Maternal dietary folate, folic acid and vitamin D intakes during pregnancy and lactation and the risk of cows' milk allergy in the offspring

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Abstract

Maternal nutrient intake during pregnancy and lactation potentially influences the development of allergic diseases. Cows' milk allergy (CMA) is often the first manifestation of atopic diseases, but the impact of early nutritional influences on CMA has not been explored. The associations between maternal intakes of folate, folic acid and vitamin D during pregnancy and lactation were addressed in a prospective, population-based birth cohort within the Finnish Type 1 Diabetes Prediction and Prevention Study. Mothers of 4921 children during pregnancy and 2940 children during lactation provided information on maternal dietary intake during the 8th month of pregnancy and the 3rd month of lactation using a detailed, validated FFQ. Information on diagnosed CMA in the offspring was obtained from a medical registry as well as queried from the parents. The Finnish food composition database was used to calculate nutrient intake. Logistic regression was applied for statistical analyses. Folate intake and folic acid and vitamin D supplement use were associated with an increased risk of CMA in the offspring, whereas vitamin D intake from foods during pregnancy was associated with a decreased risk of CMA. Thus, maternal nutrient intake during pregnancy and lactation may affect the development of CMA in offspring. Supplementation with folic acid may not be beneficial in terms of CMA development, especially in children of allergic mothers. The association between dietary supplement use and CMA risk can at least partly be explained by increased health-seeking behaviour among more educated mothers who also use more dietary supplements.

Key words: Cows' milk allergy: Lactation: Maternal diet: Nutrients: Pregnancy

Maternal nutrition during pregnancy and lactation, among other environmental factors, is suggested to have an impact on the development of allergic diseases. These early influences may be especially important in the development of cows' milk allergy (CMA), which is often the first manifestation of allergic disease. Folic acid and vitamin D have been shown to exert immunological effects, and they could potentially influence the development of allergic diseases[1]. Folic acid serves as a methyl donor

and is likely to have epigenetic effects in offspring. In mice, high intake of folic acid led to the development of allergic phenotypes and systemic allergic reactions in offspring[2]. Vitamin D has several routes through which it modifies both adaptive and innate immune responses and T-helper 1/T-helper 2 (Th1/Th2) balance, and its effects could expand to development of food allergy (recently reviewed by Bozzetto et al.[3]). Both these nutrients have been of interest with respect to risks of other

allergic diseases, but their associations with the risk of CMA have not been previously explored.

We have previously published findings of a food-based approach studying the associations between maternal food consumption during pregnancy and lactation and subsequent CMA risk in the offspring[4]. The aims of the present study were to investigate the associations between maternal dietary intake of folate, folic acid and vitamin D during pregnancy and lactation and subsequent development of CMA in the offspring.

Methods

Subjects

The Finnish Type 1 Diabetes Prediction and Prevention (DIPP) study is a multidisciplinary, prospective, population-based birth cohort study[5]. After parental informed consent, all newborn infants from the catchment areas of three university hospitals in Finland (Turku, Oulu and Tampere) were screened for human leucocyte antigen (HLA)-conferred susceptibility to type 1 diabetes from cord blood samples. Infants who carried HLA genotypes conferring high and moderate risk for type 1 diabetes (15 % of those screened) were invited to participate in the study, excluding children with severe congenital abnormalities or diseases, or whose parents were of non-Caucasian origin or did

not understand Finnish, Swedish or English. The local Ethics Committees approved the study. All families provided their written informed consent.

The DIPP Nutrition study was performed among children who were born in Oulu and Tampere university hospital areas of the DIPP study population. The present study comprised of 6288 children born between October 1997 and September 2004 and whose personal identification codes were disclosed to the study (Fig. 1). Mothers of 4921 children provided dietary data from a total of 4861 pregnancies for the pregnancy study. The lactation study was started in August 1998, collecting dietary data of the mothers who were still breast-feeding when the baby was 3 months of age. Dietary data were available for 2940 children from 2915 mothers. The numbers of mothers and children differ because of twin pregnancies. For the analysis concerning diet during breast-feeding, 120 children whose CMA had begun before completing the FFQ were omitted from the analysis, leaving us with a total of 2820 mother–child pairs for crude analysis (Fig. 1).

