Maternal folate levels in pregnancy and asthma in children at age 3 years

To the Editor:

Women are advised to increase their folate intake during early pregnancy to lower the risk of neural tube defects in their children.[1-3] Folate is involved in nucleotide synthesis, cell division, cell differentiation, and DNA methylation and is important in fetal development. Experimental data in mice and observational data in human beings have suggested possible adverse effects of folic acid supplement use in pregnancy on respiratory and allergic outcomes in offspring.[4-6] We previously reported that use of folic acid supplements in the first trimester of pregnancy was associated with an increased risk of respiratory tract infections and wheeze up to 18 months of age in children.[4] A recent study from Australia reported folic acid supplementation during late pregnancy to be associated with increased risk of asthma at age 3.5 years and with persistent asthma up to age 5 years.[5] However, none of these studies had biochemical measures of maternal folate status. In the current study, we examined the risk of asthma at age 3 years in relation to measured maternal plasma folate levels in pregnancy.

We conducted a case-control study nested within the Norwegian Mother and Child Cohort Study (MoBa), a large population based pregnancy cohort following more than 100,000 pregnant women and their offspring.[7] The current study included 1455 control children and 507 case children. Case children were children whose mothers reported that their child had asthma and had used inhalant medication in the past year on the 3-year questionnaire. Blood plasma folate was measured during the second trimester of pregnancy (median, 18 weeks). Details on the recruitment of the study population, folate measurements, and the statistical methods are contained in Fig 1 and the Methods section in this article's Online Repository at www.jacionline.org. The MoBa study has been approved by the Regional Committee for Ethics in Medical Research, the Norwegian Data Inspectorate, and the Institution Review Board of the US National Institute of Environment Health Sciences. This substudy was approved by the Regional Committee for Ethics in Medical Research.

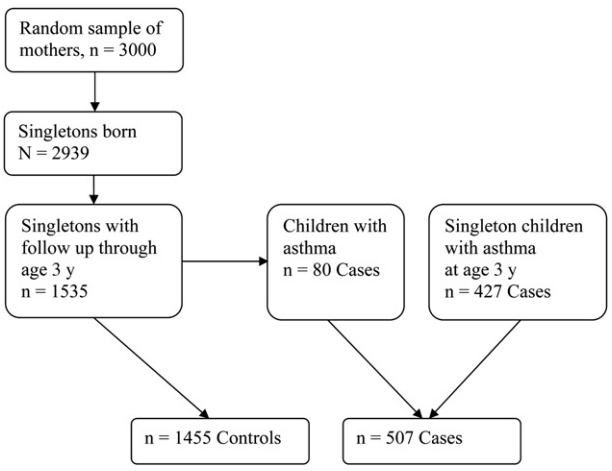

FIG 1. Flow chart illustrating the inclusion of case children and control children in MoBa.

TABLE I. Characteristics in case children (507 children with asthma) and 1455 control children in MoBa

Characteristic	n	Control children n = 1455 (%)*	Case children n = 507 (%)*
Maternal age (y)			
<25	248	12.1	14.2
25-20	945	47.2	50.9
>30	769	40.7	34.9
Maternal educational level (y)			
≤12	733	37.7	36.3
13-16	849	41.8	47.5
17+	324	17.3	14.2
Missing	56	3.2	2.0
Maternal smoking in pregnancy			
No	1752	88.5	91.7
Yes	194	10.5	8.1
Missing	16	1.0	0.2
Parity†			
0	849	44.2	40.6
1	758	36.5	44.8
>1	355	19.3	14.6
Maternal prepregnancy BMI			
<18.5	62	3.0	3.6
18.5-24.9	1233	63.4	61.1
25-29.9	412	20.6	22.3
30+	194	9.8	10.3
Missing	61	3.2	2.8
Maternal atopy†			
No	1320	70.7	57.6
Yes	642	29.4	42.4
Maternal smoking when the child is age 3 y			
No	1578	80.0	81.7
Yes	342	17.4	17.6
Missing	42	2.6	0.8
Child's use of vitamin supplements age 3 y			
No	1064	55.5	50.5
Yes	847	42.0	46.6
Missing	51	2.5	3.0
Child's use of cod liver oil supplements age 3 y			
No	881	44.1	47.1
Yes	1030	53.4	49.9
Missing	51	2.5	3.0

BMI, Body mass index.
*Percentages may not add to 100 due to rounding.
†$P < .05$.

Some baseline characteristics differed between mothers of asthma case children and control children (Table I). For example, maternal atopy was more prevalent among mothers of children with asthma. Therefore, we adjusted for potential confounding factors and present both crude and adjusted odds ratios in Table II.

