

ORIGINAL ARTICLE

Predicted vitamin D status in mid-pregnancy and child allergic disease

Ekaterina Maslova¹, Susanne Hansen¹, Andrew L. Thorne-Lyman^{2,6}, Camilla B. Jensen³, Marin Strøm¹, Arie Cohen⁴, Nina O. Nielsen⁵ & Sjurdur F. Olsen^{1,6}

¹Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; ²The WorldFish Center, Penang, Malaysia; ³Institute of Preventive Medicine, Bispebjerg and Frederiksberg Hospital, Frederiksberg, Denmark; ⁴Clinical Mass Spectrometry, Department of Clinical Biochemistry, Immunology and Genetics, Statens Serum Institut, Copenhagen, Denmark; ⁵National Institute of Public Health, University of Southern Denmark, Copenhagen K, Denmark; ⁶Department of Nutrition, Harvard School of Public Health, Boston, MA, USA

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Keywords

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Correspondence

Ekaterina Maslova, Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen, Denmark
Tel.: +4532683951
Fax: +4532683874
E-mail: kmv@ssi.dk

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Abstract

Background: Vitamin D deficiency in pregnancy may be a risk factor for child allergic disease. However, less is known about disease risk across different levels of vitamin D.

Objective: We aimed to examine the relation between a maternal vitamin D prediction score and child allergic disease.

Methods: A total of 32,456 pregnant women were enrolled in the Danish National Birth Cohort (1996–2003) and had data on a validated vitamin D prediction score based on 1497 mid-pregnancy plasma 25(OH)D samples. Child allergic disease was assessed at 18 months and at 7 years using questionnaire data and national registry extracts. We used multivariable log-binomial models to quantify risk ratios (RR) and 95% CI. Plasma 25(OH)D was examined in a stability analysis.

Results: Median (IQR) vitamin D prediction score was 58.7 (49.2–69.0) nmol/l. In main analysis, there was no association between vitamin D prediction score examined in quintiles or by restricted categories (≥ 75 nmol/l and < 25 nmol/l vs. 25–74.9 nmol/l) and child allergic disease. However, maternal vitamin D prediction score ≥ 100 nmol/l (vs. 50–79.9 nmol/l) was associated with increased risks of child asthma at 18 months (RR: 1.36, 95% CI: 1.02, 1.80) and asthma by hospital admission (RR: 1.65, 95% CI: 1.04, 2.62). For vitamin D prediction score < 25 –30 nmol/l, there were increased risks of child asthma at 18 months and by hospital admission and medication prescription at age 7, although these findings were not robust to covariate adjustment. Similar results were found for plasma 25(OH)D.

Conclusions: Our study provided little evidence for an association between maternal vitamin D prediction score and child allergic disease for scores ≥ 75 nmol/l. However, increased risks were observed for vitamin D prediction score ≥ 100 nmol/l. These associations are hypothesis generating and would need to be replicated in other cohorts.

Vitamin D is a prohormone that can be acquired from both diet and exposure to sunlight (1). Recent interest in vitamin D and its relation to human disease has led to further examination of its role in asthma and allergy development. Given that allergic disease manifests already in early childhood and that

fetal supply of vitamin D is directly dependent on maternal levels (2), exposure to vitamin D *in utero* may be associated with risk of allergic disease.

Studies on maternal dietary vitamin D intake in pregnancy have previously shown a reduced risk of wheeze and asthma (3–6), whereas studies that have used either maternal or cord serum 25-hydroxy-vitamin D (25(OH)D) have had mixed results for respiratory and allergic disease outcomes, reporting inverse (7–10), direct (11), and null (12–14) associations. Most of the inverse results were found for early wheeze and infections rather than asthma. One study found indications

Abbreviations

25(OH)D, 25-hydroxy-vitamin D; BMI, body mass index; DNBC, Danish National Birth Cohort; FFQ, food frequency questionnaire; GW, gestation week; IQR, interquartile range; LMP, last menstrual period; RR, risk ratio; SES, socioeconomic status.

of a U-shaped relation, with increased risk of atopy and sensitization in the first five years of life with cord blood 25(OH)D < 50 and ≥ 100 nmol/l, although the associations with clinical outcomes were not significant (15).

Given the inconsistency of previous findings, we explored the relationship between prenatal vitamin D status and risk of asthma in a large cohort of pregnant women, using both a validated prediction score developed from plasma samples (16) as a proxy for prenatal vitamin D status as well as a smaller subcohort of women with measured 25(OH)D levels.

Methods

Study population

The Danish National Birth Cohort (DNBC) is a large, prospective cohort study that enrolled >100,000 pregnancies in 1996–2003 during gestational week 6–10. The cohort has been described in detail elsewhere (17). In brief, data collection included 4 telephone interviews in gestation weeks (GW) 12 and 30, and 6 and 18 months postpartum; a food frequency questionnaire (FFQ) in GW 25 (18); and a 7-year questionnaire.

In addition, women and children have been followed up through national registries. We limited our data to first cohort pregnancies ($n = 84,020$) to avoid dependencies among correlated measures. The exposure information was conditional on available data on covariates ($n = 32,456$ with full covariate data), and sample size varied further depending on missing outcome data.

Mothers provided written informed consent on behalf of their children. The Regional Scientific Ethics Committee for the municipalities of Copenhagen and Frederiksberg approved all study protocols, and all procedures were in accordance with the Declaration of Helsinki.