Dietary assessment

Maternal diet during pregnancy (8th month) and lactation (3rd month) was assessed using a validated, 181-item, semi-quantitative FFQ[6]. Mothers received the FFQ concerning
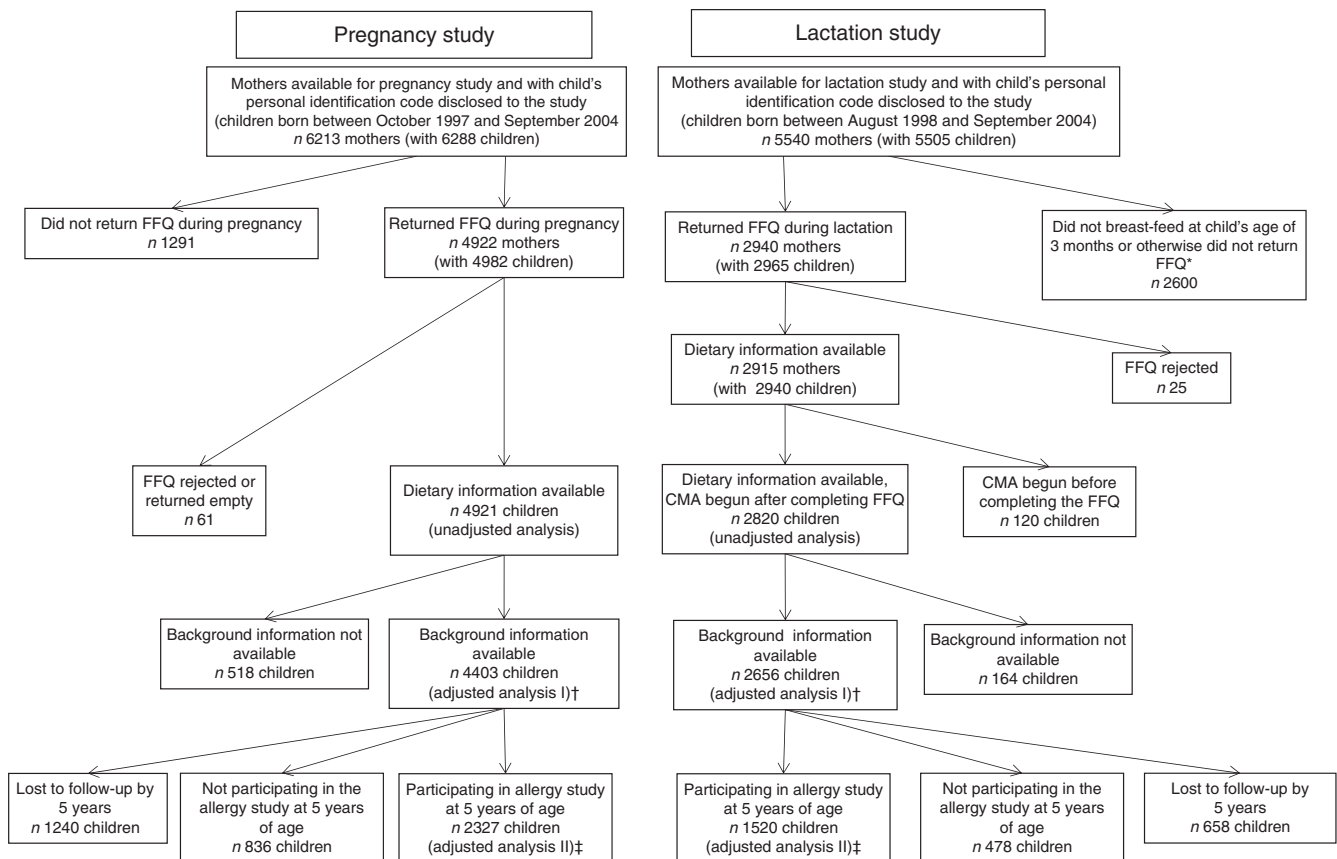

