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# Maternal folate intake during pregnancy and childhood asthma in a population based cohort

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## At a Glance Commentary

### Scientific Knowledge on the Subject

Periconceptional folic acid supplement use at 400 µg per day is internationally recommended to prevent neural tube defects and mandatory folic acid food fortification has been implemented in some countries to better target women of reproductive age at the population level. In animal models and human studies, supplementation with folic acid and other methyl donors during pregnancy alters the offspring epigenome which can influence health outcomes in offspring. Various observational studies have examined the association between maternal folate exposure and children's respiratory outcomes. This literature has been summarized in systematic reviews as inconclusive with limitations from methodological issues, including possible differential loss to follow-up. Few studies have assessed total folate intake from both food and supplements in relation to asthma at school age when the diagnosis is more certain than at preschool age.

### What This Study Adds to the Field

Virtually complete follow-up of children was enabled by linkage to the Norwegian Prescription Database. Folate intake from both food and supplements was assessed with a questionnaire validated against maternal plasma folate measurements. Our findings suggest that pregnant women who take supplements containing folic acid, at or above the recommended dose, combined with a diet rich in folate, reach a total folate intake level associated with a slightly increased risk of children's asthma.

### Online data supplement

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

## Abstract

**Rationale:** A potential adverse effect of high folate intake during pregnancy on children's asthma development remains controversial.

**Objectives:** To prospectively investigate folate intake from both food and supplements during pregnancy and asthma at age seven years when the diagnosis is more reliable than at preschool age.

**Methods:** This study included eligible children born 2002-2006 from the Norwegian Mother and Child Cohort Study, a population-based pregnancy cohort, linked to the Norwegian Prescription Database. Current asthma at age seven was defined by asthma medications dispensed at least twice in the year (1,901 cases, n=39,846) or by maternal questionnaire report (1,624 cases, n=28,872). Maternal folate intake was assessed with a food frequency questionnaire validated against plasma folate. We used log-binomial and multinomial regression to calculate adjusted relative risks with 95% confidence intervals.

**Measurements and Main Results:** Risk of asthma was increased in the highest vs. lowest quintile of total folate intake with an adjusted relative risk of 1.23 (95% confidence interval 1.06 to 1.44) that was similar for maternally reported asthma. Mothers in the highest quintile had a relatively high intake of food folate (median 308, interquartile range 241-366 μg/day) and nearly all took at least 400 μg/day of supplemental folic acid (median 500, interquartile range 400-600).

**Conclusions**: In this large prospective population based cohort with essentially complete follow-up, pregnant women taking supplemental folic-acid at or above the recommended dose,

combined with a diet rich in folate, reach a total folate intake level associated with a slightly increased risk of asthma in children.

Word count: 249

## Introduction

Nutrition in pregnancy plays a key role in fetal growth and development and may impact the long-term health of children (1). The importance of adequate folate status for preventing neural tube defects (2), has led to the current World Health Organization (WHO) recommendation that women should take a folic acid supplement of 400 micrograms per day (μg/day) from the time of planning a pregnancy until 12 weeks gestation. To better target women of reproductive age in the population as a whole, mandatory folic acid food fortification has been implemented in some countries, including the United States, and is currently under consideration in Europe (3). Although folic acid food fortification and supplementation during pregnancy at these levels are considered safe (2), there are concerns about unintended consequences (4).

Several birth cohort studies have examined the association between maternal folate exposure during pregnancy and subsequent risk of children's respiratory diseases. This literature has been summarized, in several reviews, as conflicting, with limitations due to heterogeneity in classification of folate intake, the time periods considered for exposure and disease development, and outcome definitions (5-8). Two more recent studies reported associations between asthma in children over four years of age and the use of folic acid supplements containing 1,000 μg or more during pregnancy (9, 10). This dose coincides with, or exceeds, the upper tolerable limit for daily folic acid intake in adults set by the US Institute of Medicine (11).

We previously reported that use of folic acid supplements or higher maternal plasma folate levels in pregnancy was associated with increased risk of wheeze and respiratory tract infections up to 18 months (12) and asthma at three years of age (13) from the Norwegian Mother and Child Cohort Study (MoBa).

The objective of the current study was to investigate the association between maternal total folate intake during pregnancy and asthma in MoBa children who have reached age seven years, an age when the asthma diagnosis is more reliable than at preschool age. Norway offers advantages for the assessment of folate intake from foods because very few foods are fortified with folic acid.

The primary analysis of asthma at age seven years was based on prescription registry data enabling near complete follow-up of the cohort. We performed secondary analyses using maternal report of asthma and atopy on the questionnaire mailed at age seven years for comparison with our main results and to evaluate whether associations with asthma differed by current atopy.

## Methods

See online data supplement for more detail.

### Study population

MoBa (14, 15) is a population-based pregnancy cohort (95,248 mothers and 114,761 children born 1999-2009), linked to the Norwegian birth registry and Norwegian Prescription Database (follow-up to April 1st 2014). Asthma at age seven years was defined in eligible children (sample selection and eligibility criteria, which included reaching the required age, in Figure 1) by prescription data (n=39,846, cases 1,901) or maternal report (n=28,872, cases 1,624). Both samples included births from 2002 to 2006 and partially overlapped (n=23,199). Maternal plasma folate was available for a random sample of children born 2002-2003 (16): 2,724 with maternal FFQ data (validation sample) and 2,681 with prescription follow-up and covariate information for an exploratory asthma analysis.

### Measures of maternal folate intake and folate status

Total folate intake (expressed as folic acid equivalents or dietary folate equivalents (DFEs) (11) included food folate and folic acid from supplements, estimated from a validated food frequency questionnaire (FFQ) administered at about 22 weeks gestation (17, 18). Maternal plasma folate concentrations were measured (19) at Bevital AS laboratories (www.bevital.no) in a single non-fasting venous blood sample drawn around 18 weeks gestation.

### Outcome measures of children's asthma and atopy

In separate analyses we examined current asthma in children around age seven using either at least two pharmacy dispensations of asthma medications (inhaled β2–agonists, inhaled glucocorticoids, combination inhalers with β2-agonists and glucocorticoids, and leukotriene

receptor antagonists) or maternal report of the child ever having doctor-verified asthma plus either asthma symptoms or asthma medication use in the past year (listing a valid brand). From maternal reports of the child's eczema or allergy to either pollen or animal hair (cat or dog) with symptoms in the past year we created four mutually exclusive outcome groups: "atopy only", "asthma only", "asthma with atopy", and "neither"(reference).

**Covariates**

Potential confounders and other covariates evaluated (Table 1) were based on data from the birth registry (maternal age at delivery, parity, child's sex, and birth weight) or MoBa questionnaires completed around gestational weeks 18 (baseline) and 30, and when the child was aged 6 or 18 months.

**Statistical analysis**

We used log binomial regression or multinomial logistic regression with robust cluster variance estimation to calculate relative risks (RRs) with confidence intervals (95% CIs) for children's asthma. The covariates adjusted for are listed in the footnotes of Tables 2 and 3. In sensitivity analyses we additionally adjusted for the child's birth weight (potential mediator), birth year and season, or postnatal child exposures at 6-18 months (duration of breast feeding, dietary supplement intake, and postnatal maternal smoking). Covariates were categorized as shown in Table 1 and entered as dummy variables, except for maternal age and energy intake (both continuous). We tested for multiplicative interaction between total folate intake and pre-pregnancy BMI, maternal history of atopy, and smoking. Missing values in covariates were handled by multiple imputation (10 imputations using chained equations). The statistical significance level was 5% for all tests. The analyses were conducted in Stata 13.0 (StataCorp LP, Texas, USA).

