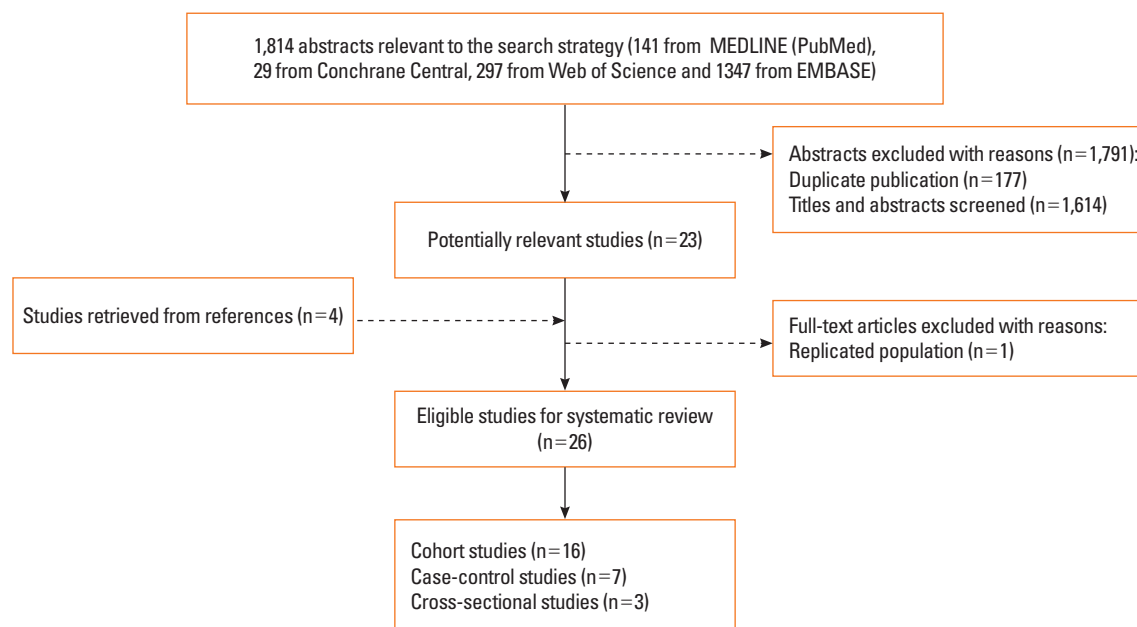


Fig. 1. Flowchart of study selection.

were cohort,^{8-13,20,24,27,31,34-38,41} 7 were case-control,^{25,26,28,29,32,33,39} and 3 were cross-sectional^{15,30,40} studies. The studies were undertaken in the US, the UK, the Netherlands, Denmark, Finland, Australia, Norway, Egypt, Korea, Japan, and India. The sample size ranged from 40 to 32,077. In the cohort studies, the follow-up duration ranged from 1 to 8 years. In birth cohort studies, the follow-up duration was more than 1 year for wheeze and 3 years for asthma. No eligible studies involving the development of hay fever were found.

Among the cohort studies, 12 (n=60,560) investigated the association between maternal folate exposure and asthma/wheeze incidence,^{8-13,24,27,31,37,38,41} while 4 (n=9,073) investigated the relationship between folate status at baseline and progress of concerned diseases.^{20,34-36} Of all the case-control studies, 5 (n=5,023) investigated the association between folate status and asthma/wheeze,^{26,28,29,33,39} and the remaining 2 (n=462) evaluated the association between folate status and AD.^{25,32}

Of the cross-sectional studies, 3 (n=10,891) investigated the association between folate status and targeted diseases.^{15,30,40} These studies mainly focused on the association of measurable folate concentration and the genotypes of subjects with specific outcomes.

The quality score of cohort studies ranged from 6 to 9 (mean 7.63, SD 1.09) (Table S1 for supplementary data), and that of case-control studies ranged from 4 to 8 (mean 6.71, SD 1.50) (Table S2 for supplementary data). All studies except 1³³ described the definition of specific outcomes.

Folate and asthma

The incidences of asthma in the offspring with maternal folic

acid supplementation during pregnancy (compared to no use) were pooled in 3 cohort studies.¹⁰⁻¹² There was an insignificant overall effect of maternal folic acid supplementation on the risk of asthma (RR=1.04, 95% CI=[0.94-1.16]). Also, no statistically significant differences in such relationships were observed before conception (RR=0.98, 95% CI=[0.73-1.33]), during early pregnancy (RR=0.98, 95% CI=[0.78-1.23]), or during other periods (RR=1.03, 95% CI=[0.92-1.16]) (Table 1).

Because of significant heterogeneity in measurement and outcome reporting, data about the relationship of the measurable folate level with asthma risk was not pooled. Two studies investigated the association between the measurable maternal folate level and asthma in the offspring. The cohort study by Magdelijns *et al.*¹² showed that higher maternal intracellular folic acid levels in pregnancy tend to decrease risk of childhood asthma in a dose-dependent manner (*P* for trend=0.05). The case-control study by Haberg *et al.*²⁶ found a trend of increasing risk of asthma for children aged 3 years across quintiles of maternal plasma folate levels in pregnancy (*P* for trend=0.006).

Five studies investigated the relationship of maternal folate concentration with risk of asthma. No significant association between the serum folate level and asthma onset^{29,30,34} or airway inflammation features of asthma (FENO, total IgE level, or the number of positive SPT)³⁶ was found in 3 studies. Thuesen *et al.*²⁰ found a significant inverse association between serum folate levels and risk of physician-diagnosed asthma, which is similar to that of a previous report.³⁹ Recently, van der Valk *et al.*³⁴ showed that folate levels of children at birth did not predict asthma-related outcomes up to the age of 6 years.