Maternal exposure – prediction score

We used a validated prediction model based on the DNBC to assign a 25(OH)D-score (in nmol/l) to each mother. The development of the prediction score has been described in detail elsewhere (16). Briefly, measures of plasma 25(OH)D in GW 25 (assay described in Data S1) and information on diet and lifestyle were available for 1497 pregnant women. Potential predictors of vitamin D status were selected on the basis of existing literature, and those with $p < 0.05$ were kept in the model, including vitamin D intake from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw. The model was developed and validated in two independent groups, and it explained 40% of the variance in 25(OH)D concentration.

Child asthma at 18 months

Asthma at 18 months was assessed from a telephone interview and defined as children with a parental report of doctor-diagnosed asthma. This outcome was considered to capture primarily transient wheeze as close to 90% of children with reported asthma at 18 months did not report current asthma at

age 7 (unpublished data). In secondary analyses, we also examined maternal report of *wheeze at 18 months* and *recurrent wheeze at 18 months* (>3 episodes vs. ≤ 3 episodes or no wheeze).

Child asthma and allergic rhinitis at 7 years

Current child asthma at 7 years was assessed from the 7-year follow-up questionnaire. Cases of current asthma were defined as children with a doctor diagnosis of asthma *and* parental report of wheeze in the past 12 months. For this comparison, non-cases were those providing at least one negative answer to either question. *Ever allergic rhinitis* was defined as children ever given a doctor diagnosis of hay fever.

Child registry-based asthma

Ever admitted asthma was assessed using data from the Danish National Patient Register that contains mandatory information about all hospitalizations and emergency room and ambulatory visits in Denmark. We defined cases of *ever admitted asthma* as children with an ICD-10 diagnosis of asthma (J45, J45.0, J45.1, J45.8, J45.9, J46, and J46.9) from the registry.

Ever prescribed asthma was assessed using data on asthma medication from the Register of Medicinal Product Statistics that registers all physician prescriptions claimed in Danish pharmacies. We defined *ever prescribed asthma* cases partly based on a previously validated definition (19) as those who had filled ≥ 2 prescriptions for beta-2-agonists or steroids or ≥ 1 prescription for any other obstructive airway disease drug, including leukotriene receptor antagonists. In contrast to the validated definition in the paper by Moth et al., we chose a capture time from birth until 7 years of age rather than in the previous 12 months.

Covariates

We evaluated information on maternal sociodemographic and lifestyle covariates from the telephone interviews and registries. The primary covariates considered were maternal age at birth of child, parental socioeconomic status (SES) according to occupation and education level, parity, maternal pre-pregnancy body mass index (BMI), month of last menstrual period (LMP), maternal paracetamol use, maternal smoking, second-hand smoke, and partner smoking during pregnancy, maternal and paternal history of asthma and allergies, breastfeeding duration, gestational weight gain, gestational age, birthweight, and child sex. Both partner smoking and secondhand smoke exposure were included because there was incomplete overlap between the variables, suggesting that they captured somewhat different exposures. Secondary covariates included postnatal exposures that have been associated with allergic disease: child antibiotic use at 6 months, child smoke exposure, day care, and pets in child's home at 18 months. The missing indicator method was used to account for missing covariate data when the proportion of missing data exceeded 1%; if $\leq 1\%$, then the missing observations were deleted.

Statistical analysis

We assessed linear associations by entering the prediction score as a continuous variable; potential non-linear relations were explored using quintiles of exposure. Vitamin D prediction score was also categorized *a priori* according to limits used in a prior study (7) (<25, 25–74.9, ≥75 nmol/l) and by the Canadian Pediatric Society (20); due to small numbers, the lowest category limit was moved to <30 nmol/l and the middle category served as reference. In secondary, exploratory, analyses, we examined associations at more extreme levels of the prediction score set *a priori* (<25, 25–49.9, 50–79.9, 80–99.9, and ≥100 nmol/l).

In the multivariable model, we introduced covariates previously identified based on current literature and biologic plausibility. Covariates initially suspected to be on the causal pathways (gestational weight gain, birthweight, gestational age) were initially excluded from the model to avoid overadjustment. Further adjustment for these covariates slightly reduced the effect estimates toward the null. Log-binomial models were used to estimate relative risks (RR) and 95% CIs (21, 22). In a few instances, the models did not converge, and log-Poisson models, which provide consistent but not fully efficient estimates of the RR and its confidence intervals, were used (23). Overall significance was calculated using a type III test. Test for interactions is described in the Data S1.

All tests were two-sided, and we used a threshold of $p < 0.05$ to denote statistical significance. The analyses were performed using the Statistical Analyses System software (release 9.4; SAS Institute, Cary, NC).

Results

Study population

In our study sample ($n = 32,465$), the median (interquartile range, IQR) of maternal vitamin D status prediction score was 58.7 (49.2–69.0) nmol/l and 58.6 (40.6–75.3) nmol/l for measured 25(OH)D ($n = 763$). Pregnant women in the highest category of the prediction score were more likely to be of high and medium SES, nulliparous, have a pre-pregnancy BMI in the normal range, and not smoke during pregnancy. Women in the lower category of vitamin D prediction score were more likely to be exposed to secondary smoke and partner smoking during pregnancy (Table 1).

There were no substantial differences in vitamin D prediction score, parental history of asthma/allergy, gestational weight gain, maternal paracetamol use, and child sex, birthweight, and gestational age between those with and without outcome data. For other covariates (parity, smoking, LMP, age, SEP, BMI), there were differences of 3–7% points between these groups.