## Results

Characteristics of the two main study samples (prescription registry sample and maternal report sample) and the validation subsample compared with those eligible for analysis, are shown in Table E1 (online data supplement). In the larger prescription registry sample, 57.6% of children (22,957/39,846) had a mother who reported any folic acid supplement use in pregnancy. Among women who took folic acid, the median intake from supplements alone was 400 (interquartile range (IQR) 200-414) μg/day. Among all women, the median intake of folate from food sources was 257 (IQR 205-321) μg/day. In the validation subsample (n=2,724), the plasma folate concentration (median 8.7, IQR 5.9-14.7) increased across quintiles of total folate intake (medians 6.5, 6.9, 8.8, 11.2 and 16.0 nmol/L for quintiles 1-5 in order, Table E2, online data supplement) and the overall Spearman correlation (continuous) was 0.45.

Women in the highest quintile of total folate intake tended to be older, lighter, and less likely to smoke. They were also more likely to be primiparous, atopic, take other dietary supplements and were more highly educated (Table 1). Children born to mothers in the highest quintile of total folate intake were more likely to have been breastfed for at least 6 months and to have received dietary supplements at 6 and 18 months (Table 1). Patterns were similar in the maternal report sample (data not shown). All patterns held for quartiles of maternal plasma folate (results not shown), with the exception of a lack of association with maternal atopy. Plasma folate was also associated with gestational week of sample collection.

The prevalence of current asthma at age seven years based on prescription registry data was 4.8% (1,901/39,846). Children born to women in the highest vs. lowest quintile of total folate intake during pregnancy had more frequent asthma. In the highest quintile of pregnancy intake

5.6% (449/8,032) of children had been dispensed asthma medications at age seven compared with 4.6% (363/7,869) in the lowest (Table 2). The relative risk after multivariable adjustment was 23% higher in the highest versus lowest quintile (Table 2). The secondary analysis of asthma by maternal report gave similar results (Table E3, online data supplement).

Sensitivity analyses are presented in Table E4 (online data supplement). Results were robust to adjustment for the child's birth weight, or birth year and season, or postnatal childhood exposures. As expected, results were virtually identical for total folate expressed in folic acid equivalents and in dietary folate equivalents. Because some other studies have examined use of higher dose supplements, we divided the top quintile into three categories: 579-799, 800-999, and ≥1,000 μg (folic acid equivalents). Within the top quintile, risk did not increase with higher intakes, but data was sparse in the upper category (Table E4, online data supplement). In the much smaller plasma folate sample there was no significant association with asthma or elevated risk in the highest category (Table E5, online data supplement).

Among children with current asthma at age seven defined by maternal report, 45% of cases (729/1,624) also had current atopy (Table 3). Compared with a reference category of neither asthma nor atopy, children born to mothers in the highest vs. lowest quintile of total folate intake during pregnancy had a higher relative risk of asthma only (33% increase) and asthma with atopy (51% increase). Although the p-value for trend across quintiles of folate intake was strongest for asthma with atopy, contrast analysis on the relative risks (highest vs. lowest quintiles) did not show statistically significant differences ($p$=0.49 for asthma with atopy vs. asthma only, and $p$=0.09 for asthma with atopy vs. atopy only, complete case analysis, n=27,369).

In stratified analyses (Table E6, online data supplement) results did not differ significantly according to pre-pregnancy BMI ($p_{interaction}$=0.22), maternal atopy ($p_{interaction}$=0.37) or maternal smoking ($p_{interaction}$=0.35). We examined the effect of food folate separately in users and nonusers of folic acid supplements and did not find a significant difference between groups ($p_{interaction}$=0.20).

## Discussion

In this population-based pregnancy cohort study, we observed a positive association between total folate intake during pregnancy and asthma at age seven in the offspring. Children born to women in the highest vs. lowest quintile of total folate intake during pregnancy had about 20% higher relative risk of asthma. Essentially all women in the highest quintile of total intake reported folic acid supplement use and took at least 400 μg/day of folic acid (Table E2, online data supplement) with many taking more; the median supplement intake was 500 μg/day, 25% above the 400 μg/day recommended both by the Norwegian government and the WHO. Women in the highest quintile of total intake, in addition to being supplement users, tended to have higher intakes from food sources; their median intake was 308 μg/day, nearly the cutpoint for the highest quintile of dietary folate intake.

In previous analyses of younger MoBa children we observed associations between maternal use of folic acid supplements and wheezing and lower respiratory illness up to 18 months of age (12). In a sample of 1,962 children with maternal plasma folate measurements during pregnancy we observed a positive association with asthma at age three years (13). The current report extends these findings on maternal folate exposure to school age when asthma is more reliably diagnosed and also includes evaluation of asthma with and without atopic illness.

### Limitations and strengths of this study

As in other large nationwide population based studies, we were not able to classify asthma based on clinical examination. Likewise we classified atopy based on questionnaire report of allergic conditions, limited to eczema and two aeroallergens, and some misclassification in our outcome measures cannot be ruled out. Another limitation of our study, as in any observational study, is

that we cannot exclude the possibility of residual or unmeasured confounding. Women who take folic acid supplements and have diets higher in folate might differ from other women in ways related to unknown causes of asthma, or health care utilization. Although we cannot exclude this possibility, Norway offers advantages because of the universal access to health care, both during pregnancy and childhood, and prescription medication coverage, coupled with a relatively narrow range of income variability. The objection could be raised that the association we are ascribing to folate could be due to correlated micronutrients from dietary supplements (21). However, adjustment for the common use of cod liver oil in Norway and other supplements, including multivitamins, had little impact on the results.

Our study has several strengths. We used a validated FFQ to estimate total folate intake from both food and supplements. Many previous studies have examined only supplement use or separately considered diet and supplements. Total folate intake from our FFQ correlated well with plasma folate. Because there was virtually no fortification with folic acid in Norway in the study period our estimation of intake of folate from foods was simplified and therefore possibly more accurate than folate estimates from populations consuming fortified foods. In addition, we had a large sample size (1,901 cases) providing good power to study asthma at school age. We also had data on a large number of potential confounders including postnatal child exposures.

Our primary outcome of asthma was defined by the filling of two prescriptions for asthma medications within a 12 month period. This is an objective outcome, requiring physician diagnosis. In a validation study of the MoBa seven year questionnaire items regarding asthma, we have found that even a single dispensing of asthma medication was very rare in the absence of the mothers report of doctor diagnosis of asthma (22). The requirement for a second prescription within 12 months in the current study decreases the possibility that the medication is

being prescribed for self-limited wheezing illness after a viral infection or as part of an evaluation to determine the child's symptoms response to asthma medication. By requiring two prescriptions we expect a higher positive predictive value which has been reported to be the most important property of an asthma definition when the goal is to estimate relative risks as in our study (23). In addition to the validation in our own MoBa study population (22), an earlier validation study in Sweden, with a similar health care system to Norway, concluded that asthma medication is a suitable proxy for asthma in older children and adults based on comparison of data from their prescription registry and national patient registries (24).

The use of this objective outcome also addresses potential selection bias caused by loss to follow-up at age seven years based on failure to return the questionnaire at this age because the prescription registry covers the entire population. Similar associations for asthma by maternal report and prescription data indicate that loss to follow-up had little influence. As expected, the prevalence of asthma at age seven years based on two dispensations of asthma medication (4.8%) is slightly lower than the reported national prevalence of around 6% for only one dispensation among children aged 6-12 years (25), but quite similar, suggesting little selection with regard to asthma among MoBa participants. Further, the prevalence among those eligible for the current study was the same as in study participants (Table E1, online data supplement).