The median maternal plasma folate level was 9.1 nmol/L, the 25th percentile was 6.2 nmol/L, and 75th percentile was 16.1 nmol/L. The median maternal plasma folate levels were 9.1 nmol/L for control children and 9.4 nmol/L for case children. As expected, plasma folate levels were substantially higher among women who reported use of folic acid supplements in pregnancy (median, 10.9 nmol/L) compared with nonusers (median, 5.8 nmol/L). The Spearman correlation was 0.46 between plasma

TABLE II. Crude and adjusted* odds ratios for asthma at 3 years of age according to maternal levels of plasma folate in the second trimester of pregnancy in 507 children with asthma (cases) and 1455 control children

			Asthma at age 3 y				
Maternal plasma folate† in pregnancy (nmol/L)	**Control children**	**Case children**	**Crude OR**	**(95% CI)**	**Adjusted OR**	**(95% CI)**	***P* value**
<5.54	293	83	1		1		
5.54-7.68	294	98	1.18	(0.84-1.64)	1.16	(0.80-1.66)	.44
7.68-10.60	283	105	1.31	(0.94-1.82)	1.48	(1.03-2.11)	.03
10.60-17.84	292	96	1.16	(0.83-1.62)	1.28	(0.89-1.85)	.18
>17.84	293	125	1.51	(1.09-2.08)	1.66	(1.16-2.37)	<.01
P trend			.03		.006		

OR, Odds ratio.

*Adjusted for maternal educational level, maternal age, parity, maternal atopy, maternal body mass index, maternal smoking in pregnancy, maternal smoking at age 3 years, and supplement use at age 3 years.

†Cutoffs based on quintiles of plasma folate levels in a random sample of 1535 women.

folate levels in the second trimester and questionnaire reports of folic acid supplement use after pregnancy week 13. Plasma folate levels were higher in nonsmokers than smokers, nulliparous than parous women, women older than 30 years, normal-weight women, and women with a higher educational level (data not shown). Table II presents the crude and adjusted odds ratios with 95% CIs for asthma at 3 years across quintiles of maternal plasma folate levels. There was an increased risk of asthma at age 3 years for children with maternal plasma folate levels in pregnancy in the highest compared with the lowest quintile (adjusted odds ratio, 1.66; 95% CI, 1.16-2.37). There was a trend of increasing risk across quintiles of plasma folate (*P* trend = .006).

Analyses with additional adjustments for maternal income, daycare attendance, the child's sex, birth weight, breast-feeding, or use of folic acid supplements did not substantially influence the results (data not shown).

As in any observational study, biases can limit causal interpretation of the associations. Accurate folate status may be difficult to obtain through questionnaires, and in the current study, biochemical measurements of pregnancy folate levels increase the accuracy of the actual fetal exposure. The measurements were conducted on nonfasting plasma samples, which may be influenced by recent intake of folate. This may have added preanalytical variation and probably attenuated associations. Although some asthma at age 3 years represents transient wheezing illness that may resolve by school age, asthma at age 3 years with use of inhalant medication in the past year is probably a better proxy for later asthma than wheezing at earlier ages. We limited our asthma case group to children for whom mothers had listed the name of a doctor-prescribed inhalation medication for asthma used within the last year, thereby increasing the validity of the asthma diagnoses. Maternal reports of specific asthma medications have been validated in MoBa against prescription records.[8] In Norway, doctor visits and prescribed medications for asthma are free for this age group, which probably decreases confounding by social class. Differential misclassification could also influence associations. If mothers with high folate levels in pregnancy were more health-aware and reported more asthma in their children, this could have introduced a positive bias of the association between higher folate levels and childhood asthma. However, proxies for health awareness, like higher maternal education, maternal body mass index, or cod liver oil in pregnancy, were not associated with asthma at age 3 years in this study, indicating such differential reporting to be unlikely. We adjusted for a number of potential confounders; however, the possibility of residual confounding cannot be excluded. We were also able to address differences in maternal plasma folate levels between subjects followed to age 3 years and those who were lost to follow-up, and the folate levels in pregnancy were similar. For details, see the Methods section in this article's Online Repository.

The modest associations between second trimester folate levels and early respiratory outcomes in children observed in this study as well as results from the small number of previous studies based on supplement use[4,5] could be subject to biases inherent of observational data. Even if these associations are confirmed in additional studies, they do not negate the value of folate supplementation in pregnancy. However, should higher folate in pregnancy pose a slight increased risk of respiratory illness in the child, additional studies might delineate levels below which adverse effects are unlikely and help fine-tune public health recommendations to maximize benefits.

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Higher-ovalbumin-content influenza vaccines are well tolerated in children with egg allergy

To the Editor:

The risk of vaccinating children who are allergic to egg with the influenza vaccine is unclear. We report our results with a 2-step influenza vaccine protocol to both the seasonal and H1N1 vaccines.