Four studies of case-control or cross-sectional design ex-

explored the association between *MTHFR* C677T polymorphism and asthma,^{15,20,27,33} but only 2 provided available effect measures.^{15,20} No significant association was found between the *MTHFR* C677T CT genotype and asthma development (OR=0.98, 95% CI=[0.82-1.17]), but the TT genotype was at risk of asthma in adults (OR=1.41, 95% CI=[1.07-1.86]) (Table 2 and Fig. 2).

Folate and wheeze

Two studies explored the relationship between maternal folic acid supplementation during pregnancy (compared to no use) and wheeze incidence in the offspring,^{11,12} and showed the insignificant overall effect of folic acid supplementation on the

Table 2. The relationship between *MTHFR* C677T polymorphism and the risk of asthma or other allergic diseases

Outcome	Genotypes	OR (95% CI)*	Heterogeneity (I^2 , %)
Asthma	CT genotype ^{15,20}	0.98 (0.82-1.17)	0.0
	TT genotype ^{15,20}	1.41 (1.07-1.86) [†]	21.5
Atopy	CT genotype ^{15,27}	1.05 (0.92-1.19)	0.0
	TT genotype ^{15,27}	0.93 (0.76-1.14)	0.0
Sensitization	CT genotype ^{15,20}	0.99 (0.89-1.10)	0.0
	TT genotype ^{15,20}	1.03 (0.86-1.23)	0.0

*vs the CC genotype; [†] $P=0.014$. OR, odds ratio; CI, confidence intervals.

Table 1. Effects of folate status on the development of asthma, wheeze, or other allergic diseases

Outcome	Exposure timing types of folic acid	Timing of assessment	Folic acid use, RR (95% CI)*	Heterogeneity (I^2 , %)
Asthma	Any use ^{10,12}	At 6 yr ¹⁰ , 3-8 yr ¹¹ , at 6-7 yr ¹²	1.04 (0.94-1.16)	0.0
	Pre-pregnancy ^{9,10}	At 3.5 yr and 5.5 yr ⁹ , at 6 yr ¹⁰	0.98 (0.73-1.33)	0.0
	Early pregnancy ^{9,10,12}	At 3.5 yr and 5.5 yr ⁹ , at 6 yr ¹⁰ , at 6-7 yr ¹²	0.98 (0.78-1.23)	0.0
	Other period in pregnancy ^{11,12}	3-8 yr ¹¹ , at 6-7 yr ¹²	1.03 (0.92-1.16)	0.0
Wheeze	Any use ^{11,12}	1-8 yr ¹¹ , until 6-7 yr ¹²	1.05 (0.95-1.15)	0.0
	Early pregnancy ^{8,12,13}	6-18 mo ⁸ , until 6-7 yr ¹² , up to 4 yr ¹³	1.06 (1.02-1.09) [†]	0.0
	Other period in pregnancy ^{8,11,12}	6-18 mo ⁸ , 1-8 yr ¹¹ , until 6-7 yr ¹²	1.01 (0.98-1.03)	0.0
Atopic Dermatitis	Early pregnancy ^{12,13}	at 2 yr ¹² , up to 4 yr ¹³	1.15 (0.91-1.45)	0.0
Eczema	Other period in pregnancy ^{11,12}	1-8 yr ¹¹ , until 6-7 yr ¹²	0.99 (0.89-1.10)	0.0
Sensitization	Other period in pregnancy ^{11,12}	At 8 yr ¹¹ , at 2 yr ¹²	0.88 (0.75-1.03)	0.0

*vs no folic acid use; [†] $P=0.001$. RR, risk ratio; CI, confidence intervals.

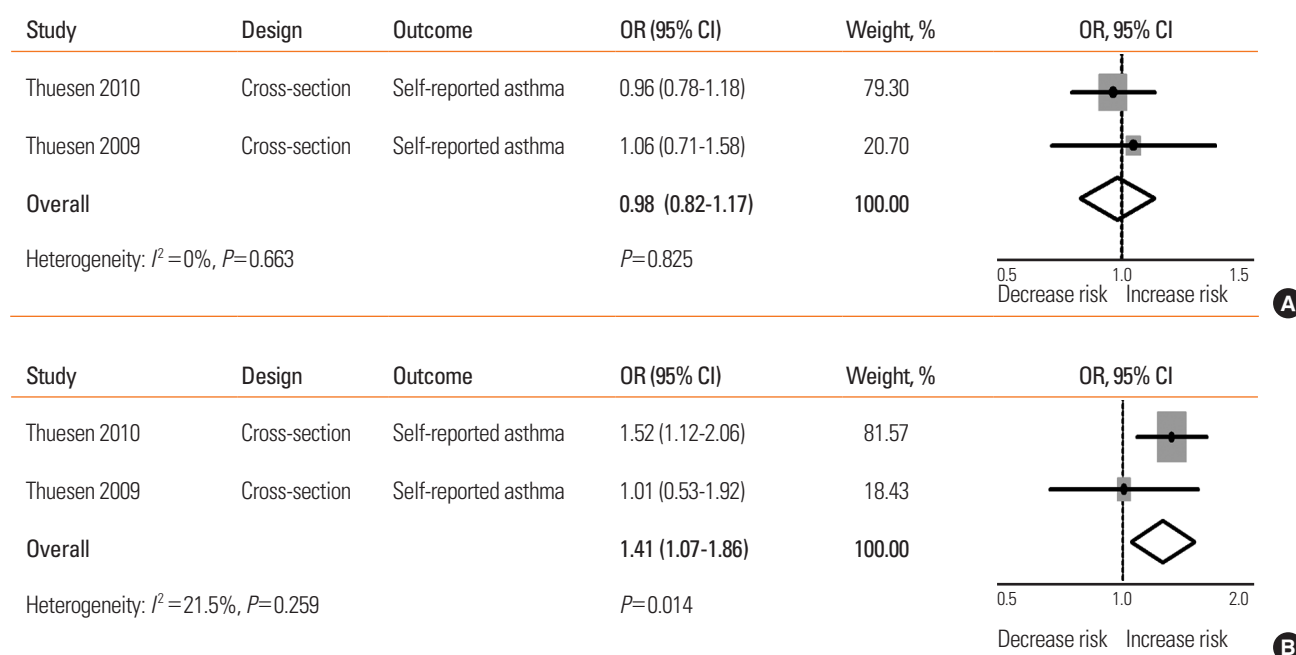


Fig. 2. The relationship between *MTHFR* C677T polymorphism and asthma risk. (A) CT vs CC; (B) TT vs CC. OR, odds ratio; CI, confidence intervals.