**Comparison with other studies and potential mechanism**

The current study is the largest to date that examines total folate intake during pregnancy in relation to children's asthma at school age. The total folate intake in these Norwegian women is very similar to that of women of reproductive age in the US, which practices mandatory folic acid food fortification. Specifically, the median total folate intake in dietary folate equivalents (Table E1, online data supplement) was 482 versus 490 reported for nonpregnant women aged

19-30 years in the US NHANES study from 2003-2006 (26). Two recent studies reported 20-40% increased risk of asthma at school age (4.5 to 8 years) associated with dispensing of high dose maternal folic acid supplements: 1,000 μg in the study of Veeranki et al. (9) and 5,000 μg in the study of Zetstra-van der Woude et al. (10). Our study suggests that a lower intake of total folate is associated with increased risk of asthma. We only observed an increased risk in the top quintile, which could suggest a threshold effect. This level of intake (≥578 μg/day of folic acid equivalents) can be reached by taking the WHO recommended dose of 400 μg folic acid combined with either a multivitamin containing folic acid or at least 300 μg/day of food folate. Four prospective studies of childhood asthma at school age (5 to 8 years), have examined folic acid supplement use during pregnancy without finding an association (27-30); sizes ranged from 130 to 605 cases. Thus, differences may be due in part to the greater power in the current study with 1,901 cases. However, because these studies did not include a measure of total folate intake, from both foods and supplements, their null findings may not be in conflict with ours. The US study (30) recruited pregnant women from 1997 to 2000, mostly after folic acid food fortification was implemented. Thus, the folate intake among the reference group of women, who did not take folic acid supplements, could already be above a threshold where risk of asthma does not increase much further. Of note, a US study found no evidence for protection against neural tube defects from folic acid supplement use in pregnancy and interpreted this result as evidence that fortification had already increased intake sufficiently to prevent these birth defects (31).

Studies with null results from European countries without mandatory folic acid fortification of the food supply (27-29) only analyzed food folate or folic acid supplements, but not the combined intake, and thus may not have identified the pregnancies with the highest total intake of folate. Two previous studies of children's asthma at school age used FFQs to assess folate

intake amounts from food and supplements, as in our study, during pregnancy (32, 33). Nwaru et al. (32) studied children at age five years in Finland and found no association of total folate intake with asthma. Although the dietary folate intake was relatively high (mean 364 μg/day), the folic acid intake from supplements was very low (mean 48 μg/day). In contrast, the Australian study population of Withrow et al. (33) took higher dose folic acid supplements during early pregnancy (median 658 μg/day) and increased risks of asthma at age 3.5 years, and persistent asthma at ages 3.5 and 5.5 years were observed.

Few studies to date have investigated maternal circulating folate levels in relation to asthma in school age children. Magdelijns et al. (28) reported a tendency of an inverse association between red blood cell folate in late pregnancy (week 35) and asthma risk at age six to seven years (n=837, 43 cases in total). The lack of association with plasma folate in the current study likely reflects the much lower statistical power in the plasma subsample with only 127 cases compared with 1,901 in the primary analysis of folate intake and 507 cases in our previous case-control study of asthma at age three (13). Very large studies with measurements of red blood cell folate, which reflects longer term status than plasma folate, may be necessary to more definitely answer the question of whether maternal folate status in the first trimester is associated with children's asthma development, because other lifestyle factors, metabolism and genetics all can affect folate levels in addition to intake.

Folate (natural or as synthetic folic acid) provides, with vitamin B12 as cofactor, methyl groups for the synthesis of methionine and S-adenosyl-methionine, which acts as a methyl donor (4). Epigenetic modification, more specifically DNA methylation, provides one potential mechanism for an effect of *in utero* exposures to folate and other methyl donors on the offspring (34). Although it did not address relevance to asthma, a recent study identified differential methylation

at various loci in newborns in relation to maternal folate levels in pregnancy (35). In an *Agouti* mouse model, supplementation of mothers with folate and other methyl donors just before and during gestation and during lactation, abrogated effects of an environmental contaminant (bisphenol-A) on methylation in offspring (36). Interest in this mechanism with respect to asthma was stimulated by the report of Hollingsworth et al. in 2008 (34) that *in utero* supplementation with methyl donors in pregnant mice, altered locus-specific DNA methylation and predisposed to allergic airway disease by directing the differentiation of T lymphocytes toward a TH2 phenotype. One of the top differentially methylated loci, *RUNX3*, in a previous knock out mouse model (37), displayed an allergic asthma phenotype. However, this paper was recently retracted because of problems with the airway hyperresponsiveness data; the other data in the paper were not affected (38). We acknowledge that the mechanistic data on periconceptional folate, or other methyl donors, and asthma pathogenesis is very limited.

Folic acid supplementation during pregnancy has an established role in the prevention of neural tube defects but the mechanism of this protection remains largely unknown (2). Two MoBa studies have found the use of folic acid supplements during pregnancy to protect against other neurological outcomes including language delay (39) and autism spectrum disorders (40). Taken together with our results, these findings point towards multiple folate-dependent pathways in the fetus of importance to development of neurologic and immune systems.

## Conclusion

In this large prospective pregnancy cohort, pregnant women taking folic-acid containing supplements at or above the recommended dose, combined with a diet rich in folate, reach a total folate intake level that was associated with a slightly increased asthma risk in children. We note that increased risk of asthma was only seen in the highest quintile of intake in which women also had a generally folate rich diet. In populations with very low folate intake, women taking the WHO recommended dose of folic acid may not reach this level of intake. On the other end of the spectrum, the specific associations might not be observable in populations where mandatory folic acid food fortification has already been implemented.