Influenza infections account for significant morbidity and mortality in the United States and abroad. The influenza vaccine decreases symptoms, outpatient visits, and hospitalizations[1,2] and is now recommended for all children aged 6 months to 18 years.[3] Because the influenza vaccine contains egg protein, whether patients with egg allergy can be safely vaccinated is unclear. The American Academy of Pediatrics and US Centers for Disease Control and Prevention[4,5] recommend not vaccinating children with severe egg allergy, which is defined by the American Academy of Pediatrics as a history of hives, angioedema, allergic asthma, or anaphylaxis.

Although anaphylaxis has been described, recent studies suggest vaccination might be safe in patients with egg allergy. In 1998, James et al[6] vaccinated 83 subjects with egg allergy (23 of whom had egg-induced anaphylaxis) with a 2-step protocol to the influenza vaccine (0.01-0.6 μg of ovalbumin/0.5-mL dose) without any significant reactions. More recently, Esposito et al[7] vaccinated children with egg allergy and asthma and children with asthma alone in a 1-dose protocol (1 ng of ovalbumin/0.5-mL dose) after having negative skin prick test (SPT) results, with 1 wheezing episode in each group. Gagnon et al[8] vaccinated 830 children with egg allergy (<8 ng of ovalbumin/0.5-mL dose) and had no cases of anaphylaxis. Chung et al[9] found a 3% to 5% systemic reaction rate among children with egg allergy given the influenza vaccine by means of a 2-step challenge protocol regardless of whether SPTs were performed.[9]

We report our experience using a 2-dose graded protocol to administer influenza vaccine in patients with egg allergy. We conducted a retrospective chart review of patients with egg allergy who received the influenza vaccine, either seasonal, H1N1, or both, in our allergy/immunology clinic during 2009-2010. We included all known patients with egg allergy who received an influenza vaccine during that time using a 2-step protocol. Written consent was obtained before vaccine administration. Egg allergy was defined as having (1) a clinical history of egg reaction with confirmatory positive results on either egg-specific IgE measurement or positive SPT results (ie, egg-specific IgE level >0.35 kUA/L or an SPT wheal response 3 mm or larger than that elicited by the saline control) or (2) positive test results (SPT or egg-specific IgE measurement) without a history of egg consumption. Patients currently consuming egg directly (eg, scrambled eggs) were excluded from this study because they were not considered to have egg allergy. We recorded parameters of age, sex, atopic history, asthma, previous egg reaction, SPTs, egg-specific IgE levels, total IgE levels, type of influenza vaccine (seasonal or H1N1), type of reaction, and vaccine lot number and producer for all patients, if available. Three patients were excluded from our study, 1 who received the vaccine in a single dose and 2 who received the vaccine in a graded protocol using more than 2 steps. None of these patients had a reaction. The 2 patients who received a greater than 2-step protocol did not appear to be at higher risk for an allergic reaction based on history and were vaccinated early in the season before implementation of the 2-step protocol.

Our vaccine protocol provided the influenza vaccine in a 2-step protocol first as a 10% aliquot and then the remaining 90% with a 15- to 30-minute observation period between doses and after the final dose. The Sanofi-Pasteur seasonal (Fluzone) and H1N1 influenza vaccine were exclusively used in our clinic for vaccination. All patients were observed for signs of both local

TABLE I. Demographics and reaction details for patients experiencing an allergic reaction to the influenza vaccine

Age (y)	Sex	Asthma	Other food allergies	Previous egg reaction	SPT egg	IgE egg (kUA/L)	Eats egg	Total IgE (kUA/L)	Reaction and timing	Dose
1	Male	No	Peanut	Vomiting	Not done	7.02	Baked goods	Not done	Small area of erythema with papule at diaper line, resolved with observation	90% Seasonal
1	Male	No	Milk	Hives	3-mm Wheal/5-mm flare	1.28	Baked goods	25	Multiple hives on chest, given diphenhydramine with resolution	Simultaneous administration of 10% H1N1 and 10% seasonal
6	Female	No	None	Hives, vomiting	Not done	>100	None	>2000	Large local erythema, resolved with observation.	10% H1N1
3	Male	No	Peanut, tree nut, milk	None	Not done	6.26	Baked goods	1099	Single prominent hive, resolved with diphenhydramine	10% Seasonal

METHODS

Study population

MoBa is a cohort with more than 100,000 pregnant women included between June 1999 and December 2008. The MoBa study was conducted by the Norwegian Institute of Public Health. Women were included around the time of their routine ultrasound examination offered freely to all pregnant women in Norway, and all geographic areas of Norway were represented in the study. Around 44% of invited women agreed to participate, and around 60% of participating women returned the questionnaire at age 3 years of the child. The questionnaires are available at the MoBa Web site: http://www.fhi.no/morogbarn.