Fig. 1. Flow chart of the participants in the pregnancy and lactation studies. CMA, cows' milk allergy. *The number of breast-fed children at the age of 3 months was 3757 – that is, 68 % of the children. †Background information variables: study centre, sex, birth weight of the child, maternal age and education, maternal smoking status during pregnancy, mode of delivery, number of older siblings, duration of gestation, season of birth and length of breast-feeding. ‡Variables that were queried at 5 years of age: maternal asthma or allergic rhinitis, urbanity of the living environment, visits to a stable and pet keeping during the first year of life.

the diet at the 8th month of pregnancy after delivery by mail, and it was returned at a visit to the study centre and checked by a trained study nurse when the child was 3 months old. The questionnaire concerning diet during lactation was administered at the 3-month visit and returned at the 6-month visit to the study centre. The FFQ assessed the consumption frequency of foods or food groups (not at all, number of times per day, week or month) as common serving sizes such as a glass, a plateful or decilitre. The questionnaire was specifically designed to reflect Finnish food consumption habits. Types of fat used for cooking and baking were taken into account individually. The food consumption data were double-entered. If there were ten or more missing frequencies or the form was inadequately filled in, the FFQ was rejected (n 61, 1·2 % of FFQ concerning diet during pregnancy and n 25, 0·9 % of FFQ concerning diet during lactation). Daily intakes of nutrients were calculated using the Finnish national food composition database[7] by an in-house software of the National Institute for Health and Welfare. The detailed content of the FFQ and data processing have been described elsewhere[6,8]. We used two updated versions of the database: the first version for the study years 1997–2002 and the second version for the years 2003/2004. Recipe compositions were changed from the first version to the second, in order to reflect changes in food consumption habits and changes in the food market. The changes in recipes were mainly based on food consumption information of women aged 25–44 years from the national dietary surveys FINDIET 1997[9] and FINDIET 2002[10]. Vitamin D concentrations were systematically changed in milk and fat products because of changes in fortification in 2003. Recording of the FFQ and the accuracy of the nutrient database of the National Public Health Institute were checked at dietary analysis. The use of dietary supplements was determined with a question in the FFQ form, asking about the type, brand name and manufacturer's name, the amount of each supplement taken per day or per week and the pregnancy weeks during which the supplements were used. The nutrient contents of the dietary supplements were obtained from the National Food Administration, the manufacturers and from the Finnish pharmacopeia for supplements registered as drugs.

End points

Information on CMA from the registers of the Social Insurance Institution, complemented with parental reports, was used as the end point. In Finland, infants who need special infant formulae for the management of diagnosed CMA (ICD-10 codes L27.2 or K52.2) are entitled to special reimbursement for costs up to 2 years of age. Information on this physician-diagnosed CMA was received from a register maintained by the Social Insurance Institution. Paediatricians have agreed on the criteria for the diagnosis (a response to an elimination diet and an open challenge; rarely, a response to an elimination diet with positive skin prick test or specific IgE; in select rare cases double-blind, placebo-controlled challenge is performed). In addition, CMA up to the age of 3 years was queried with open questions from parents at the age of 6 months and 1 and 2 years and with a structured, validated questionnaire at the age of 3 years[11,12].

Background factors

Families were asked for information on parental vocational education, age and place of residence at recruitment. Information on pregnancy and delivery complications, gestational age, birth weight and height, earlier deliveries and maternal smoking status during pregnancy were received from the National Medical Birth Registry. Information on breast-feeding was obtained from parents at study visits. Parental atopic history, pet keeping and farm animal contacts during the child's 1st year of life were queried when the child was 5 years old with questions on parental history of asthma, allergic rhinitis and atopic eczema among the families who participated in the allergy study at the 5-year follow-up[13].