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## Figure legends

Figure 1 Sample selection

## Tables/Figures

Figure 1

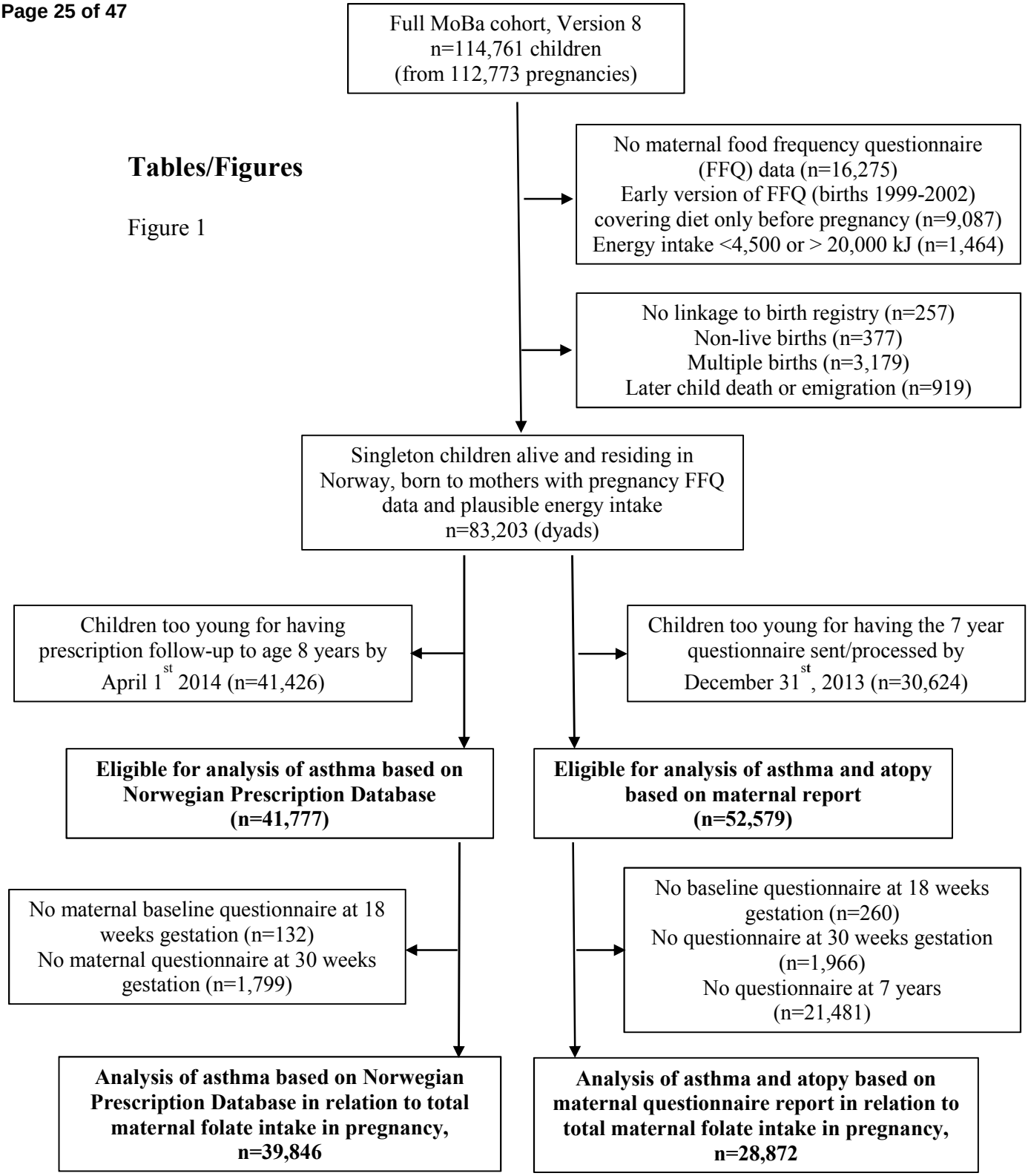

Table 1 Distribution of maternal and child characteristics according to total maternal folate intake in pregnancy, by quintiles (n=39,846)

| | Total folate intake (folic acid equivalents, µg/day) | | | | |
|---|---|---|---|---|---|
| | Q1 ≤146 | Q2 147-216 | Q3 217-391 | Q4 392-577 | Q5 ≥578 |
| N | 7,869 | 8,046 | 7,981 | 7,918 | 8,032 |
| Mean maternal age at delivery, years | 29.8 | 30.1 | 30.0 | 30.0 | 30.2 |
| No. of previous children (%) | | | | | |
| 0 | 36.4 | 37.9 | 43.5 | 47.1 | 52.0 |
| 1 | 39.5 | 36.9 | 34.3 | 35.2 | 32.5 |
| ≥2 | 24.1 | 25.2 | 22.2 | 17.7 | 15.5 |
| Maternal education* (%) | | | | | |
| Less than high school | 11.8 | 9.9 | 9.4 | 6.3 | 5.5 |
| High school | 38.0 | 33.7 | 32.9 | 27.7 | 28.1 |
| Up to 4 years of college | 36.7 | 38.9 | 40.0 | 44.8 | 44.7 |
| More than 4 years of college | 13.5 | 17.5 | 17.7 | 21.2 | 21.6 |
| Maternal pre-pregnancy BMI*, $kg/m^2$ (%) | | | | | |
| <18.5 | 2.2 | 2.6 | 3.1 | 2.7 | 3.0 |
| 18.5-24.9 | 59.0 | 63.5 | 65.1 | 65.4 | 67.0 |
| 25.0-29.9 | 25.4 | 24.3 | 21.7 | 22.8 | 21.2 |
| ≥30 | 13.3 | 9.6 | 10.1 | 9.2 | 8.7 |
| Mean maternal energy intake (kJ/day) | 8,150 | 10,174 | 10,680 | 9,387 | 10,439 |
| Maternal history of atopy (% Yes) | 31.1 | 30.5 | 33.2 | 34.4 | 36.8 |
| Cod liver oil taken in pregnancy (% Yes) | 29.5 | 36.6 | 39.0 | 44.0 | 46.3 |
| Other supplements taken in pregnancy† (% Yes) | 34.7 | 40.6 | 65.1 | 61.6 | 79.2 |
| Maternal smoking in pregnancy* (% Yes) | 12.8 | 10.6 | 10.1 | 7.1 | 7.0 |
| Postnatal maternal smoking at 18 months* (% Sometimes/daily) | 20.3 | 17.6 | 17.7 | 13.7 | 14.3 |
| Child's sex (% Boys) | 52.3 | 51.0 | 50.7 | 50.3 | 50.7 |
| Child's birth weight*, grams (%) | | | | | |
| <2,500 | 2.6 | 2.6 | 2.7 | 2.6 | 2.6 |
| 2,500-4,500 | 92.6 | 92.6 | 92.7 | 93.0 | 92.7 |
| >4,500 | 4.8 | 4.8 | 4.6 | 4.4 | 4.7 |
| Any breast feeding ≥ 6 months duration (% Yes) | 80.5 | 84.3 | 84.0 | 86.0 | 86.2 |
| Child given supplements at 6 months† (% Yes) | 56.2 | 60.8 | 62.3 | 61.8 | 64.5 |
| Child given supplements at 18 months† (% Yes) | 68.9 | 72.5 | 76.9 | 76.8 | 80.6 |

Pearson's $chi^2$ p-value ≤0.001 for all associations except with child's sex (p=0.1) and birth weight (p=0.98).

*Missing values: from <0.05% (birth weight) to 20% (child supplements at 18 months) as indicated in Table E1 (online data supplement).

†Other supplements taken in pregnancy contains at least one of the vitamins B2, B6, B12, C, A, D, and E. Children's supplements include cod liver oil and/or liquid multivitamins.

Table 2 Crude and adjusted relative risk (RR) estimates (95% confidence interval) for current asthma at age 7 years based on the Norwegian Prescription Database, by quintiles of total maternal folate intake in pregnancy (n=39,846)

| Total folate* | Cases/total (n) | Crude RR | Adjusted RR† |
|---|---|---|---|
| Q1 | 363/7,869 | 1.00 (ref) | 1.00 (ref) |
| Q2 | 351/8,046 | 0.95 (0.82,1.09) | 0.97 (0.83,1.13) |
| Q3 | 393/7,981 | 1.07 (0.93,1.23) | 1.07 (0.92,1.24) |
| Q4 | 345/7,918 | 0.94 (0.82.1.09) | 0.98 (0.85,1.14) |
| Q5 | 449/8,032 | 1.21 (1.06,1.39) | 1.23 (1.06,1.44) |
| $p_{trend}$ | | 0.01 | 0.01 |

*Quintile limits for total folate intake from food and supplements (folic acid equivalents, µg/day): Q1 (≤146), Q2 (147-216), Q3 (217-391), Q4 (392-577), Q5 (≥578).

†Adjusted for maternal age at delivery (continuous), parity (0, 1, ≥2), maternal education (less than high school, high school, up to 4 years of college, more than 4 years of college), pre-pregnancy BMI (<18.5, 18.5-24.9, 25.0-29.9, ≥30 kg/m$^2$), maternal history of atopy (no, yes), maternal smoking in pregnancy (no, yes), and use of cod liver oil (no, yes), other dietary supplements (no, yes), and maternal energy intake (continuous) in pregnancy. Missing values in covariates (Table E1, online data supplement) handled by multiple imputation (m=10) using chained equations.