Maternal vitamin D prediction score and child asthma at 18 months and 7 years

The prevalence of *asthma at 18 months* was 17% (4127/24,662) and 4% (819/21,194) for *current asthma at 7 years*. For *ever admitted asthma* and *ever prescribed asthma*, prevalence was 7% (1473/21,194) and 31% (6614/21,194), respectively.

There was no association with any of the child asthma outcomes when evaluating prediction score continuously, in quintiles (data not shown), or when comparing predefined categories of the prediction score <30 and ≥75 nmol/l (vs. 30–74.9 nmol/l) (Fig. 1, Table S1). A finer categorization of the exposure revealed a higher risk of child *asthma at 18 months* (1.36, 95% CI: 1.02, 1.80) and *ever admitted asthma* diagnosis (1.65, 95% CI: 1.04, 2.62) for maternal prediction score ≥100 vs. 50–79.9 nmol/l (Fig. 2, Table S2), but the overall tests of significance were insignificant ($p = 0.20$ and $p = 0.27$, respectively). There was no indication of increased risk for *current asthma at 7 years* or *ever prescribed asthma*. An increased risk of all measures of child asthma was observed in the <25 nmol/l category although these were not statistically significant after adjustment for confounders. Further adjustment for child covariates did not alter the effect estimates (data not shown).

In secondary analyses of *wheeze at 18 months* (6094/24,764) and *recurrent wheeze at 18 months* (1985/24,729), we found a direct association for a prediction score <30 nmol/l (vs. 30–74.9 nmol/l) (1.22, 95%CI: 1.04, 1.44) with *wheeze at 18 months* only.

The results for interactions can be found in the Data S1.

Maternal vitamin D prediction score and ever child allergic rhinitis at 7 years

Child *ever allergic rhinitis* was reported by 5% (1019/21,090) of mothers, and there were no associations with child *ever allergic rhinitis* when examining prediction score as continuous, in quintiles (data not shown), or by categories (Fig. 3, Table S3). There were modest but non-significant elevated risks of child *ever allergic rhinitis* in the <30 nmol/l and the ≥100 nmol/l categories (Fig. 3, Table S4). Further adjustment for child covariates did not alter the effect estimates (data not shown). The results for interactions can be found in the Data S1.

Stability analysis: maternal 25OH vitamin D levels and child allergic disease

The results from analyses in the subset with biomarker measurements available ($n = 763$) were consistent with those from the prediction score analysis: maternal 25(OH)D level ≥100 nmol/l (vs. 50–79.9 nmol/l) was associated with higher risk of child *asthma at 18 months* (2.40, 95% CI: 1.29, 4.46) and *ever prescribed asthma* (1.75, 95% CI: 1.13, 2.69), but the overall tests of significance were insignificant ($p = 0.21$ and $p = 0.44$, respectively) (Table S6). Similar patterns were observed for other child outcomes in the ≥100 nmol/l category, but these were non-significant (Tables S6, S8). A potential U-shaped curve was also noted for *current asthma at 7 years* and *ever allergic rhinitis* (Tables S6–S8), although the rise in risk in the <30 nmol/l category was not statistically significant for the latter.

Discussion

In a longitudinal cohort study of over 30,000 women enrolled during pregnancy, there was no overall association between

Table 1 Age-standardized covariates in relation to vitamin D¹ prediction score among 32,456 pregnant Danish women

	Vitamin D prediction score		
	<30 nmol/l n = 372 %	30–74.9 nmol/l n = 27,094 %	≥75 nmol/l n = 4990 %
Maternal age (years)*			
≤25	30	20	20
>25–30	35	45	48
>30–35	26	28	26
>35	9	7	5
Parental socioeconomic status and SES			
High	14	23	24
Medium	25	32	33
Skilled	28	28	26
Unskilled	25	13	12
Student	4	3	3
Unemployed	5	2	1
Parity			
Nulliparous	43	53	62
Pre-pregnancy body mass index (kg/m ²)			
18.6–24.9	62	65	69
Season at LMP			
Winter	5	21	56
Spring	10	24	24
Summer	59	25	8
Fall	26	30	12
Maternal paracetamol use (% yes)	1	0	1
Smoking during pregnancy			
Non-smoker	41	76	75
Occasional smoker	4	12	17
Daily smoker	55	12	7
Secondhand smoking during pregnancy (% yes)	60	46	44
Partner smoking during pregnancy (% yes)	45	29	28
Maternal asthma (% yes)	11	9	10
Maternal allergy (% yes)	30	32	32
Paternal asthma (% yes)	9	8	8
Paternal allergy (% yes)	21	24	26
Breastfeeding duration (months)			
No breastfeeding	4	2	2
0–1	21	10	10
2–3	15	11	11
4–6	16	18	20
7–9	22	27	26
≥10	22	32	31
Gestational weight gain (g/week)	426 (242)	468 (214)	472 (203)
Gestational age (days)	280 (13)	281 (12)	280 (12)
Birthweight (kg)	3.4 (0.5)	3.6 (0.6)	3.6 (0.6)
Child sex (% male)	48	51	51
Child antibiotics use at 6 months (% yes)	13	15	15
	38	12	9

Table 1. (Continued).

	Vitamin D prediction score		
	<30 nmol/l n = 372 %	30–74.9 nmol/l n = 27,094 %	≥75 nmol/l n = 4990 %
Child smoke exposure in first 18 months (% yes)			
Day care during the first 18 months (% yes)	11	7	6
Pets at 18 months (% yes)	38	35	34

Values are means (s.d.) or percentages and are standardized to the age distribution of the study population.