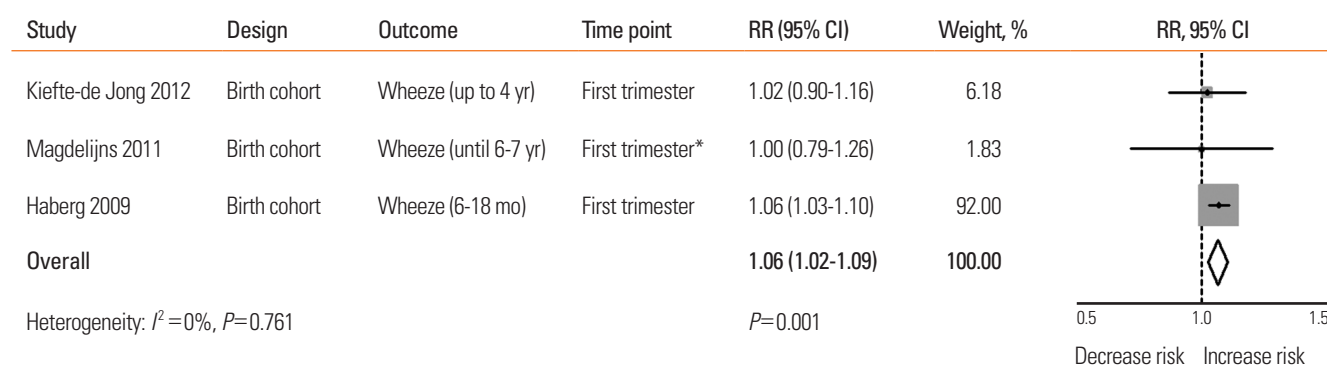


Fig. 3. The relationship between maternal folic acid supplementation during early pregnancy and risk of wheeze in childhood. *Four weeks before conception until 8 weeks after conception and/or during the first trimester of pregnancy. RR, risk ratio; CI, confidence intervals.

risk of wheeze (RR=1.05, 95% CI=[0.95-1.15]) (Table 1).

Three cohort studies investigated the relation of maternal folic acid supplementation during early pregnancy with the occurrence of wheeze in the offspring^{8,12,13} and found a significant pooled effect of maternal folic acid supplementation during early pregnancy on the incidence of offspring wheeze (RR=1.06, 95% CI=[1.02-1.09]) (Fig. 3). No association was found between maternal folic acid supplementation during other periods and risk of wheeze in childhood (RR=1.01, 95% CI=[0.98-1.03]).

No significant dose-response relationship was observed between maternal folic acid supplementation during pregnancy and the risk of wheeze.^{10,24,31} Furthermore, a recently published study did not find that early-life folate levels measured at ages 2, 4, 6, and 8 years were associated with asthma or wheeze at 6 years.³⁵ Kiefte-de Jong *et al.*¹³ found no association between maternal *MTHFR* C677T polymorphism and wheeze in the offspring.

Folate and other allergic diseases

No association of maternal folic acid supplementation during early pregnancy with the occurrence of offspring AD was found (RR=1.15, 95% CI=[0.91-1.45]) (Table 1). For eczema and sensitization reflected by sIgE status in the offspring, the association also did not reach statistically significant difference (Table 1).

Magdelijns *et al.*¹² also did not observe the relationship between maternal use of folic acid supplements during pregnancy and increased risk of AD at 2 years. A study by Oh *et al.*²⁵ found that intake of antioxidants nutrients, including folic acid, was negatively associated with AD development (OR=0.37, 95% CI=[0.18-0.73]). A cohort study by Kim *et al.*⁴¹ found that a maternal serum folate level of ≥ 9.5 ng/mL during mid-pregnancy was inversely associated with AD risk at 24 months (OR=0.52, 95% CI=[0.31-0.88]). A case-control study by Shaheen *et al.*³² found that serum folic acid levels were lower in AD patients than in the control subjects, although this did not reach statistical significance. Kiefte-de Jong *et al.*¹³ found no as-

sociation between maternal *MTHFR* C677T polymorphism and AD in the offspring.

Two studies investigated the association of the *MTHFR* C677T polymorphism with atopy or sensitization.^{15,20} Pooled analyses did not indicate the association between the *MTHFR* C677T polymorphism and atopy or sensitization (Table 2).

Assessment of the quality of evidence for the association between folate status and the development of asthma/wheeze or other allergic diseases

Evidence from observational studies was graded from low quality in term of the relatively higher level of heterogeneity or higher risk bias compared to randomized controlled trials.⁴² Furthermore, we performed the analyses at consistent exposure time that would reduce unexplained heterogeneity of the results. As a result, the quality of evidence related to the estimation of association between maternal folic acid supplementation and asthma/wheeze in childhood was very low (Table 3). Likewise, although genotyping is an objective definition, the quality of evidence related to the estimation of the association between *MTHFR* C667T polymorphisms and asthma, atopy, or sensitization risk was graded as low (Table 4). The overall quality of evidence was not upgraded due to lack of large effects or dose-response relationships.