Table 3 Crude and adjusted multinomial logistic regression relative risk (RR) estimates (95% confidence interval) for current asthma and/or current atopy at age 7 years based on maternal questionnaire report by quintiles of total maternal folate intake in pregnancy (n=28,872)

| | Neither asthma nor atopy n=22,788 | Current atopy only n=4,460 | | | Current asthma only n=895 | | | Current asthma with atopy n=729 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Total folate* | Reference n | Cases n | Crude RR | Adj RR† | Cases n | Crude RR | Adj RR† | Cases n | Crude RR | Adj RR† |
| Q1 | 4,190 | 758 | 1.00 (ref) | 1.00 (ref) | 160 | 1.00 (ref) | 1.00 (ref) | 116 | 1.00 (ref) | 1.00 (ref) |
| Q2 | 4,425 | 872 | 1.09 (0.98,1.21) | 1.11 (1.00,1.25) | 167 | 0.99 (0.79,1.23) | 1.07 (0.85,1.34) | 109 | 0.89 (0.68,1.16) | 0.96 (0.73,1.26) |
| Q3 | 4,461 | 819 | 1.01 (0.91,1,13) | 0.99 (0.88,1.11) | 165 | 0.97 (0.78,1.21) | 1.02 (0.80,1.29) | 133 | 1.08 (0.84,1.39) | 1.11 (0.84,1.47) |
| Q4 | 4,878 | 972 | 1.10 (0.99,1.22) | 1.06 (0.95,1.19) | 173 | 0.93 (0.75,1.16) | 0.99 (0.79,1.25) | 176 | 1.30 (1.03,1.66) | 1.36 (1.06,1.74) |
| Q5 | 4,834 | 1,039 | 1.19 (1.07,1.32) | 1.12 (1.00,1.26) | 230 | 1.25 (1.01,1.53) | 1.33 (1.05,1.68) | 195 | 1.46 (1.15,1.84) | 1.51 (1.16,1.96) |
| $p_{trend}$ | | | 0.001 | 0.11 | | 0.03 | 0.02 | | <0.0001 | <0.0001 |

*Quintile limits for total folate intake from food and supplements (folic acid equivalents, µg/day): Q1 (≤146), Q2 (147-216), Q3 (217-391), Q4 (392-577), Q5 (≥578).

†Adjusted for maternal age at delivery (continuous), parity (0, 1, ≥2), maternal education (less than high school, high school, up to 4 years of college, more than 4 years of college), pre-pregnancy BMI (<18.5, 18.5-24.9, 25.0-29.9, ≥30 kg/m$^2$), maternal history of atopy (no, yes), maternal smoking in pregnancy (no, yes), and use of cod liver oil (no, yes), other dietary supplements (no, yes), and

maternal energy intake (continuous) in pregnancy. Missing values in covariates (Table E1, online data supplement) handled by multiple imputation (m=10) using chained equations.

**Online Data Supplement: Maternal folate intake during pregnancy and childhood asthma in a population based cohort**

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## Unabridged methods section

### Study population

We used data from MoBa, a population-based prospective pregnancy cohort administered by the Norwegian Institute of Public Health (1, 2). MoBa recruited pregnant women nationwide between 1999 and 2008 at approximately 18 weeks gestation. Mothers could participate with more than one pregnancy, resulting in 95,248 mothers and 114,761 children born 1999-2009. The follow-up of children is ongoing through postal questionnaires and registry linkages using the national 11-digit personal identification number.

For the current study we analyzed MoBa data with linkage to two national health registries: the Norwegian birth registry and the Norwegian Prescription Database (henceforth referred to as the "prescription registry", end of follow-up April 1st 2014). The primary analysis of asthma at age seven years was based on prescription registry data (n=39,846, 1901 cases), which enabled near complete follow-up of the cohort to limit potential selection bias. In a secondary analysis we used maternal questionnaire report (n=28,872, cases 1,624). The sample selection and eligibility criteria are shown in Figure 1.

To define asthma using the prescription registry, children had to be old enough to have prescription data recorded 12 months past age seven years. This definition required longer follow-up (to eight years) than asthma based on maternal report at age seven years, but the prescription registry sample was larger because the follow-up was near complete. Both study samples included births from 2002 to 2006 and partially overlapped (n=23,199). Of the prescription registry sample 42% had no follow-up through the 7-year questionnaire; of the maternal report sample 20% were younger than eight years by the end of prescription follow-up.

Maternal plasma folate was available for a random sample of children born 2002-2003 (3): 2,724 with maternal FFQ data (validation sample) and 2,681 with prescription follow-up and covariate information for an exploratory asthma analysis.

**Ethical approval**

All participants in MoBa gave written informed consent at the time of enrolment. The Norwegian Data Inspectorate approved the ongoing data collection in MoBa (reference number 01/4325-69/HTL), and the linkage between MoBa and the Norwegian Prescription Database (reference number 08/00854-2/IUR). Ethical approval was obtained by the Regional Committee for Medical and Health Research Ethics of South/East Norway (reference number 2011/2313b).

**Exposure measures of maternal folate intake**

Maternal total folate intake included folate from foods and folic acid from supplements, estimated from a validated food frequency questionnaire (FFQ) administered at about 22 weeks gestation (4, 5). Starting in 2002, women reported their intake since becoming pregnant. Folic acid supplement use was defined as folic acid intake >0 µg/day from any supplement (folic acid only, multivitamin, or other). Total folate was expressed in µg of folic acid equivalents using the established conversion factor of 0.6 × food folate + supplemental folic acid (6). For comparability with some other studies we also calculated total folate in dietary folate equivalents (DFEs) as food folate + 1.7 × supplemental folic acid. Subject classification into quintiles of total folate intake was almost identical for folic acid equivalents and dietary folate equivalents (>99% agreement).

**Exposure measure of maternal folate status in mid-pregnancy**

A single non-fasting venous blood sample was drawn from mothers (median 18, interquartile range 17-19 gestational weeks) at a routine ultrasound examination offered to all pregnant

women, and processed as previously described (2). Plasma was mailed at ambient temperature to the MoBa biobank and stored at −80°C until analysis at Bevital AS laboratories (www.bevital.no). The plasma folate concentration was measured by microbiological assay (*Lactobacillus casei*) (7). The within- and between day coefficients of variation are 4% and 5% respectively and the lower limit of detection (LOD) is 2.0 nmol/L.

**Outcome measures of children's asthma and atopy**

Our primary asthma outcome was registry based and required that the child had at least two pharmacy dispensations of asthma medications in a year (at least one dispensation in the past 12 months at seven years in addition to a second dispensation within 12 months of the first). The ascertainment period was 12 months for all eligible children. The medication groups and Anatomical Therapeutic Chemical (ATC) classification codes included were inhaled β2–agonists (R03AC), inhaled glucocorticoids (R03BA), combination inhalers with β2-agonists and glucocorticoids (R03AK), and leukotriene receptor antagonists (R03DC). The active substances in each medication group are listed elsewhere (8). Noncases were all children who did not meet the criteria of having at least two dispensations of these medications around age seven.

As a secondary outcome we used maternal report of asthma on the follow-up questionnaire when the child was seven years old. The mother had to confirm all of the following: her child ever having asthma verified by a doctor and, in addition, either asthma symptoms or asthma medication use in the past year (listing at least one brand). Noncases were children with no asthma verified by a doctor regardless of symptoms or medication use.

To evaluate whether associations differed by the presence of atopy, we used a surrogate measure based on maternal report on the seven year questionnaire of the child's eczema or allergy to either pollen or animal hair (cat or dog) with symptoms in the past year. Using this

surrogate for atopy, we created four mutually exclusive groups: “atopy only”, “asthma only”, “asthma with atopy”, and “neither” (reference).

**Covariates**

Potential confounders and other covariates evaluated in the analysis (Table 1) were identified *a priori* based on the literature (9-11) or included for consistency with our previous study results (12-14). Variables were based on data from the birth registry (maternal age at delivery, parity, child’s sex, and birth weight) or MoBa questionnaires completed at about gestational weeks 18 (baseline) and 30, and when the child was aged 6 or 18 months. Maternal history of atopy was defined as ever reported asthma, allergy to pollen, animal hair, or “other” allergy, or atopic dermatitis. “Other” dietary supplements taken in pregnancy included supplements other than folic acid and cod liver oil that contained at least one of the vitamins B2, B6, B12, C, A, D, and E. Children’s supplement intake included cod liver oil and liquid multivitamins. Because the majority of Norwegian women breastfeed and have more than six months maternity leave, we dichotomized any breast feeding into two categories based on duration using a cutpoint of 6 months. Maternal smoking during pregnancy or at 18 months was treated as yes versus no because most women who smoked reported low amounts of smoking (median (interquartile range) = 5 (1-8) cigarettes per day during pregnancy and 10 (5-10) at 18 months). Gestational week of sample collection (categorized as ≤16, 17, 18, 19, ≥20 weeks) was included as a covariate in the analysis of maternal plasma folate.