Statistical methods

Differences in background characteristics between cases and controls and between mothers with and without allergic history were assessed using χ^2 or Fisher's exact tests. Wilcoxon's signed-rank test was used for pairwise comparison for difference between intakes during pregnancy and lactation. The Mann–Whitney U test was used to assess differences in nutrient intakes between mothers with and without allergic history. Logistic regression was applied to study the associations between maternal nutrient intakes and the risk of CMA in the offspring. The possible dependence among siblings was accounted for by using the generalised estimating equations with the sandwich estimator of variance to estimate regression coefficients in the logistic regression analysis[14]. The dietary nutrient intakes were adjusted for energy intake by the residual method[15] after logarithmic transformation; supplemental intake was not adjusted for energy. The total intake of each nutrient was calculated as the sum of intake from foods and supplements. Total and dietary nutrient intake variables were used as continuous explanatory variables in the analysis. Nutrient intakes from supplements were categorised into users and non-users. The sex and birth weight of the child, maternal age and education, maternal smoking status during pregnancy, duration of gestation, type of delivery, number of older siblings, season of birth, length of breast-feeding, atopic family history, study centre, urbanity of the living environment (urban/semi-rural/rural), visits to a stable and pet keeping during the 1st year of life were adjusted for in the analysis. Those whose CMA diagnosis was confirmed before completing the FFQ during lactation were excluded from the analysis concerning diet during lactation. Interaction between maternal allergic history (mother has asthma or allergic rhinitis) and nutrient intakes was tested, and analyses were carried out separately for allergic and non-allergic mothers if the interaction was significant ($P < 0·05$). Multiple testing was addressed using the P-value adjustment method that controls the false discovery rate[16]. SAS version 9.2 (SAS Institute Inc.) was used for the analysis.

Results

Male sex, high maternal education level and parental allergic history were associated with an increased risk of CMA in offspring (Table 1). On the other hand, maternal smoking and having pets indoors during the 1st year of life were associated

Table 1. Distribution of cows' milk allergy by background characteristics in the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) nutrition study (Numbers and percentages; n 6288)

| | Distribution | | Cows' milk allergy* | | |
| | | | Cases | | |
	%	n	%	n	P†
Sex					<0·001
Boys	52·5	3304	9·8	324	
Girls	47·5	2984	6·9	205	
Season of birth					0·360
Spring (April–May)	18·2	1142	7·5	86	
Summer (June–August)	27·2	1709	7·9	135	
Autumn (September–November)	22·2	1394	8·8	122	
Winter (December–March)	32·5	2043	9·1	186	
Age of the mother at delivery (years)					0·002
<25	19·4	1221	5·8	71	
25–29	33·8	2127	8·7	186	
30–34	28·7	1806	9·6	174	
≥35	18·0	1134	8·6	98	
Maternal vocational education					<0·001
No professional education	7·7	469	5·1	24	
Vocational school or course	28·4	1725	6·3	108	
Upper secondary vocational education	41·9	2541	9·7	246	
Academic education	22·0	1333	10·4	138	
Missing information		220		13	
Maternal smoking status during pregnancy					0·009
No	89·8	5449	8·7	476	
Yes	10·2	620	5·7	35	
Missing information	3·5	219	8·2	18	
Urbanity of the place of living					0·045
Rural	13·3	831	6·5	54	
Semi-urban	9·7	606	10·1	61	
Urban	77·0	4800	8·5	410	
Missing information		51		4	
Maternal asthma or allergic rhinitis					<0·001
No	54·7	1544	7·8	120	
Yes	45·3	1278	12·1	155	
Missing information		3469		254	
Paternal asthma or allergic rhinitis					<0·001
No	60·0	1659	8·0	133	
Yes	40·0	1104	12·2	135	
Missing information		3525		261	
Pets inside home during the 1st year of life					0·006
No	68·5	1991	10·7	213	
Yes	31·5	917	7·4	68	
Missing information		3380		248	
Maternal use of vitamin D supplements during pregnancy (including multivitamin supplements)					0·003
No	67·2	3307	8·2	272	
Yes	32·8	1614	10·9	176	
Missing information		1367		81	
Maternal use of folic acid supplements during pregnancy (including multivitamin supplements)					<0·001
No	64·0	3151	8·1	254	
Yes	36·0	1770	11·0	194	
Missing information		1367		81	
Maternal use of vitamin D supplements during lactation (including multivitamin supplements)					0·004
No	64·7	1901	8·6	164	
Yes	35·3	1039	11·9	124	
Missing information		3348		241	
Maternal use of folic acid supplements during lactation (including multivitamin supplements)					0·006
No	68·1	2001	8·8	175	
Yes	31·9	939	12·0	113	
Missing information		3348		241	

* Cumulative incidence of cows' milk allergy by the age of 3 years.
† Comparison with χ^2 or two-sided Fisher's exact test comparing distributions of cows' milk allergy across categories.

with a lower risk of CMA. The cumulative incidence of CMA was 8·4 % by the age of 3 years. About the same proportion, one-third of the mothers, received folic acid and vitamin D from

supplements both during pregnancy and lactation (Table 1). Most of them received both supplements: during pregnancy 26·7 % and during lactation 29·3 % of the mothers had used both

folic acid and vitamin D supplements. Energy intake reduced from pregnancy to lactation, and the quality of the diet, as assessed by the energy-adjusted intakes of the studied nutrients from food, was poorer during lactation (Table 2).