DISCUSSION

Along with an increasing number of studies investigating the association between folate status and allergic outcomes, conflicts in this field have been on the rise. Thus, current meta-analyses provide no evidence for the relationship of maternal folic acid supplementation during pregnancy with asthma, AD, eczema, or sensitization in the offspring except that an increased risk of wheeze in childhood related to maternal folic acid supplementation during early pregnancy was observed. In addition, our meta-analysis found no evidence for any association between *MTHFR* C677T polymorphism and targeted out-

Table 3. The quality of evidence assessment of association between maternal folic acid supplementation and asthma/wheeze in childhood by the GRADE approach

Outcomes	Exposure timing	Relative effect (95% CI)	No. of participants (studies)	Quality of evidence (GRADE)
Asthma in childhood	Pregnancy ^{10,12}	RR=1.04 (0.94-1.16)	7,925 (n=3)	⊕⊕⊕⊕ Low 1
	Pre-pregnancy ^{9,10}	RR=0.98 (0.73-1.33)	2,056 (n=2)	⊕⊕⊕⊕ Very Low 2
	Early pregnancy ^{9,10,12}	RR=0.98 (0.78-1.23)	4,696 (n=3)	⊕⊕⊕⊕ Low 3
	Other period in pregnancy ^{11,12}	RR=1.03 (0.92-1.16)	6,426 (n=2)	⊕⊕⊕⊕ Low 4
Wheeze in childhood	Pregnancy ^{11,12}	RR=1.05 (0.95-1.15)	6,426 (n=2)	⊕⊕⊕⊕ Low 5
	Early pregnancy ^{8,12,13}	RR=1.06 (1.02-1.09)	43,459 (n=3)	⊕⊕⊕⊕ Low 6
	Other period in pregnancy ^{8,11,12}	RR=1.01 (0.98-1.03)	38,503 (n=3)	⊕⊕⊕⊕ Low 7
Atopic Dermatitis	Early pregnancy ^{12,13}	RR=1.15 (0.91-1.45)	11,382 (n=2)	⊕⊕⊕⊕ Very Low 8
Eczema	Other period in pregnancy ^{11,12}	RR=0.99 (0.89-1.10)	6,426 (n=2)	⊕⊕⊕⊕ Low 9
Sensitization	Other period in pregnancy ^{11,12}	RR=0.88 (0.75-1.03)	6,426 (n=2)	⊕⊕⊕⊕ Low 10

1 No factors exist that can reduce or increase the quality of evidence.

2 (-1 imprecision) 95% CI overlaps no effect (CI includes RR of 1.0) and CI fails to exclude 0.75 and 1.25 though the sample size is very large.

3 No factors exist that can reduce or increase the quality of evidence.

4 No factors exist that can reduce or increase the quality of evidence.

5 No factors exist that can reduce or increase the quality of evidence.

6 No factors exist that can reduce or increase the quality of evidence.

7 No factors exist that can reduce or increase the quality of evidence.

8 (-1 imprecision) 95% CI overlaps no effect (CI includes RR of 1.0) and CI fails to exclude 1.25 though the sample size is very large.

9 No factors exist that can reduce or increase the quality of evidence.

10 No factors exist that can reduce or increase the quality of evidence.

RR, risk ratio; CI, confidence intervals.

Table 4. The quality of evidence assessment of association *MTHFR* C667T polymorphisms and asthma risk by the GRADE Approach

Outcomes	Comparisons	Relative effect (95% CI)	No. of participants (studies)	Quality of evidence (GRADE)
Asthma	CT vs CC ^{15,20}	OR=0.98 (0.82-1.17)	7,991 (n=2)	⊕⊕⊕⊕ Low 1
	TT vs CC ^{15,20}	OR=1.41 (1.07-1.86)	7,991 (n=2)	⊕⊕⊕⊕ Low 2
Atopy	CT vs CC ^{15,27}	OR=1.05 (0.92-1.19)	6,571 (n=2)	⊕⊕⊕⊕ Low 3
	TT vs CC ^{15,27}	OR=0.93 (0.76-1.14)	6,571 (n=2)	⊕⊕⊕⊕ Low 4
Sensitization	CT vs CC ^{15,20}	OR=0.99 (0.89-1.10)	7,991 (n=2)	⊕⊕⊕⊕ Low 5
	TT vs CC ^{15,20}	OR=1.03 (0.86-1.23)	7,991 (n=2)	⊕⊕⊕⊕ Low 6

Quality of evidence (GRADE) as in Table 3.

OR, odds ratio; CI, confidence intervals.

comes except that the TT genotype was associated with asthma.

Blatter *et al.*¹⁶ reviewed the relationship between folate and asthma and did not support excessive worry about the adverse effect of maternal folate supplementation during pregnancy on asthma, although there was no adequate data to exclude a weak or modest effect. Brown *et al.*¹⁹ summarized epidemiologic evidence from 10 large prospective cohort studies supporting or refuting the relationship between maternal folate exposure in pregnancy and asthma/allergy in the offspring. A previous meta-analysis conducted by Crider *et al.*¹⁸ did not support the association between folic acid supplementation in the periconceptional period or the first trimester and risk of asthma, but the sensitivity analysis of combined asthma and wheeze showed a significant association. Given a diagnosis of asthma is

difficult to establish in infants and toddlers but a diagnosis of symptoms, such as wheeze, are more suitable, it seems better to evaluate the effect of maternal folic acid supplementation during pregnancy on wheeze rather than asthma. A study by Haberg *et al.*⁸ reviewed in the 2 meta-analyses reported an increased risk of wheeze between the ages of 6 and 18 months, which is consistent with the result of Bekkers' study¹¹ in which an increased risk of wheeze was reported at age 1 year but not at later ages. Bekkers *et al.*¹¹ evaluated the effects of maternal folic acid supplementation during pregnancy on wheeze or asthma, respectively, from age 1 or 3 years to 8 years and reported an increased risk association with wheeze but not asthma. Thus, we speculated that not all children with wheeze in early life has a diagnosis of asthma in later life and as time goes on,

the effect of maternal folate status on respiratory health in the offspring goes down. However, both of these findings were driven by the large cohort study conducted by Haberg *et al.*,⁸ which weighted 89% of the summary estimate in the Crider's study¹⁸ and 92% in this study. To address the question of a possible causal association between maternal folic acid status in pregnancy and risk of asthma or wheeze in children, a number of additional studies or analyses should be undertaken considering the misclassification of wheeze and asthma with age factor and follow-up duration.