**Statistical analysis**

We used log binomial regression to calculate relative risks (RRs) for children’s asthma in relation to quintiles of maternal total folate intake. Maternal plasma folate levels were analyzed as quartiles using a similar approach as for total folate intake.

For asthma with atopy we used multinomial logistic regression. Confidence intervals (95% CIs) were derived by robust cluster variance estimation to account for correlation between siblings. All adjusted models included the following prenatal maternal factors showing a univariate association with both exposure and outcome (confounders by classical definition): age at delivery, parity, education, pre-pregnancy BMI, smoking in pregnancy, history of atopy, and use of cod liver oil, other supplements (containing at least one of the vitamins B2, B6, B12, C, A, D, or E), and energy intake in pregnancy. In sensitivity analyses we additionally adjusted for the child's birth weight as a potential mediator, or birth year and season (January-March, April-June, July-September, October-December), or postnatal child exposures (duration of breastfeeding, supplement intake at 6 months and 18 months, and maternal smoking at 18 months). Covariates were categorized as shown in Table 1 and entered as dummy variables, except for maternal age and energy intake (both continuous). We tested for multiplicative interaction between total folate intake (highest vs. all lower quintiles) and the following variables: pre-pregnancy BMI ($\geq 25$ vs $< 25$ kg/m$^2$) (15), maternal history of atopy (no/yes) and smoking. Missing values in covariates were handled by multiple imputation using chained equations to generate 10 datasets. The statistical significance level was 5% for all tests. The analyses were conducted in Stata 13.0 (StataCorp LP, Texas, USA).

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E1: Distribution of maternal and child characteristics among eligible participants and study samples in the Norwegian Mother and Child Cohort Study

| | Asthma based on Norwegian Prescription Database | | | Asthma based on maternal report | |
|---|---|---|---|---|---|
| | Eligible | Study sample | Validation subsample | Eligible | Study sample |
| N | 41,777 | 39,846 | 2,724 | 52,579 | 28,872 |
| Maternal age at delivery, years (%) | | | | | |
| <25 | 11.6 | 11.3 | 12.1 | 11.3 | 8.7 |
| 25-30 | 42.6 | 42.8 | 43.9 | 42.4 | 41.8 |
| >30 | 45.8 | 45.9 | 44.0 | 46.3 | 49.5 |
| No. of previous children (%) | | | | | |
| 0 | 43.2 | 43.4 | 41.2 | 43.9 | 44.2 |
| 1 | 35.8 | 35.7 | 37.7 | 35.9 | 36.0 |
| ≥2 | 21.1 | 20.9 | 21.1 | 20.1 | 19.8 |
| Maternal education (%) | | | | | |
| Less than high school | 8.9 | 8.6 | 8.9 | 8.2 | 5.8 |
| High school | 31.9 | 31.9 | 35.4 | 30.9 | 27.9 |
| Up to 4 years of college | 40.4 | 40.9 | 38.8 | 40.6 | 44.3 |
| More than 4 years of college | 18.1 | 18.2 | 16.6 | 19.4 | 21.7 |
| Missing | 0.7 | 0.4 | 0.4 | 0.9 | 0.3 |
| Maternal pre-pregnancy BMI, $kg/m^2$ (%) | | | | | |
| <18.5 | 2.8 | 2.7 | 2.5 | 2.8 | 2.5 |
| 18.5-24.9 | 61.9 | 62.2 | 62.4 | 62.1 | 64.3 |
| 25.0-29.9 | 22.3 | 22.4 | 20.9 | 22.1 | 22.0 |
| ≥30 | 9.8 | 9.9 | 10.6 | 9.8 | 8.8 |
| Missing | 3.3 | 2.9 | 3.5 | 3.3 | 2.4 |
| Maternal history of atopy (%) | | | | | |
| No | 66.7 | 66.8 | 67.3 | 66.2 | 66.3 |
| Yes | 33.0 | 33.2 | 32.7 | 33.3 | 33.7 |
| Missing | 0.3 | 0.0 | 0.0 | 0.5 | 0.0 |
| Cod liver oil taken in pregnancy (%) | | | | | |
| No | 58.1 | 60.9 | 61.6 | 57.6 | 58.0 |
| Yes | 37.3 | 39.1 | 38.4 | 38.2 | 42.0 |
| Missing | 4.6 | 0.0 | 0.0 | 4.2 | 0.0 |
| Other supplements taken in pregnancy (%) | | | | | |
| No | 41.7 | 43.7 | 48.3 | 41.3 | 41.5 |
| Yes | 53.7 | 56.3 | 51.7 | 54.5 | 58.5 |
| Missing | 4.6 | 0.0 | 0.0 | 4.2 | 0.0 |
| Maternal smoking in pregnancy (%) | | | | | |
| No | 89.5 | 90.1 | 87.1 | 90.1 | 92.8 |
| Yes | 9.8 | 9.5 | 12.2 | 8.9 | 6.7 |
| Missing | 0.8 | 0.4 | 0.7 | 1.0 | 0.5 |
| Child's sex (% boys) | 51.1 | 51.0 | 50.9 | 51.1 | 51.3 |
| Child's birth weight, grams (%) | | | | | |

| | Asthma based on Norwegian Prescription Database | | | Asthma based on maternal report | |
|---|---|---|---|---|---|
| | Eligible | Study sample | Validation subsample | Eligible | Study sample |
| <2,500 | 2.9 | 2.6 | 2.1 | 2.7 | 2.6 |
| 2,500-4,500 | 92.4 | 92.7 | 92.8 | 92.7 | 92.9 |
| >4,500 | 4.7 | 4.7 | 5.2 | 4.5 | 4.5 |
| Missing | 0.04 | 0.04 | 0.0 | 0.04 | 0.03 |
| Birth season | | | | | |
| Jan-Mar | 23.6 | 23.6 | 21.4 | 23.3 | 24.0 |
| Apr-Jun | 21.9 | 21.9 | 7.3 | 24.5 | 25.0 |
| Jul-Sep | 28.5 | 28.4 | 27.4 | 28.9 | 28.9 |
| Oct-Dec | 26.0 | 26.0 | 43.9 | 23.2 | 22.1 |
| Birth year | | | | | |
| 2002 | 10.2 | 10.1 | 50.7 | 7.6 | 8.0 |
| 2003 | 27.0 | 26.9 | 49.3 | 21.0 | 20.7 |
| 2004 | 28.6 | 28.5 | - | 22.4 | 22.9 |
| 2005 | 31.0 | 31.3 | - | 24.5 | 24.7 |
| 2006 | 3.1 | 3.2 | - | 24.5 | 23.7 |
| Duration of any breast feeding (%) | | | | | |
| < 6 months | 15.6 | 15.8 | 15.9 | 15.3 | 14.8 |
| ≥ 6 months | 84.4 | 84.2 | 84.1 | 84.7 | 85.2 |
| Child given supplements at 6 months (%) | | | | | |
| No | 34.9 | 35.7 | 32.0 | 36.0 | 38.3 |
| Yes | 54.9 | 56.3 | 60.4 | 53.8 | 59.0 |
| Missing | 10.2 | 8.0 | 7.6 | 10.2 | 2.7 |
| Child given supplements at 18 months (%) | | | | | |
| No | 19.3 | 19.9 | 19.2 | 19.8 | 22.8 |
| Yes | 58.6 | 60.4 | 61.5 | 57.5 | 68.8 |
| Missing | 22.1 | 19.7 | 19.3 | 22.7 | 8.4 |
| Postnatal maternal smoking at 18 months (%) | | | | | |
| No | 64.3 | 66.3 | 63.1 | 64.2 | 77.8 |
| Sometimes/daily | 12.9 | 13.2 | 16.6 | 12.3 | 13.1 |
| Missing | 22.8 | 20.5 | 20.3 | 23.5 | 9.2 |
| **Folate exposures from food frequency questionnaire at about 22 weeks of pregnancy** | | | | | |
| Food folate, median (µg/day) | 258 | 257 | 255 | 259 | 258 |
| Folic acid from supplements in users only, median (µg/day) | 400 | 400 | 400 | 400 | 400 |
| Folic acid supplement use (%) | | | | | |
| No | 41.6 | 41.3 | 44.8 | 40.7 | 38.5 |
| Yes | 57.2 | 57.6 | 53.9 | 58.2 | 60.7 |
| Missing | 1.2 | 1.1 | 1.3 | 1.1 | 0.8 |
| Total folate as folic acid equivalents*, median (µg /day) | 284 | 287 | 251 | 295 | 319 |
| Total folate as dietary folate | 477 | 482 | 421 | 495 | 536 |