¹Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

*Value is not age adjusted.

maternal vitamin D prediction score and child allergic disease in quintiles or in categories <30, 25–74.9, ≥75 nmol/l. In secondary, more exploratory, analyses, there was a trend toward an increased risk of child *asthma at 18 months* and asthma by hospital admission during the first 7 years of life in offspring of mothers with prediction score >100 nmol/l. Although not statistically significant, an elevated risk of child asthma and allergic rhinitis was also present for score <25–30 nmol/l. These results were reproducible using plasma 25(OH)D for a subset of the study population. We found effect modification by breastfeeding, where both low prediction score and shorter duration of breastfeeding, and high prediction score and longer breastfeeding duration appeared to increase risk of child *asthma at 18 months*. Findings from the present study are partly in line with previous studies that have examined maternal or fetal 25(OH)D levels in relation to child allergic disease and have reported no associations (7, 8, 10, 12, 14). However, our findings of a potential U-shaped relation between maternal vitamin D levels and offspring allergic disease are new. They are in support of Rothers et al. (15) who found that 25(OH)D ≥ 100 nmol/l was associated with an increased risk of detectable allergen-specific IgE. Rothers et al. also found increased levels of IgE with vitamin D levels <50 nmol/l. U- or J-shaped associations for vitamin D have been observed with other outcomes including PPD (24), all-cause mortality (25, 26), and birth size (27); however, in some of these studies, subclinical disease and intractable confounding by lifestyle factors may account for the association.

One possible explanation for the inconsistency between our results and those of earlier studies is the use of different cutoff points to define the upper category (7, 9, 10, 14) or the failure to examine non-linear relationships in the case of studies that have only used a continuous exposure (12). Our main exposure, the vitamin D prediction score, is somewhat novel and does not represent actual biologic levels but rather modeled estimations of 25(OH)D (16). The prediction score explained 40% of the variance in plasma 25(OH)D, which is higher than has been previously reported by studies examining other chronic disease

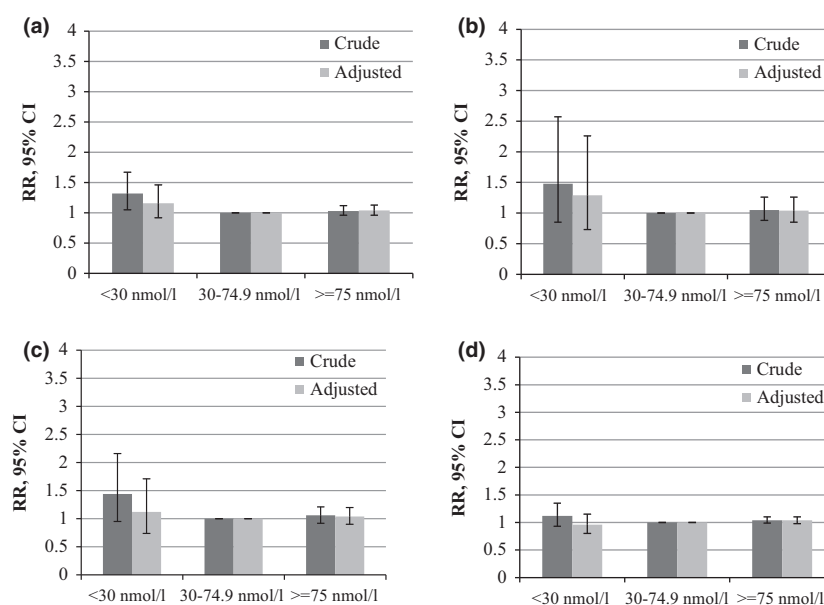


Figure 1 Multivariable¹ relation (RR, 95% CI) between maternal vitamin D prediction scores² and (a) child asthma at 18 months; (b) current asthma at 7 years; (c) ever admitted asthma; and (d) ever prescribed asthma. ¹Adjusted for maternal age, socioeconomic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex. ²Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

associations (25–33% in (28–30)). This may be due to better predictive power for a shorter exposure window like pregnancy. As the modeling is based on potential mismeasured covariates, some uncertainty in the prediction score is inevitable, which generally would be expected to attenuate associations to the null. The main point from the prediction score validation was that it did well in ranking women when compared to plasma levels (16), but that estimation of absolute levels of the prediction score should only be performed with caution. In our study, we found no association by ranking the women in quintiles. As our significant results are based on grouping using absolute levels of the prediction score, these need to be interpreted in light of the above-mentioned limitations. However, our conclusions are strengthened by similar results found for plasma 25(OH)D in the subset with biomarker measurements available.

Findings from animal and *in vitro* studies point to a complex immune response to vitamin D. Studies have found that 1,25(OH)2D enhances (31, 32) and suppresses (33) an allergy-prone T-helper (Th)2 response while others found that vitamin D influenced both Th1 and Th2 pathways (34, 35). Vitamin D has also been found to activate and maintain Treg cells (reviewed in (36)). Treg cells assure peripheral tolerance and immune homeostasis by depressing Th1 and Th2 responses, inducing tolerance in antigen-presenting cells, and reducing lung inflammation (24). Results from our study suggested an elevated risk of *asthma at 18 months* and *ever admitted asthma* with high levels of vitamin D prediction score. As the vast majority of children in our study reporting

asthma at 18 month did not report later asthma, this outcome most likely encompasses primarily transient wheeze, viral bronchitis, and bronchiolitis. This suggests that vitamin D may influence both a Th1 response in early life and a more established Th2 profile.