To better understand the possible association, timing of folate intake during pregnancy should be taken into consideration. Since most clinical trials reviewed in this article provided direct evidence that there is no association between maternal folic acid supplementation during pregnancy and asthma,¹⁰⁻¹² wheeze,¹¹⁻¹³ shortness of breath,¹³ AD,^{12,13} or eczema,^{11,12} objective measurements, including allergic sensitization and bronchial hyperresponsiveness, should be made.¹¹ Nevertheless, studies referred to maternal folic acid supplementation at different periods during pregnancy seemed to have different results. Whitrow *et al.*⁹ reported that maternal folic acid supplementation in late pregnancy (30-34 weeks) increased the risk of childhood asthma at 3.5 years. Bekkers *et al.*¹¹ assessed the risk at similar exposure time (median 33 weeks) and reported an increased risk of wheeze at age 1 year. Moreover, Haberg *et al.*⁸ found that early pregnancy (any 4-week-period during weeks 0-12 in pregnancy) increased the risk of childhood wheeze in infants up to 18 months. Because widespread DNA methylation programming occurs in the early embryo, the association between maternal folate status during early pregnancy and respiratory health in early childhood might receive more attention.

Although several studies measured direct or indirect folate exposure and investigated a dose-response association of folate with allergic outcomes, it is not possible to do quantitative analyses due to diverse exposure assessments. Both positive^{13,26,31} and negative^{12,20,30} correlations were observed in current evidence. It is possible that these inconsistencies reflect complex relationships between direct or indirect folate exposure and the risk of asthma/wheeze or other allergic outcomes. Recent studies showed that folate levels measured in early life did not affect asthma-related outcomes.^{34,35} Interestingly, another study conducted by Lin *et al.*³⁶ found "bell-shaped" relationships between serum folate and some inflammatory biomarkers of asthma. Likewise, nonlinear relationships were found between serum folate levels and fractional exhaled nitric oxide, total IgE, or the frequency of positive SPTs. The folate level in the second quartile was significantly associated with increased total IgE when compared to the first quartile (the lowest). However, the levels of inflammatory biomarkers in the third or fourth (the highest) quartiles were similar to those in the first quartile and had no association with folate levels. Therefore, high-quality evidence is needed to interpret this complex network. For fu-

ture studies, objective measurements can be included, and plasma folate concentration might be a better biomarker. In addition, measurements should be taken into account to evaluate not only indirect folate exposure resulting from mothers during pregnancy, but also the folate status of subjects themselves. The dose, timing, and source of folate exposure should also be considered.

The *MTHFR* gene encodes the protein for making an enzyme called methylene tetrahydrofolate reductase that catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is required for the conversion of homocysteine to methionine. *MTHFR* C677T polymorphism was assumed to be associated with the risk of asthma. An association was reported by Zhou *et al.*³³ that the TT genotype significantly increases the risk of atopic asthma compared to all of the CC and CT genotypes. However, subsequent studies found no association between maternal *MTHFR* C677T polymorphism and asthma,²⁷ wheeze,¹³ or AD¹³ in the offspring or mothers themselves.²⁷ Our meta-analysis found no evidence of the association between *MTHFR* C677T polymorphism and targeted outcomes except that the TT genotype was associated with asthma. In addition, a previous study found a significant interaction between *MTHFR* C677T and folate levels.³⁴ Because DNA genotyping is assigned at birth and as an objective measurement, utilizing such methods could help reduce measurement biases and help explore a causal association. Thus, intrinsic genetic factors that could impair folate metabolism for a long time might play an important role in the occurrence and development of asthma. However, because folate status is largely influenced by dietary intake, this hypothesis still needs to be confirmed by further studies, and interpretations of these complex relationships should be presented with caution.

Although this study has referred to almost all of the existing evidence we could acquire, limitations of this study should be noted. First, since the articles included in this study were not randomized controlled trials, some obvious or hidden confounding factors may be unavoidable more or less. Second, potential risk of bias resulting from differences in study design, outcomes assessment, measurements or follow-up performance might produce heterogeneity. Third, the dose-response relationship was not analyzed due to limited eligible studies. Fourth, although asthma or other allergic diseases are associated with both genetic and environmental effects,⁴³ it is difficult to analyze potential interactions between *MTHFR* C677T polymorphism and environmental factors, such as dietary intake, due to lack of data.

In conclusion, our study indicates that maternal folic acid supplementation during early pregnancy might increase the risk of wheeze in early childhood and that the TT genotype of *MTHFR* C677T polymorphism impairing folic acid metabolism would be at high risk of asthma development. These results can provide additional information for recommendations regard-

ing forced folate consumption or folic acid supplementation in pregnancy based on its well-established benefits for the prevention of congenital malformations. Further studies are needed to elucidate whether there is a critical window of susceptibility that may reduce risk of neural tube defects without increased risk of asthma or other allergic diseases.

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Table S1. The characteristics of studies included in the systematic review on the relationship between folate status and asthma, wheeze or other allergic diseases (cohort study)