Maternal dietary supplement use was associated with risk of CMA: children had a higher risk of CMA if their mothers had used folic acid or vitamin D supplementation during pregnancy or lactation (users *v.* non-users; Table 1). After adjusting for potential confounding factors, only vitamin D supplementation remained significantly associated with increased risk of CMA (Table 3). Higher folate intake by the mother both during pregnancy and lactation was associated with an increased risk of CMA in the offspring (Table 3). Vitamin D intake from food during pregnancy was not associated with the risk of CMA in the crude analysis, but the association was statistically significant after adjusting for background factors ($P = 0.033$ in the first adjustment and $P = 0.048$ in the second adjustment)

(Table 3). Adjusting dietary intake during lactation for the intake during pregnancy did not change the results (data not shown). After controlling for the false discovery rate, only the unadjusted findings of an association between folic acid supplement use during pregnancy and increased risk of CMA remained significant.

Stratifying according to maternal history of asthma or allergic rhinitis revealed that higher maternal intake of folic acid was associated with a higher risk of CMA only in children of allergic mothers (OR 1·34; CI 1·14, 1·57, $P = 0.007$ for allergic and OR 0·89; CI 0·69, 1·16 for non-allergic mothers; $P = 0.008$ for interaction).

Allergic mothers differed from non-allergic mothers in some respects: they were younger and they had used folic acid and vitamin D supplements more often during lactation (online Supplementary Table S1). The amount of folic acid and vitamin D intake from dietary supplements was higher for

Table 2. Maternal daily intake of energy and nutrients during pregnancy and lactation
(Mean values and standard deviations)

	During pregnancy (*n* 4861)		During lactation (*n* 2915)			Reference value[39]	
	Mean	SD	Mean	SD	*P**	Pregnancy	Lactation
Energy (kJ)	11 430	3370	9956	2884	<0·001		
Protein (energy %)	16·4	2·2	16·7	2·3	<0·001	10–20	10–20
Carbohydrate (energy %)	49·6	5·1	48·7	5·6	<0·001	45–60	45–60
Fat (energy %)	33·8	4·8	34·3	5·0	<0·001	25–40	25–40
Vitamin D total (µg)	6·9	4·0	7·3	5·0	<0·001	10	10
Vitamin D diet (µg)	5·5	2·9	5·0	2·7	<0·001		
Vitamin D supplements (µg)	1·4	2·6	2·3	4·0	<0·001		
Folate + folic acid total (µg)	409·8	154·0	359·5	157·4	<0·001	500	500
Folate diet (µg)	364·0	120·5	302·0	101·3	<0·001		
Folic acid supplements (µg)	45·8	93·0	57·5	120·2	<0·001		

* For the pairwise comparison, information on maternal nutrient intake during both pregnancy and lactation was available for 2838 mothers. Pairwise comparison for difference between energy-adjusted intakes during pregnancy and lactation was carried out using Wilcoxon's signed-rank test.

Table 3. Associations between maternal daily intake of nutrients during pregnancy and lactation with the risk of cows' milk allergy in the offspring by 3 years of age
(Odds ratios and 95 % confidence intervals)