On the basis of data from MoBa, we constructed a case-control study. We drew a random sample of 3000 mothers who gave birth in the MoBa cohort between July 2002 and December 2003. These women had donated a blood sample in the second trimester of pregnancy, were registered in the Medical Birth Registry of Norway, and had returned the baseline questionnaire from pregnancy. Of the children born to this random sample of 3000, a total of 1535 were singletons and had questionnaire follow-up through age 3 years. Eighty of these children fulfilled our asthma definition at age 3 years and were classified as case children, leaving 1455 children to be controls. Children were defined as asthma cases if the mother reported current asthma at age 3 years and had listed a name of an inhalation medication for asthma when asked to list medications used by their child during the last 12 months. In addition to the 80 asthma case children born to mothers within the random sample, we selected all asthma cases born between July 2002 and June 2004 with maternal samples and similar follow-up, giving 427 more case children, resulting in a study population of 1962 children, composed of 507 asthma case children and 1455 control children (Fig 1).

Blood sampling and biochemical analyses of plasma folate

The median gestational week for blood sample collection was 18 weeks. Nonfasting blood samples were collected at the hospitals in EDTA tubes. The samples were centrifuged within 30 minutes after collection and placed in the hospital's refrigerator at 4°C until shipped overnight to the Biobank of MoBa at the Norwegian Institute of Public Health in Oslo. On the day of receipt (usually within 1-2 days), plasma were aliquoted onto polypropylene microtiter plates and stored at –80°C. Plasma folate concentration was measured by using the *Lactobacillus casei* microbiological assay.

Folic acid supplement use

Information on use of folic acid supplements in pregnancy was obtained from the baseline pregnancy questionnaire around week 18 of pregnancy, administered around the same time the blood samples were drawn. Subjects with no report of folic supplement use were regarded as nonusers of folic acid supplements. Folic acid supplement use was reported in 4-week intervals in pregnancy: weeks 0 to 4, weeks 5 to 8, weeks 9 to 12, and after week 13. Use of folic acid supplement was coded as a dichotomous variable, with any intake reported in the first pregnancy questionnaire versus no intake.

Categorizations of plasma folate

Plasma folate levels were divided into quintiles on the basis of levels in the sample of 1535 women initially drawn from the cohort (Fig 1).

Covariates

Information on covariates was based on data from the Medical Birth Registry of Norway and MoBa questionnaires in pregnancy and when the child was 3 years old. Covariates were selected *a priori* on the basis of factors assumed to be associated with folate levels in early pregnancy (for example, factors that might influence use of folic acid supplements) and also possibly related to respiratory disease in childhood. Covariates included maternal atopy (history of or current asthma, hay fever, eczema, or urticaria), maternal educational level (years of completed education), parity (based on records in the birth registry), maternal prepregnancy body mass index calculated from height and prepregnancy weight reported in the first questionnaire, maternal smoking in pregnancy (report of smoking in the questionnaire around week 18), maternal smoking when the child was 3 years old, and the child's use of vitamin supplements or cod liver oil at 3 years old.

Statistical analyses

Data were analyzed by using the statistical software STATA (Stata Corp, College Station, Tex). We estimated odds ratios with 95% CIs for asthma at age 3 years across quintiles of maternal plasma folate. We used univariate and multivariate logistic regression analyses, with the lowest quintile as the reference category. *P* values for trend were obtained by treating the quintile variable as linear term in the logistic regression analyses. Missing data on covariates were not included in analyses. The inclusion period for case children was 6 months longer (July 2002 to July 2004) than for the control children (July 2002 to Dec 31, 2003) to increase the number of case children and the power. However, we also conducted the analyses without the case children from this added 6-month period (children born in 2004), and the results are similar.

Loss to follow-up

The follow-up rate through age 3 years was around 55% in this substudy, and loss to follow-up could have biased results if folate levels and asthma prevalence were different in our study population of children with follow-up at age 3 years and children whose mothers did not return the 3-year questionnaire. Fortunately we have data to evaluate this possibility from random samples of 2939 singleton mothers with folate levels measured in pregnancy. Folate levels were similar between the 1535 mothers who were followed to their children's age 3 years and the 1404 mothers who were not. For the 1404 children who were lost to follow-up, the maternal plasma folate quintiles were 5.2, 7.2, 10.0, and 16.0 nmol/L. For the 1535 children followed to age 3 years, the maternal plasma quintiles were 5.5, 7.7, 10.6, and 17.8 nmol/L (Table II).