Study	Study Design; follow-up time	Definition of Outcomes	Measurement of Folate/ Folic Acid Status	Main Findings	Study Limitations	Adjusted Factors	Quality Assessment Score
Kim 2014 ¹	Birth cohort study of 917 children in South Korea, carried out from 2006-2011, embed in the MOCEH study; 2 yr	Asthma: physician-diagnosed or treatment for asthma in past year AD: physician-diagnosed or treatment for AD in past year	Serum folate level	Increased maternal folate level during mid-pregnancy was associated with a decreased risk of LRTIs at 6 mo and AD at 24 mo	Potential selection bias due to the substantial loss of follow-up (~50%) at 24 mo, no assessment of maternal folate status in the early pregnancy	Baby's sex, birth weight, gestational age, and duration of breastfeeding, and maternal age, the history of allergic diseases such as AD, asthma, allergic rhinitis, and allergic conjunctivitis, the urinary cotinine levels in mid- and late-pregnancy, and the prepregnancy BMI	7
van der Valk 2013 ²	Birth cohort study of 2001 children in Netherlands, carried out from 2002-2006, embedded in the Generation R study; 6 yr	Asthma: physician-diagnosed Wheeze: using questions from the ISAAC Eczema: physician-diagnosed or self-reported	Cord blood folate, <i>MTHFR</i> C677T polymorphism	No significant association between folate level at birth and wheeze or eczema until 4 yr, or asthma and eczema ever. Genetic mutations in <i>MTHFR</i> C677T and higher folate levels were associated with increased risk of eczema until 4 yr	Lack of data on maternal folate status, potential selection bias due to missing cord blood samples	Maternal age, BMI, educational level at intake, history of maternal atopy or asthma, parity, smoking, folic acid supplement use and pet keeping during pregnancy, and children's sex, gestational age, birth weight and day care attendance, based on the significance of their associations with repeated wheezing ($P < 0.05$), or a change in effect estimate of $> 10\%$	8
Okupa 2013 ³	Cohort study of 138 children (2-9 yr) in USA, carried out from 1998, embedded in the COAST study; 8 yr	Asthma: physician-diagnosed and/or the use of asthma medication Sensitization: 1 or more positive (> 0.34) specific IgE results	Plasma folate level	Higher plasma folate levels at or before age 6 yr were associated with allergic sensitization but not with IgE production, wheeze, or asthma at age 6 yr	Small sample size, no assessment of dietary intake, and limited adjustment for covariates	Gender and socioeconomic status	6
Lin 2013 ⁴	Cohort study of 150 children (5-17 yr) in USA; 1 yr	Asthma: long-term controller medication for asthma or meeting National Asthma Education and Prevention Program guideline Atopy: at least 1 positive skin prick test (SPT) result	Serum folate level	Serum folate level was not significantly associated with FENO, the number of positive SPTs, lung function, or hospitalizations for asthma. A folate level in the second quartile was associated with increased total IgE when compared with the lowest folate level. Increased serum folate level was associated with less exercise-related symptoms	Small sample size, no assessment of dietary intake, lack of multiple testing corrections, and limited adjustment for covariates	Age, sex, and education	6

(Continued to the next page)

Table S1. Continued

Study	Study Design; follow-up time	Definition of Outcomes	Measurement of Folate/Folic Acid Status	Main Findings	Study Limitations	Adjusted Factors	Quality Assessment Score
Martinussen 2012 ⁵	Birth cohort study of 1,499 children in USA, carried out from 1997-2000, embedded in the AIP and later the PRAM study; 6 yr	Asthma: physician diagnosed and current symptoms	Maternal folic acid supplement	No significant association between maternal folate supplement in the first trimester and childhood asthma at 6 age yr	Lack of data on maternal dietary intake	Maternal marital status, family income, and maternal asthma.	9
Bekkers 2012 ⁶	Birth cohort of 3,786 children in Netherlands, carried out from 1996-1997, embedded in the PIAMA study; 8 yr	Asthma: symptoms and/or doctor-diagnosed Wheeze: at least one attack of wheeze Eczema: an itchy rash that came and went on typical eczema sites Sensitization: serum IgE ≥ 0.70 IU/mL ⁻¹	Maternal folic acid supplement	Maternal folic acid use was associated with wheeze at age 1 yr and eczema at age 7 yr. No overall (from 1 to 8 yr of age) associations between maternal folic acid supplementation and (frequent) asthma symptoms, wheeze, LRIs and eczema	Lack of data on maternal dietary intake	Maternal education, maternal allergy, maternal smoking during pregnancy and number of older siblings	9
Kieffte-de 2012 ⁷	Birth cohort study of 8,742 children in Netherlands, carried out from 2002-2006, embedded in the Generation R study; 4 yr	Wheeze: symptoms, ISAAC AD: doctor-diagnosed	Maternal <i>MTHFR</i> C677T polymorphism, maternal folic acid supplement, and maternal plasma folate	Higher maternal plasma folate level in early pregnancy was associated with increased prevalence of AD but not with wheeze and shortness of breath at age 4 yr	No assessment of maternal dietary intake, and measured plasma folate level at a very early stage in pregnancy (13 ± 2.0 wk of gestation)	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 birth weight SD score derived from generalized estimation equations.	8
Dunstan 2012 ⁸	Birth cohort of 484 children in Australia; 1 yr	Asthma: recurrent wheeze to be responsive to bronchodilator medications Eczema: typical skin lesions Food Allergy: IgE-mediated food allergy and a positive SPT to the implicated food Sensitization: SPT positive	Folate consumption from foods and folic acid supplement, maternal and cord blood serum folate level	Maternal folate taken as a supplement in higher doses during the third trimester was significant associated with eczema at age 1 yr. With cord folate levels <50 nmol/L and >75 nmol/L was associated with greater sensitization risk than levels between 50 and 75 nmol/L	Short duration of follow-up and limited adjustment of co-variables	Maternal allergy and infant postnatal diet	6