	Maternal intake during pregnancy						Maternal intake during lactation					
	Unadjusted (*n* 448†/4921‡)		Adjusted§ (*n* 409†/4403‡)		Adjusted‖ (*n* 234†/2327‡)		Unadjusted (*n* 168†/2820‡)		Adjusted§ (*n* 159†/2656‡)		Adjusted‖ (*n* 92†/1520‡)	
	OR¶	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI
Vitamin D total	1·01	0·92, 1·11	0·98	0·88, 1·08	0·99	0·86, 1·13	1·07	0·91, 1·25	1·01	0·85, 1·20	1·01	0·81, 1·26
Vitamin D diet	0·92	0·83, 1·03	0·89*	0·79, 1·00	0·86*	0·74, 1·01	0·96	0·80, 1·16	0·92	0·75, 1·13	0·95	0·73, 1·24
Vitamin D supplement use (users *v.* non-users)	1·34**	1·10, 1·65	1·30*	1·05, 1·61	1·37*	1·034, 1·81	1·41*	1·03, 1·92	1·29	0·93, 1·79	1·18	0·76, 1·83
Folate and folic acid total	1·14**	1·05, 1·24	1·11	1·00, 1·23	1·15	1·00, 1·32	1·17*	1·03, 1·32	1·16*	1·01, 1·34	1·15	0·98, 1·35
Folate diet	1·11*	1·01, 1·23	1·05	0·94, 1·17	1·07	0·92, 1·24	1·19	1·03, 1·37	1·22*	1·05, 1·42	1·30	1·09, 1·56
Folic acid supplement use (users *v.* non-users)	1·40***	1·15, 1·71	1·33**	1·08, 1·64	1·31	0·99, 1·73	1·40*	1·01, 1·93	1·35	0·95, 1·91	0·97	0·60, 1·56

* $P < 0.05$ but not statistically significant after controlling for the false discovery rate; ** $P < 0.01$ but not statistically significant after controlling for the false discovery rate; *** $P < 0.01$ with the association remaining statistically significant after controlling for the false discovery rate.
† Number of children with cows' milk allergy.
‡ Number of children in the analysis.
§ Adjusted for energy intake by the Willett residual method, study centre, sex, birth weight of the child, maternal age and education, maternal smoking status during pregnancy, mode of delivery, number of older siblings, duration of gestation, season of birth and length of breast-feeding.
‖ Adjusted for variables that were queried at 5 years of age: maternal asthma or allergic rhinitis, urbanity of the living environment, visits to a stable and pet keeping during the 1st year of life, as well as for those variables in the first adjustment.
¶ OR describes change in risk, when the energy-adjusted variable is changed to an extent corresponding to its standard deviation.

allergic mothers compared with non-allergic mothers both during pregnancy and lactation. Otherwise the diets did not differ between these groups (online Supplementary Table S2).

There were some differences between children who participated in the allergy study and those who declined to participate or had dropped out of the study by the age of 5 years when the allergy study was performed. Participating children compared with non-participants had older, more educated and less frequently smoking mothers who had used more often folic acid and vitamin D supplements (data not shown). Moreover, the amount of folic acid supplementation during pregnancy and lactation was higher among mothers of participating than non-participating children, whereas no difference was seen in the amount of vitamin D supplementation (data not shown). No differences according to participation were observed in maternal energy or energy-yielding nutrient intakes or in folate or vitamin D intake from food (data not shown).

Discussion

The results of this study suggest that maternal nutrient intake during pregnancy and lactation is associated with the development of CMA in offspring. High maternal intake of folate appears to be associated with a higher risk of CMA in the offspring. On the other hand, maternal intake of vitamin D from food during pregnancy seems to be associated with a decreased risk of CMA in the offspring. Then again, supplemental intakes of both folic acid and vitamin D were associated with an increased risk of CMA. The association between dietary supplement use and CMA risk can at least partly be explained by increased health-seeking behaviour among more educated mothers who also use more dietary supplements. However, after considering multiple comparisons by using the false discovery rate, these associations became marginal.

To our knowledge, this is the first study to examine the associations between maternal folate, folic acid and vitamin D intake during pregnancy and lactation and subsequent CMA in offspring.

A major strength of our study is that data were collected prospectively from a large number of mothers and children. In addition, a major advantage is the good quality of dietary information, which was gathered by a detailed, validated FFQ. In the validation of the FFQ, conducted by Erkkola et al.[6], Pearson's correlations with food records were 0·48 for folic acid and 0·44 for vitamin D. Cross-classification in quintiles revealed decent associations for both nutrients.