An alternative explanation of the U-shaped relation could be genetic polymorphisms of proteins governing vitamin D metabolism and transport (25, 26). High 25(OH)D may entail low intracellular 1,25(OH)2D as a consequence of reduced or inefficient hydroxylation of 25(OH)D (25). Alternatively, or in addition, high 25(OH)D may reflect lower transport to the fetus or poor 1,25(OH)2D conversion at the placenta. Future studies need to clarify a potential dual response of 25(OH)D and the role of genetic polymorphisms in relation to allergic disease development.

In our study, we found that low vitamin D prediction score and shorter breastfeeding duration, and high vitamin D prediction score and longer breastfeeding duration both increased the risk of asthma in early childhood. As ~95% of women in our study population who breastfed <7 months reported some use of formula, both groups may represent high exposure. While some indications exist that dietary and supplemental vitamin D in infancy may increase risk of allergic disease (27, 37), higher child 25(OH)D has also been associated with a reduced risk of asthma (38, 39). An increased risk of *ever admitted asthma* among those with no maternal history of asthma is intriguing but requires verification.

The greatest strength of this study was the inclusion of both early and later manifestations of allergic disease. Asthma

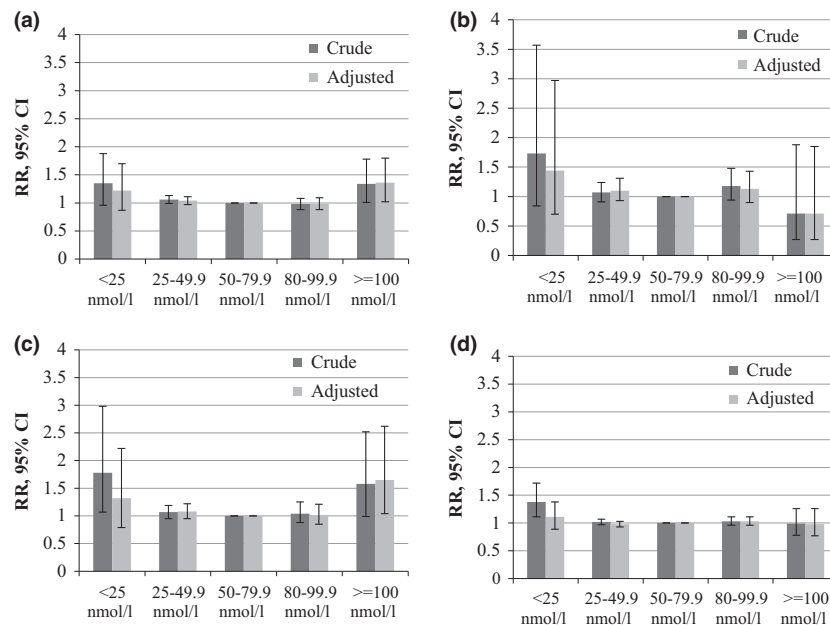


Figure 2 Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal vitamin D prediction scores² and (a) child asthma at 18 months; (b) current asthma at 7 years; (c) ever admitted asthma; and (d) ever prescribed asthma. ¹Adjusted for maternal age, socioeconomic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex. ²Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

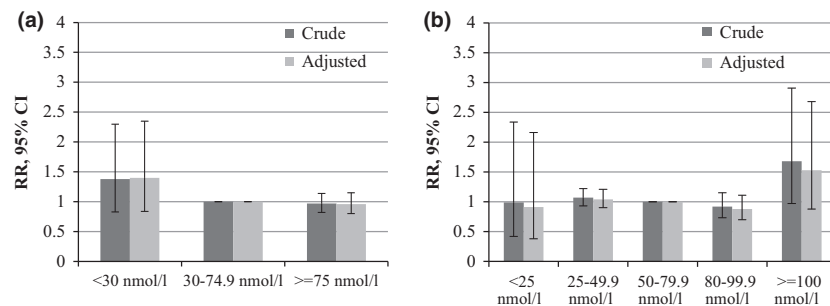


Figure 3 Multivariable¹ relation (RR, 95% CI) between maternal vitamin D prediction scores² and child ever allergic rhinitis (a) primary and (b) secondary classification. ¹Adjusted for maternal age, socioeconomic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex. ²Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

identified before age of 5 years is a conglomerate measure of transient and viral-induced wheeze, and some persistent asthma (40), while asthma assessed at age 7 may represent more clinically established asthma and may vary in severity. If these outcomes result from potentially different etiology, for example, as it relates to the Th1: Th2: Treg balance and relation to early life vitamin D, this justifies separate examination of the different outcomes. We employed both self-reported and registry-based outcomes. Self-reported asthma

may be prone to misclassification, but we would argue that such misclassification would most likely be non-differential given the prospective design and the lack of emphasis on any specific disease hypothesis. The registry-based asthma outcomes most likely represent different phenotypes, from more severe asthma admitted to the hospital, which also represents an outcome with high specificity, to mild-to-moderate asthma requiring some form of prescribed medication (41). Both ever admitted and ever prescribed asthma have been well validated

against medical examinations (42) and discharge diagnosis (19), respectively. In this study, we found an increased risk of child admitted but not prescribed asthma with high vitamin D prediction score. This may suggest that vitamin D status plays a role in severity of asthma. In a longitudinal cohort study, we cannot exclude possible residual confounding and selection bias, although we found little change in our findings in sensitivity analyses ‘... adjusted for both parental and child covariate..’ and observed few differences in the characteristics of those with and without outcome data. Additionally, given the number of analyses, we cannot completely exclude the possibility of significant associations occurring by chance alone.