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Table S1. Continued

Study	Study Design; follow-up time	Definition of Outcomes	Measurement of Folate/ Folic Acid Status	Main Findings	Study Limitations	Adjusted Factors	Quality Assessment Score
Magdalijs 2011 ⁹	Birth cohort of 2,640 children in Netherlands, carried out from 2000, embedded in the KOALA Birth Cohort Study, 6-7 yr	Asthma: physician-diagnosed with clinical symptoms and/or asthma medication use Wheeze: parents report Eczema: parents report AD: symptoms, UK Working Party criteria Sensitization: sIgE >0.3 IU/mL for ≥ 1 of the tested allergens	Maternal folic acid supplement and intracellular folic acid level	No significant association between maternal folic acid supplement use during pregnancy and AD and sensitization at age 2 yr or wheeze, lung function, asthma, and eczema at age 6-7 yr. Maternal folate level in erythrocytes in late pregnancy was inversely associated with asthma at age 6-7 yr.] ($P=0.05$)	No assessment of maternal dietary intake, potential selection bias due to loss of follow-up, and no assessment of folate level in early pregnancy	Maternal antibiotic use during pregnancy, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, mode and place of delivery, birthweight, gender of the child, treatment with antibiotics during the first 6 mo of life, breastfeeding during the first 2 yr of life, exposure to domestic animals during pregnancy and the first 2 yr of life, exposure to environmental tobacco smoke in the first 6 to 7 yr of life, siblings, family history, recruitment group, maternal education level, daycare, and other supplement use during pregnancy.	8
Nwaru 2011 ¹⁰	Birth cohort of 2,441 children in Finland, carried out from 1996-1997, embedded in the Finnish Type 1 Diabetes Prediction and Prevention study; 5 yr	Asthma: physician-diagnosed plus either any wheezing symptom or asthma medication use in the past 12 mo, ISAAC Eczema: parents report	Maternal folate consumption from foods and folic acid supplement	No significant associations between maternal folate intake and eczema at age 5 yr	Potential recall bias	Sex of child, place of birth, season of birth, gestational age at birth, maternal age at birth, maternal basic education, maternal smoking during pregnancy, mode of delivery, number of siblings, parental asthma, parental allergic rhinitis, pets at home at 1 yr of age, and atopic eczema by 6 mo of age.	9
Miyake 2011 ¹¹	Birth cohort of 763 children in Japan, carried out from 2001-2003, embedded in the OMCHS study; 16-24 mo	Wheeze: had symptom 12 mo pre based on the International Study of Asthma and Allergies in Childhood (ISAAC) criteria Eczema: had symptom, ISAAC	Maternal folate consumption from foods	No significant association between maternal consumption of folate during pregnancy and wheeze or eczema at age 16-24 mo	No assessment of maternal folate supplementation, lack of analysis of folate intake at different stage of pregnancy, and potential selection bias	Maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema, and allergic rhinitis, changes in maternal diet in the previous 1 mo, season when data at baseline were collected, maternal smoking during pregnancy, baby's older siblings, baby's sex, baby's birth weight, household smoking in same room as infant, breastfeeding duration, age at which solid foods were introduced, age of infant at the third survey, and maternal intake of docosahexaenoic acid, n-6 polyunsaturated fatty acids, vitamin D, calcium, vitamin E, and b-carotene during pregnancy.	8

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Table S1. Continued

Study	Study Design; follow-up time	Definition of Outcomes	Measurement of Folate/ Folic Acid Status	Main Findings	Study Limitations	Adjusted Factors	Quality Assessment Score
Thuesen 2010 ¹²	Cohort study of 6,794 adults (30-60 yr) in Denmark, carried out from 1999-2001, embedded in the Inter99 study; 5 yr	Asthma: self-reported doctor-diagnosed Atopy: specific IgE $\geq$ 0.35kU/l to at least one of the four allergens	Dietary intake of folate, serum folate level and <i>MTHFR</i> C677T polymorphism	Low serum folate level and the TT genotype of the <i>MTHFR</i> C677T polymorphism were associated with increased prevalence of self-reported doctor-diagnosed asthma at baseline	Lake of data on vitamin supplements, limited analysis of longitudinal data with 5-yr follow-up, and potential bias due to loss of follow-up	Age, sex, total energy intake, smoking, alcohol intake, BMI, and socioeconomic status	7
Whitrow 2009 ¹³	Birth cohort study of 557 children in Australia, carried out from 1998-2000, embedded in the Generation 1 cohort study; 5.5 yr	Asthma: physician-diagnosed	Maternal folate consumption from foods and folic acid supplement	Maternal folic acid supplementation in late pregnancy significantly increase the risk of asthma 3.5 yr and of persistent asthma at 3.5 and 5.5 yr	No assessment of folate level, and potential selection bias due to loss of follow-up	Maternal education, maternal age, parity, gravida, gestational age, maternal asthma status, and breastfeeding (partial or full for <3 mo)	9
Haberg 2009 ¹⁴	Birth cohort study of 32,077 children in Norway, carried out from 2000-2005, embedded in the MoBa study; 1.5 yr	Wheeze: had symptom	Maternal folic acid supplement	Maternal folate supplementation in the first trimester was associated with increased risk of wheeze and LRI up to 18 mo of age	No assessment of dietary intake	Other vitamin supplements and cod liver oil in pregnancy, vitamin supplements and cod liver oil at 6 mo of age, and for maternal age, maternal atopy, maternal smoking in pregnancy, maternal educational level, postnatal parental smoking, sex, parity, birth weight, season born, breast feeding and type of day care	7
Granel 2008 ¹⁵	Birth cohort study of 5,364 children in UK, carried out from 1991-1992, embedded in the ALSPAC study; 7-8 yr	Asthma: physician-diagnosed and current symptom Atopy: SPT positive	Maternal and child <i>MTHFR</i> C677T polymorphism, Maternal and child folate consumption from foods, and maternal folic acid supplement	No significant association between <i>MTHFR</i> C677T genotype and asthma or atopy in mothers or children	No assessment of dietary intake in early pregnancy, and potential selection bias due to loss of follow-up	Exposure to pre-natal and post-natal smoking, maternal education and social class	7
Litonjua 2006 ¹⁶	Birth cohort of 1,290 children in USA, carried out from 1999-2002, embedded in the Project Viva study; 2 yr	Wheeze: mother-reported symptom Eczema: health care professional diagnosed	Maternal folate consumption from foods and folic acid supplement	No significant association between maternal folate intake in the first and second trimesters of pregnancy and wheeze or eczema at age 2 yr	Limited assessment of folate status at different stage of pregnancy, and potential selection bias due to loss of follow-up	Sex, maternal age, maternal asthma, paternal asthma, family income, passive smoke exposure, breastfeeding, and other	8