| | Asthma based on Norwegian Prescription Database | | | Asthma based on maternal report | |
|---|---|---|---|---|---|
| | Eligible | Study sample | Validation subsample | Eligible | Study sample |
| equivalents (DFE)*, median (µg /day) | | | | | |

*Food folate assumed to be 60% bioavailable relative to folic acid from supplements in the calculation of folic acid equivalents and dietary folate equivalents (DFE). Maternal folic acid supplement intake was missing for 0.8-1.1% in the study samples and only food folate was included in total folate intake.

Table E2: Relationship between quintiles (Q1 to Q5) of total folate intake, food folate, folic acid from supplements, and plasma folate during pregnancy in the study sample based on the Norwegian Prescription Database (n=39,846)

| | Total folate intake (folic acid equivalents, µg/day)* | | | | |
|---|---|---|---|---|---|
| | Q1 ≤146 | Q2 147-216 | Q3 217-391 | Q4 392-577 | Q5 ≥578 |
| N | 7,869 | 8,046 | 7,981 | 7,918 | 8,032 |
| Median (interquartile range) | | | | | |
| FFQ intake | | | | | |
| Food folate (µg/day) | 195 (166, 219) | 282 (257, 313) | 298 (231, 390) | 241 (199, 286) | 308 (241, 366) |
| Supplemental folic acid (µg/day), users only (n=22,957) | 22 (12, 29) | 57 (29, 86) | 171 (100, 200) | 400 (314, 400) | 500 (400, 600) |
| Supplemental folic acid (µg/day), all (n=39,403)* | 0 (0, 0) | 0 (0, 0) | 100 (0, 200) | 400 (300, 400) | 500 (400, 600) |
| % Contribution of folic acid to total folate intake (n=39,403)* | 0 (0, 0) | 0 (0, 0) | 38 (0, 57) | 73 (67, 77) | 74 (67, 81) |
| Total folate (folic acid equivalents)* | 118 (101, 132) | 176 (161, 194) | 287 (246, 335) | 517 (472, 546) | 680 (618, 780) |
| Total folate (dietary folate equivalents, DFE)* | 197 (168, 221) | 293 (268, 323) | 481 (411, 564) | 875 (798, 922) | 1,150 (1,044, 1,319) |
| % Folic acid supplement use (n=39,403) | 4 | 16 | 70 | 99 | >99 |
| Median (interquartile range) plasma folate (nmol/L), (n=2,724) | 6.5 (4.6, 9.3) | 6.9 (5.2, 10.1) | 8.8 (6.2, 13.3) | 11.2 (7.8, 18.6) | 16.0 (9.9, 23.4) |

*Food folate assumed to be 60% bioavailable relative to folic acid from supplements in the calculation of folic acid equivalents and dietary folate equivalents (DFE). Maternal folic acid supplement intake was missing for n=443 and only food folate was included in total folate intake.

Table E3: Crude and adjusted relative risk (RR) estimates (95% confidence interval) for children having current asthma at age 7 years based on the Norwegian Prescription Database compared with maternal questionnaire report, by quintiles (Q1 to Q5) of total folate intake (diet and supplements) in pregnancy

| | Prescription database N=39,846 | | | Maternal report N=28,872 | | |
|---|---|---|---|---|---|---|
| Total folate* | Cases/total (n) | Crude RR | Adj RR† | Cases/total (n) | Crude RR | Adj RR† |
| Q1 | 363/7,869 | 1.00 (ref) | 1.00 (ref) | 276/5224 | 1.00 (ref) | 1.00 (ref) |
| Q2 | 351/8,046 | 0.95 (0.82,1.09) | 0.97 (0.83,1.13) | 276/5,573 | 0.94 (0.80,1.10) | 1.00 (0.85,1.18) |
| Q3 | 393/7,981 | 1.07 (0.93,1.23) | 1.07 (0.92,1.24) | 298/5,578 | 1.01 (0.86,1.19) | 1.05 (0.89,1.25) |
| Q4 | 345/7,918 | 0.94 (0.82.1.09) | 0.98 (0.85,1.14) | 349/6,199 | 1.07 (0.91,1.24) | 1.13 (0.96,1.32) |
| Q5 | 449/8,032 | 1.21 (1.06,1.39) | 1.23 (1.06,1.44) | 425/6,298 | 1.28 (1.10,1.48) | 1.35 (1.14,1.59) |
| $p_{trend}$ | | 0.01 | 0.01 | | <0.0001 | <0.0001 |

*Food folate assumed to be 60% bioavailable relative to folic acid from supplements in the calculation of folic acid equivalents. Maternal folic acid supplement intake was missing for n=443 and only food folate was included in total folate intake. Quintile limits for total folate intake (folic acid equivalents, µg /day): Q1 (≤146), Q2 (147-216), Q3 (217-391), Q4 (392-577), Q5 (≥ 578).

†Adjusted for maternal age at delivery (continuous), parity (0, 1, ≥2), maternal education (less than high school, high school, up to 4 years of college, more than 4 years of college), pre-pregnancy BMI (<18.5, 18.5-24.9, 25.0-29.9, ≥30 kg/m$^2$), maternal history of atopy (no, yes), maternal smoking in pregnancy (no, yes), and use of cod liver oil (no, yes), other dietary supplements (no, yes), and maternal energy intake (continuous) in pregnancy.

Missing values in covariates (Web Table 1) handled by multiple imputation (m=10) using chained equations.

Table E4: Sensitivity analysis of adjusted relative risk (RR) estimates (95% confidence interval) for children having current asthma at age 7 years based on the Norwegian Prescription Database by quintiles (Q1 to Q5) of total folate intake (diet and supplements) in pregnancy (n=39,846)

| Total folate[*] | Cases/total (n) | Initial larger[†] model | Main model[‡] | Main model, complete cases[‡] | Main model, total folate in DFE[*] | Main model + child's birth weight[§] | Main model + birth year and season[§] | Main model + postnatal child exposures[§] |
|---|---|---|---|---|---|---|---|---|
| Q1: ≤146 | 363/7,869 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2:147-216 | 351/8,046 | 0.98 (0.84,1.14) | 0.97 (0.83,1.13) | 0.95 (0.82,1.11) | 0.98 (0.84,1.14) | 0.97 (0.84,1.13) | 0.98 (0.84,1.13) | 0.98 (0.83,1.15) |
| Q3:217-391 | 393/7,981 | 1.08 (0.93,1.25) | 1.07 (0.92,1.24) | 1.07 (0.92,1.25) | 1.07 (0.92,1.25) | 1.07 (0.92,1.24) | 1.08 (0.93,1.26) | 1.07 (0.91,1.26) |
| Q4:392-577 | 345/7,918 | 0.99 (0.85,1.16) | 0.98 (0.85,1.14) | 0.94 (0.81,1.11) | 0.99 (0.85,1.15) | 0.98 (0.85,1.14) | 0.99 (0.85,1.16) | 1.02 (0.87,1.19) |
| Q5: ≥ 578 | 449/8,032 | 1.24 (1.06,1.44) | 1.23 (1.06,1.44) | 1.22 (1.04,1.42) | 1.24 (1.06,1.45) | 1.23 (1.06,1.44) | 1.25 (1.07,1.46) | 1.23 (1.05,1.45) |
| $p_{trend}$ | | 0.01 | 0.01 | 0.03 | 0.01 | 0.01 | 0.007 | 0.01 |
| **Splitting Q5** | | | | | | | | |
| 578-799 | 350/6,248 | | 1.24 (1.06,1.46) | | | | | |
| 800-999 | 68/1,126 | | 1.30 (1.00,1.68) | | | | | |
| ≥ 1,000 | 31/658 | | 1.03 (0.71,1.48) | | | | | |