In this large prospective cohort study, there was little evidence of an association between vitamin D status prediction score based on maternal plasma measurements in mid-pregnancy and child allergic disease. Exploratory analyses of the highest and lowest values of the prediction score revealed indications of a U-shaped relation for child asthma at 18 months and ever admitted asthma, which was corroborated by analyses of 25(OH)D plasma levels. Our findings are hypothesis generating and therefore need to be replicate in other cohorts. While addressing vitamin D deficiency should still be a priority, potential adverse effects of high vitamin D in early life may also need to be considered and explored.

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Authors’ contribution

The authors’ responsibilities were as follows – EM: study concept and design, and data analysis; EM, SH, CBJ, and MS: data composition and drafting the manuscript; SFO: led data collection on dietary intake in pregnancy. AC: plasma 25(OH)D measurements on maternal plasma; MS and NON: contribution of data on maternal plasma 25(OH)D levels; EM, SH, ATL, CBJ, MS, AC, NON, SFO: critical revising and final accept of manuscript.

Conflict of interest

All authors had full access to study data. None of the authors had a conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Multivariable¹ relation (RR, 95% CI) between maternal vitamin D prediction scores² and child asthma at 18 months and 7 years.

Table S2. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal vitamin D prediction scores² and child asthma at 18 months and 7 years.

Table S3. Multivariable¹ relation (RR, 95% CI) between maternal vitamin D prediction scores² and child ever allergic rhinitis.

Table S4. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal vitamin D prediction scores² and child ever allergic rhinitis.

Table S5. Multivariable¹ relation (RR, 95% CI) between maternal plasma 25OH-vitamin D and child asthma at 18 months and 7 years.

Table S6. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal plasma 25OH-vitamin D and child asthma at 18 months and 7 years.

Table S7. Multivariable¹ relation (RR, 95% CI) between maternal plasma 25OH-vitamin D and child ever allergic rhinitis.

Table S8. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal plasma 25OH-vitamin D and child ever allergic rhinitis.

Table S9. P-values for interaction for selected effect modifiers with vitamin D prediction score in relation to child allergic disease.

Data S1. Supplementary document contains additional Methods, Results and References.

Supplemental Table 1. Multivariable¹ relation (RR, 95% CI) between maternal vitamin D prediction scores² and child asthma at 18 months and 7 years.

Exposure/Outcome		Cases/N	Asthma at 18 months N=24,662 N=24,240 RR (95% CI)	Cases/N	Current asthma at 7 years N=21,194 N=20,800 RR (95% CI)	Cases/N	Ever admitted asthma N=21,194 N=20,800 RR (95% CI)	Cases/N	Ever prescribed asthma N=21,194 N=20,800 RR (95% CI)
<30 nmol/L	Crude	57/260	1.32 (1.05, 1.67)	12/213	1.48 (0.85, 2.57)	21/213	1.44 (0.95, 2.16)	74/213	1.12 (0.93, 1.35)
	Adjusted	57/257	1.16 (0.92, 1.46)	12/210	1.29 (0.73, 2.26)	21/210	1.12 (0.74, 1.71)	73/210	0.96 (0.80, 1.15)
30-74.9 nmol/L	Crude	3,423/20,629	1.00	675/17,694	1.00	1,214/17,694	1.00	5,481/17,694	1.00
	Adjusted	3,370/20,272	1.00	657/17,355	1.00	1,182/17,355	1.00	5,370/17,355	1.00
>=75 nmol/L	Crude	647/3,773	1.03 (0.96, 1.12)	132/3,287	1.05 (0.88, 1.26)	238/3,287	1.06 (0.92, 1.21)	1,059/3,287	1.04 (0.99, 1.10)
	Adjusted	635/3,711	1.04 (0.96, 1.13)	131/3,235	1.04 (0.85, 1.26)	234/3,235	1.04 (0.90, 1.20)	1,034/3,235	1.04 (0.98, 1.10)
P-value ³	Crude		0.07		0.39		0.21		0.20
	Adjusted		0.30		0.71		0.74		0.38

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

³Overall test of significance using likelihood ratio statistics.

Supplemental Table 2. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal vitamin D prediction scores² and child asthma at 18 months and 7 years.