MOCEH, Mothers and Children's Environmental Health study; AD, Atopic dermatitis; BMI, body mass index; *MTHFR*, methylene-tetrahydrofolate reductase; ISAAC, International Study of Asthma and Allergies in Childhood; COAST, Childhood Origins of Asthma project; AIP, Asthma in Pregnancy; PRAM, Perinatal Risk of Asthma in Infants of Asthmatic Mothers; PLAMA, Prevention and Incidence of Asthma and Mite Allergy; OMCHS, Osaka Maternal and Child Health Study; MoBa, Norwegian Mother and Child Cohort Study; ALSPAC, Avon Longitudinal Study of Parents and Children

Table S2. The characteristics of included study (case-control study)

Study	Study Design	Definition of Outcomes	Measurement of Folate/Folic Acid Status	Main Findings	Study Limitations	Adjusted Factors	Quality Assessment Score
Haberg 2011 ¹⁷	Case-control study of 1,962 children (case/control: 507/1455) in Norway, carried out in 2002-2004, embedded in the MoBa study, and followed up to age 3 yr	Asthma: mother report and had used inhalant medication	Maternal blood plasma folate level	Maternal plasma folate in the second trimester of pregnancy was linearly associated with increased risk of asthma at age 3 yr	Lack of data on dietary intake, and nonassessment of folate status in early pregnancy	Maternal educational level, maternal age, parity, maternal atopy, maternal body mass index, maternal smoking in pregnancy, maternal smoking at age 3 yr, and supplement use at age 3 yr	7
Farres 2011 ¹⁸	Case-control study of 180 adults (case/control: 120/60) in Egypt, carried out in 2009-2010	Asthma: doctor-diagnosed, GINA 2007 Atopic: at least one positive SPT	Serum folate level	No significant association between serum folate levels and asthma or FEV1. Among atopic asthmatics, serum folate levels were inversely associated with total IgE levels, and the number of positive SPTs.	Small sample size, nonassessment of dietary intake, and limited adjustment for potential confounding factors	Age and sex	6
Bueso 2011 ¹⁹	Case-control study of 169 children (case/control: 93/76) aged 13-14 yr in Norway, carried out in 2005-2006, embedded in the ECA study	Asthma: doctor-diagnosed and/or symptoms and/or medication use, at least fulfilled two of the three criteria	folate consumption from foods and folic acid supplement	No significant association between dietary intake of folate and asthma	Small sample size, and limited adjustment for potential confounding factors	Age and sex	8
Shaheen 2011 ²⁰	Case-control study of 40 children (case/control: 20/20) in India, carried out in 2009-2010	AD: physician diagnosed	Serum folic acid level	No significant association between serum folic acid level and AD	Small sample size, lack of data on dietary intake, and lack of adjustment for potential confounding factors	NA	6
Oh 2010 ²¹	Case-control study of 422 children (case/control: 180/242) in Korea, carried out in 2006-2007	AD: ISAAC	folate consumption from foods and folic acid supplement	Intake of folic acid was inversely associated with AD risk	Lack of data on folate levels, and potential selection bias	Monthly household income, parental histories of allergic diseases, and the child's age, gender, body mass index, supplement intake (ye s/no), and total energy intake	8
Patel 2006 ²²	Case-control study of 1030 adults (case/control: 515/515) in UK, carried out from 1993-1998, embedded in the EPIC-Norfolk study	Asthma: physician diagnosed	folate consumption from foods	Increased intake of folate was associated with reduced risk of asthma	Nonassessment of folate supplementation	Pack years smoked, social class, BMI, increasing level of physical activity and level of education	8
Zhou 2003 ²³	Case-control study of 1,682 adults (case/control: 433/1,249) in Japan, carried out in 2001-2002	Asthma: NA	<i>MTHFR</i> C677T polymorphism	The TT genotype of <i>MTHFR</i> C677T was significant associated with increased risk of atopic asthma	Lack of data on folate levels, lack of clear asthma definition, and lack of adjustment for potential confounding factors	NA	4

MoBa, Norwegian Mother and Child Cohort Study; GINA, Global Initiative for Asthma; SPT, Skin Prick Test; AD, Atopic dermatitis; EPIC-Norfolk, Norfolk arm of the European Prospective Investigation of Cancer; *MTHFR*, methylene-tetrahydrofolate reductase.

Table S3. The characteristics of included study (cross-section study)

Study	Study Design	Definition of outcomes	Measurement of folate/folic acid status	Main Findings	Study Limitations	Adjusted factors
Thuesen 2009 ²⁴	Cross-sectional study of 1,207 adults (15-77 yr) in Denmark, carried out in 1997-1998	Asthma: diagnose of allergic asthma, and self-reported allergy and asthma SPT reactivity: at least one positive SPT slgE positivity: a positive test to at least one of the six inhalant allergens	<i>MTHFR</i> C677T polymorphism	No significant associations between <i>MTHFR</i> C677T polymorphism and asthma or allergy	Lack of data on folate levels, and potential selection bias	Sex, age, BMI, smoking, social status and alcohol intake
Matsui 2009 ²⁵	Cross-sectional study of 8,083 individuals (≥ 2 yr) in USA, collected data from the 2005-2006 National Health and Nutrition Examination Survey Wheeze: symptom Atopy: at least 1 positive allergen-specific IgE level	Asthma: doctor-diagnosed	Serum folate level	Higher serum folate levels were inversely associated with a lower risk of wheeze and atopy, but not with asthma	Nonassessment of the effect with different age group, and lack of data on dietary intake	Age, sex, race/ethnicity, and poverty income ratio
Woods 2003 ²⁶	Cross-sectional study of 1,601 adults in Australia, carried out in 1999 Atopy: a ≥ 3 mm wheal diameter in response to any allergen in SPT	Asthma: self-reported or doctor-diagnosed	folate consumption from foods	No consistent associations between intakes of folate and asthma, BHR, or atopy	Potential selection bias	Age, sex, body mass index, smoking status, region of birth, and family history of asthma

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