[*]Food folate assumed to be 60% bioavailable relative to folic acid from supplements in the calculation of folic acid equivalents and dietary folate equivalents (DFE). Maternal folic acid supplement intake was missing for n=443 and only food folate was included in total folate intake. Quintile limits for DFEs: Q1 (≤245), Q2 (246-361), Q3 (362-659), Q4 (660-976), Q5 (≥ 977).

[†]Adjusted for all factors listed in Table 1 (related to exposure and/or outcome): maternal age at delivery (continuous), parity (0, 1, ≥2), maternal education (less than high school, high school, up to 4 years of college, more than 4 years of college), pre-pregnancy BMI (<18.5, 18.5-24.9, 25.0-29.9, ≥30 kg/$m^2$), maternal energy intake (continuous, kj/day), maternal history of atopy (no, yes), use of cod liver oil (no, yes) and other dietary supplements (no, yes) in pregnancy, maternal smoking in pregnancy (no, yes), child's sex, child's birth weight (<2500, 2500-4500, >4500 grams), duration of any breast feeding (< 6 months, ≥ 6 months), dietary supplements given at 6 months (no, yes) and 18 months (no, yes), and postnatal maternal smoking at 18 months (no, sometimes/daily).

[‡]Main model is adjusted for maternal characteristics and prenatal factors related to both exposure and outcome: age at delivery (continuous), parity (0, 1, ≥2), maternal education (less than high school, high school, up to 4 years of college, more than 4 years of college), pre-pregnancy BMI (<18.5, 18.5-24.9, 25.0-29.9, ≥30 kg/$m^2$), maternal history of atopy (no, yes), maternal smoking in pregnancy (no, yes), and use of cod liver oil (no, yes) and other dietary supplements (no, yes) in pregnancy. Additional adjustment for maternal energy intake (continuous, kJ/day). Complete cases include the subsample with complete data for all covariates (n=38,398).

[§]Child's birth weight categorized as: <2500, 2500-4500, >4500 grams. Birth years 2002 to 2006 (categorical) and birth season (Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec). Postnatal child exposures: duration of any breast feeding (< 6 months, ≥ 6 months), dietary supplement intake at 6 months (no, yes) and 18 months (no, yes), postnatal maternal smoking at 18 months (no, sometimes/daily).

Table E5: Crude and adjusted relative risk (RR) estimates (95% confidence interval) for children having current asthma at age 7 years based on the Norwegian Prescription Database, by quartiles (Q1-Q4) of maternal plasma folate concentrations in mid-pregnancy (n=2,681)

| Plasma folate (nmol/L) | Cases/total (n) | Crude RR | Adj. RR* |
|---|---|---|---|
| Q1: ≤5.91 | 27/653 | 1.00 (ref) | 1.00 (ref) |
| Q2: 5.92-8.68 | 39/668 | 1.41 (0.87,2.28) | 1.53 (0.94,2.49) |
| Q3:8.69-14.69 | 38/679 | 1.35 (0.84,2.19) | 1.52 (0.92,2.51) |
| Q4: ≥14.70 | 23/681 | 0.82 (0.47,1.41) | 0.97 (0.54,1.76) |
| $p_{trend}$ | | 0.44 | 0.99 |

*Adjusted for maternal age at delivery (continuous), parity (0, 1, ≥2) maternal education (less than high school, high school, up to 4 years of college, more than 4 years of college), pre-pregnancy BMI (continuous), maternal history of atopy (no, yes), maternal smoking in pregnancy (no, yes), use of cod liver oil (no, yes) and other dietary supplements (no, yes) in pregnancy, and gestational week of sample collection (≤16, 17, 18, 19, ≥20 weeks).

Table E6: Stratified multivariable relative risk (RR) estimates (95% confidence interval) for children having current asthma at age 7 years based on the Norwegian Prescription Database by highest (Q5) vs. all lower quintiles (Q1-Q4) for intake of total folate or food folate in pregnancy (n=39,846)

| Folate exposure and stratification variable | Cases (Q1-Q4) | Cases (Q5) | Adj RR† Missing value imputation n=39,846 | Adj RR† Complete cases n=38,398 |
|---|---|---|---|---|
| **Total folate*** | | | | |
| Pre-pregnancy BMI, | | | | |
| < 25 kg/m$^2$ (n=25,839) | 863 | 274 | 1.16 (1.01,1.33) | 1.16 (1.01,1.33) |
| ≥ 25 kg/m$^2$ (n=12,864) | 550 | 164 | 1.33 (1.12,1.58) | 1.33 (1.12,1.58) |
| Missing (n=1,143) | 39 | 11 | | |
| $P_{interaction}$ | | | 0.22 | 0.20 |
| Maternal history of atopy | | | | |
| No (n=26,613) | 773 | 207 | 1.16 (0.99,1.35) | 1.16 (0.1.11,1.36) |
| Yes (n=13,233) | 679 | 242 | 1.28 (1.10,1.48) | 1.27 (1.10,1.48) |
| $P_{interaction}$ | | | 0.36 | 0.41 |
| Smoking in pregnancy | | | | |
| No (n=35,894) | 1,268 | 405 | 1.20 (1.07,1.35) | 1.21 (1.08,1.36) |
| Yes (n=3,774) | 178 | 43 | 1.40 (1.02,1.93) | 1.31 (0.94,1.83) |
| Missing (n=178) | 6 | 1 | | |
| $P_{interaction}$ | | | 0.37 | 0.66 |
| **Food folate*** | | | | |
| Folic acid supplement use | | | n=39,846 | n=37,989 |
| No (n=16,446) | 600 | 165 | 1.13 (0.94,1.35) | 1.13 (0.94,1.35) |
| Yes (n=22,957) | 841 | 277 | 1.29 (1.12,1.50) | 1.28 (1.10,1.49) |
| Missing (n=443) | 11 | 7 | | |
| $P_{interaction}$ | | | 0.20 | 0.24 |

*Upper quintile limit for total folate intake (folic acid equivalents, µg/day): Q5 (≥ 578).

Upper quintile limit for food folate (µg/day): Q5 (≥ 308).

[†]Adjusted for maternal age at delivery (continuous), parity (0, 1, ≥2), maternal education (less than high school, high school, up to 4 years of college, more than 4 years of college), pre-pregnancy BMI (<18.5, 18.5-24.9, 25.0-29.9, ≥30 kg/m2), maternal history of atopy (no, yes), maternal smoking in pregnancy (no, yes), use of cod liver oil (no, yes) and other dietary supplements (no, yes) in pregnancy, and maternal energy intake (continuous).

Missing values in stratification variables and other covariates handled by multiple imputation (m=10) using chained equations.

Complete cases include the subsample with complete data for all covariates (n=38,398) or for all covariates and folic acid supplement use (n=37,989).