Exposure/Outcome		Cases/N	Asthma at 18 months N=24,662 N=24,240 RR (95% CI)	Cases/N	Current asthma at 7 years N=21,194 N=20,800 RR (95% CI)	Cases/N	Ever admitted asthma N=21,194 N=20,800 RR (95% CI)	Cases/N	Ever prescribed asthma N=21,194 N=20,800 RR (95% CI)
<25 nmol/L	Crude	27/122	1.35 (0.96, 1.88)	7/108	1.73 (0.84, 3.57)	13/108	1.78 (1.07, 2.98)	46/108	1.38 (1.11, 1.72)
	Adjusted	27/120	1.22 (0.87, 1.70)	7/107	1.44 (0.70, 2.97)	13/107	1.32 (0.79, 2.22)	45/107	1.11 (0.89, 1.38)
25-49.9 nmol/L	Crude	1,119/6,428	1.06 (0.99, 1.13)	218/5,474	1.07 (0.91, 1.24)	394/5,474	1.07 (0.95, 1.19)	1,725/5,474	1.02 (0.97, 1.07)
	Adjusted	1,100/6,303	1.04 (0.97, 1.11)	214/5,352	1.10 (0.93, 1.31)	382/5,352	1.08 (0.95, 1.22)	1,676/5,352	0.98 (0.93, 1.03)
50-79.9 nmol/L	Crude	2,604/15,831	1.00	509/13,620	1.00	920/13,620	1.00	4,209/13,620	1.00
	Adjusted	2,564/15,568	1.00	495/13,377	1.00	898/13,377	1.00	4,135/13,377	1.00
80-99.9 nmol/L	Crude	339/2,109	0.98 (0.88, 1.08)	81/1,842	1.18 (0.94, 1.48)	130/1,842	1.04 (0.88, 1.25)	588/1,842	1.03 (0.96, 1.11)
	Adjusted	334/2,082	0.98 (0.88, 1.09)	80/1,818	1.13 (0.90, 1.43)	128/1,818	1.01 (0.85, 1.21)	577/1,818	1.03 (0.96, 1.11)
>=100 nmol/L	Crude	38/172	1.34 (1.01, 1.78)	4/150	0.71 (0.27, 1.88)	16/150	1.58 (0.99, 2.52)	46/150	0.99 (0.78, 1.26)
	Adjusted	37/167	1.36 (1.02, 1.80)	4/146	0.71 (0.27, 1.85)	16/146	1.65 (1.04, 2.62)	44/146	0.98 (0.77, 1.26)
P-value ³	Crude		0.05		0.35		0.10		0.11
	Adjusted		0.20		0.52		0.27		0.84

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

³Overall test of significance using likelihood ratio statistics.

Supplemental Table 3. Multivariable¹ relation (RR, 95% CI) between maternal vitamin D prediction scores² and child ever allergic rhinitis.

Exposure/Outcome		Cases/N	Ever allergic rhinitis N=21,090 N=20,702 RR (95% CI)
<30 nmol/L	Crude	14/210	1.38 (0.83, 2.30)
	Adjusted	14/207	1.40 (0.84, 2.35)
30-74.9 nmol/L	Crude	852/17,611	1.00 (ref)
	Adjusted	840/17,275	1.00 (ref)
≥75 nmol/L	Crude	153/3,269	0.97 (0.82, 1.14)
	Adjusted	150/3,220	0.96 (0.80, 1.15)
P-value ³	Crude		0.46
	Adjusted		0.45

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

³Overall test of significance using likelihood ratio statistics.

Supplemental Table 4. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal vitamin D prediction scores² and child ever allergic rhinitis.

Exposure/Outcome		Cases/N	Ever allergic rhinitis N=21,090 N=20,702 RR (95% CI)
<25 nmol/L	Crude	5/106	0.99 (0.42, 2.34)
	Adjusted	5/105	0.91 (0.38, 2.16)
25-49.9 nmol/L	Crude	276/5,441	1.07 (0.93, 1.22)
	Adjusted	271/5,319	1.04 (0.90, 1.21)
50-79.9 nmol/L	Crude	646/13,567	1.00 (ref)
	Adjusted	638/13,328	1.00 (ref)
80-99.9 nmol/L	Crude	80/1,826	0.92 (0.73, 1.15)
	Adjusted	79,1,804	0.88 (0.70, 1.11)
≥100 nmol/L	Crude	12/150	1.68 (0.97, 2.91)
	Adjusted	11/146	1.53 (0.88, 2.68)
P-value ³	Crude		0.35
	Adjusted		0.48

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Prediction scores based on a model including intake of vitamin D from diet and supplements, outdoor physical activity, tanning bed use, smoking, and month of blood draw.

³Overall test of significance using likelihood ratio statistics.

Supplemental Table 5. Multivariable¹ relation (RR, 95% CI) between maternal serum 25OH-vitamin D and child asthma at 18 months and 7 years.

Exposure/Outcome		Cases/N	Asthma at 18 mo	Cases/N	Current asthma at 7 years	Cases/N	Ever admitted asthma	Cases/N	Ever prescribed asthma
			N=561 N=538 RR (95% CI)		N=488 N=465 RR (95% CI)		N=488 N=465 RR (95% CI)		N=488 N=465 RR (95% CI)
<30 nmol/L	Crude	13/74	1.23 (0.70, 2.15)	4/57	2.66 (0.83 8.53)	4/57	0.92 (0.33, 2.57)	23/57	1.22 (0.86, 1.74)
	Adjusted	9/65	0.80 (0.43, 1.47)	4/51	4.49 (1.19, 17.0)	3/51	0.88 (0.27, 2.87)	18/51	1.07 (0.72, 1.59)
30-74.9 nmol/L	Crude	48/336	1.00 (ref)	8/303	1.00 (ref)	23/303	1.00 (ref)	100/303	1.00 (ref)
	Adjusted	47/325	1.00 (ref)	6/288	1.00 (ref)	21/288	1.00 (ref)	92/288	1.00 (ref)
≥75 nmol/L	Crude	33/151	1.53 (1.03, 2.28)	5/128	1.48 (0.49, 4.44)	6/128	0.62 (0.26, 1.48)	46/128	1.09 (0.82, 1.44)
	Adjusted	32/148	1.59 (1.04, 2.43)	5/126	3.59 (0.97, 13.3)	6/126	0.66 (0.30, 1.47)	46/126	1.27 (0.95, 1.69)
P-value ²	Crude		0.12		0.30		0.52		0.53
	Adjusted		0.13		0.06		0.66		0.47

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Overall test of significance using likelihood ratio statistics.