Collection of dietary information with a FFQ also comes with some limitations: it is subject to recall bias as well as having the tendency to overestimate food intake. Despite these shortcomings, the aforementioned validation study showed the method to be valid in studying maternal dietary intakes during pregnancy and lactation. The exactly same setting was used in the present study[6].

A limitation of the present study is also that the subjects carried HLA-conferred genetic susceptibility to type 1 diabetes, representing about 15 % of all newborn infants in Finland. An increased intestinal permeability in those with type 1 diabetes or genetic disease susceptibility has been proposed[17]. This may limit the generalisability of our results to the overall population, although a similar incidence of CMA has been reported in a genetically unselected Finnish cohort of children[18].

Although being in the same magnitude with other birth cohort studies, dropouts may have led to bias. Children who participated in the allergy study at the age of 5 years had older and more highly educated mothers, who were more often non-smokers, than those who did not participate. These factors are also associated with the child's risk of CMA, which could potentially confound the results. The study period expanding over several years could be a limitation; however, the diagnostic criteria of CMA have remained the same throughout the study years.

We took several possible confounders into the statistical model in order to specifically evaluate the effects of the selected nutrients and to take into account putative confounding by some lifestyle factors associated with the intake of these nutrients. Nutrient intake depends not only on diet but also on the use of dietary supplements; we found that both vitamin D and folic acid supplement use by mothers was associated with an increased risk of CMA in the offspring in this cohort. Supplement users were more highly educated, health conscious and smoked less than non-users, which also may confound the results[19]. Maternal education level also correlated with the risk of CMA in the offspring, possibly at least partly explaining the findings, because highly educated mothers may be more prone to seek for a CMA diagnosis for their children. Maternal education level was adjusted for in the analysis to reduce this confounding. Unfortunately, the diet of the children themselves could not be controlled for. A further limitation is that the age of onset of symptoms of CMA was not known, and only the age at diagnosis was known, although these two should coincide. When studying the association between maternal diet during lactation and CMA in the offspring, those whose CMA diagnosis was confirmed before completing the FFQ during lactation were excluded from the analysis in an attempt to rule out reverse causation.

Our findings on background factors and risk of CMA are in line with a previous, large, register-based study, which found that maternal smoking, younger age and low socio-economic status of the mother were associated with a lower risk of CMA[20]. We do not believe that smoking would in fact decrease the risk of CMA; it is assumed that smoking is a surrogate for other environmental factors or may be related to lower levels of health-seeking behaviour. The lower risk of CMA with pet keeping, on the other hand, follows the principles of the hygiene hypothesis and is in line with previous reports on the aetiology of food allergy[21].

Evidence for an association between maternal dietary intake of vitamin D during pregnancy and lactation and atopic outcomes is equivocal. Gale et al. and Weisse et al. have reported a higher risk of eczema, asthma, food allergy or sensitisation against food allergens in children with high maternal 25-OH-vitamin D levels[22,23], whereas Camargo et al.[24] found an inverse association between maternal vitamin D intake and asthma, but not with eczema. Furthermore, Devereux et al.[25] found no association between maternal vitamin D intake during pregnancy with risk of atopic

sensitisation or asthma in children. In addition, two previous reports from our cohort[26,27] have found an inverse association between maternal vitamin D intake during pregnancy and allergic outcomes. From our cohort, we have reported a lower risk of asthma and allergic rhinitis by the age of 5 years with high maternal intake of vitamin D during pregnancy[26] as well as a lower risk of atopic sensitisation, measured by specific IgE, to any of the most common food allergens[27]. Meta-analysis of observational birth cohort studies showed that increasing intake of dietary vitamin D during pregnancy was associated with a reduced risk of asthma symptoms in the offspring[28].

Recently, two trials showed that prenatal vitamin D supplementation with a daily dose of 60 and 100 μg, respectively, as compared with the 10 μg usual care, did not significantly affect the absolute incidence of asthma-like symptoms[29,30]. However, these trials evaluated the effect of additional high dosage of vitamin D on top of usual care, whereas the observational studies have compared sub-optimal *v.* optimal intake in relation to dietary recommendations. In the present study, higher maternal intake of vitamin D from food tended to be associated with a lower risk of CMA in offspring, but the association was not consistent after all the adjustments.

Adding to the confusion, in the present study, vitamin D intake from supplements was associated with a higher risk of CMA in offspring. It seems unlikely that the small amount of vitamin D derived from supplements would actually be harmful, and the association is probably explained by other lifestyle factors. This association between supplement use and risk of CMA is likely to dilute the potential real association between vitamin D intake from food and CMA risk. As a limitation, sunlight exposure as a source of vitamin D or 25-OH-vitamin D levels was not studied. However, there was no variation in CMA incidence by birth season (Table 1), suggesting that the influence of sunlight exposure on CMA development is inconsequential. Potential mechanisms on how vitamin D deficiency could contribute to the development of food allergy include increased T cell proliferative responses, IL leading to Th1-type reactions as well as decreasing tolerogenic dendritic cells, regulatory T cell production and IL-10, in addition to compromising intestinal barrier function (recently reviewed by Vassallo *et al.*[31]).

Folate metabolism is important in DNA methylation, and has therefore been implicated to play a role in epigenetics[32]. A recent meta-analysis found no evidence of association between folic acid supplementation in the first trimester and risk of asthma in offspring[33]. One cohort looking at maternal folate intake during the first trimester did not find associations with risk of eczema[34], whereas another cohort did not find associations between maternal intake of folate or folic acid supplements during the last trimester and risk of atopy in offspring[35]. However, another report found that not dietary folate intake but supplementation with folic acid above 500 μg/d to be associated with the development of eczema in children[36]. Although the association between maternal folate and folic acid intake and risk of CMA in offspring found in our study is only modest and the cohort design cannot rule out all the confounding factors, we cannot rule out the potential real association either.

There is a strong hereditary component in allergic diseases. The immunological environment *in utero* is determined by the immunology of the mother and the interaction between the mother and the *fetus*. It is possible that the immunological effects of nutrients differ in allergic individuals from those without atopic diseases. Therefore, we examined the associations between nutrients and CMA in children separately with allergic and non-allergic mothers. The associations did, in fact, differ somewhat: maternal intake of folic acid during pregnancy was associated with a higher risk of CMA in offspring of allergic mothers only. Granell *et al.*[35] assessed a genetic polymorphism in gene MTHFR C677T and its association with atopic disease; their study did not support the hypothesis that impaired folate metabolism would be associated with allergy. They did, however, discover that folic acid supplementation by mothers during pregnancy was related to an increased risk of atopy in children carrying a certain T allele, which has previously been linked to an increased risk of allergic disease[37]. Further studies are needed to explore the immunological mechanisms behind this finding.

Mothers who use dietary supplements differ in other aspects of lifestyle from those who do not use supplements: they have higher educational levels and smoke less[38]. It is possible that those with an overall healthier lifestyle may be more prone to seek medical assistance, thus increasing the likelihood of receiving a CMA diagnosis for their child, which could confound the associations observed in this study. Even though the associations prevailed after adjusting for maternal level of education, it is possible that there remains residual confounding and the association between supplemental folic acid and vitamin D intake and higher risk of CMA in children that we found is not causal.

Average folate, folic acid and vitamin D intakes from food and supplements of the mothers during pregnancy and lactation were below the recommended 10 and 500 μg/d, respectively[39]. As a substantial amount of these vitamins is derived from supplements, it is not feasible to adjust for supplement use in the regression model. A majority of the mothers used both folic acid and vitamin D supplements, making it difficult to differentiate between their separate associations with CMA risk. In the end, we cannot be certain whether the association between supplemental intake and the risk of CMA is causal or whether it acts as a proxy for some other lifestyle factor/s.

In conclusion, these results are the first to link maternal folate, folic acid and vitamin D intakes during pregnancy and lactation with the development of CMA in offspring. The associations found are marginal and need to be confirmed or rejected in further studies. The association between maternal intake of folic acid and CMA in offspring calls for precaution in the use of supplements. However, the slightly higher risk of CMA with high intake of folic acid does not warrant changes in recommending higher intake of folic acid during pregnancy to prevent conditions such as neural tube defects. Adhering to the dietary guidelines for pregnant and lactating women leads to sufficient, balanced intake of nutrients; excessive intake of nutrients is not advised, as it may have adverse immunological effects.

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Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114516002464

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