Supplemental Table 6. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal serum 25OH-vitamin D and child asthma at 18 months and 7 years.

Exposure/Outcome		Cases/N	Asthma at 18 mo	Cases/N	Current asthma at 7 years	Cases/N	Ever admitted asthma	Cases/N	Ever prescribed asthma
			N=561 N=538 RR (95% CI)		N=488 N=465 RR (95% CI)		N=488 N=465 RR (95% CI)		N=488 N=465 RR (95% CI)
<25 nmol/L	Crude	7/45	1.01 (0.48, 2.13)	0/31	-	2/31	0.82 (0.20, 3.38)	12/31	1.10 (0.68, 1.78)
	Adjusted	5/38	0.65 (0.28, 1.53)	0/26	-	2/26	1.16 (0.20, 6.64)	8/26	0.83 (0.45, 1.55)
25-49.9 nmol/L	Crude	27/171	1.03 (0.65, 1.62)	6/144	1.29 (0.46, 3.63)	10/144	0.88 (0.42, 1.87)	50/144	0.99 (0.74, 1.32)
	Adjusted	24/164	0.91 (0.55, 1.50)	5/135	0.87 (0.27, 2.85)	8/135	0.92 (0.38, 2.24)	45/135	0.96 (0.69, 1.34)
50-79.9 nmol/L	Crude	36/234	1.00 (ref)	8/216	1.00 (ref)	17/216	1.00 (ref)	76/216	1.00 (ref)
	Adjusted	36/228	1.00 (ref)	7/209	1.00 (ref)	16/209	1.00 (ref)	72/209	1.00 (ref)
80-99.9 nmol/L	Crude	14/85	1.07 (0.61, 1.88)	2/70	0.88 (0.19, 4.06)	1/70	0.18 (0.02, 1.34)	18/70	0.73 (0.47, 1.13)
	Adjusted	13/82	1.11 (0.61, 2.02)	2/68	1.28 (0.26, 6.24)	1/68	0.21 (0.03, 1.36)	18/68	0.86 (0.54, 1.36)
≥100 nmol/L	Crude	10/26	2.50 (1.41, 4.43)	1/27	1.14 (0.15, 8.80)	3/27	1.41 (0.44, 4.51)	13/27	1.37 (0.89, 2.11)
	Adjusted	10/26	2.40 (1.29, 4.46)	1/27	4.78 (0.41, 56.5)	3/27	2.01 (0.71, 5.71)	13/27	1.75 (1.13, 2.69)
P-value ²	Crude		0.11		0.64		0.23		0.30
	Adjusted		0.21		0.55		0.28		0.44

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Overall test of significance using likelihood ratio statistics.

Supplemental Table 7. Multivariable¹ relation (RR, 95% CI) between maternal serum 25OH-vitamin D and child ever allergic rhinitis.

Exposure/Outcome		Cases/N	Ever allergic rhinitis N=483 N=460 RR (95% CI)
<30 nmol/L	Crude	4/56	1.79 (0.60, 5.34)
	Adjusted	4/50	2.75 (0.83, 9.16)
30-74.9 nmol/L	Crude	12/300	1.00 (ref)
	Adjusted	11/285	1.00 (ref)
≥75 nmol/L	Crude	5/127	0.98 (0.35, 2.74)
	Adjusted	5/125	1.01 (0.33, 3.10)
P-value ²	Crude		0.60
	Adjusted		0.32

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Overall test of significance using likelihood ratio statistics.

Supplemental Table 8. Multivariable¹ relation (RR, 95% CI) between a secondary classification of maternal serum 25OH-vitamin D and child ever allergic rhinitis.

Exposure/Outcome		Cases/N	Ever allergic rhinitis N=483 N=483 RR (95% CI)
<25 nmol/L	Crude	2/31	1.37 (0.31, 5.95)
	Adjusted	2/26	2.47 (0.56, 10.9)
25-49.9 nmol/L	Crude	6/144	0.88 (0.33, 2.38)
	Adjusted	5/135	0.68 (0.23, 2.02)
50-79.9 nmol/L	Crude	10/212	1.00 (ref)
	Adjusted	10/205	1.00 (ref)
80-99.9 nmol/L	Crude	1/69	0.31 (0.04, 2.36)
	Adjusted	1/67	0.25 (0.03, 2.35)
≥100 nmol/L	Crude	2/27	1.57 (0.36, 6.79)
	Adjusted	2/27	2.03 (0.34, 12.2)
P-value ²	Crude		0.60
	Adjusted		0.30

¹Adjusted for maternal age, socio-economic position, parity, prepregnancy BMI, smoking during pregnancy, secondary smoking exposure during pregnancy, partner's smoking during pregnancy, maternal paracetamol use during pregnancy, maternal history of asthma, maternal history of allergies, paternal history of asthma, paternal history of allergies, month of last menstrual period, breastfeeding duration, and child sex.

²Overall test of significance using likelihood ratio statistics.

Supplemental Table 9. P-values for interaction for selected effect modifiers with vitamin D prediction score in relation to child allergic disease.

Effect modifier/Outcome	Asthma at 18 months	Current asthma at 7 years	Ever admitted asthma	Ever prescribed asthma	Ever allergic rhinitis
Maternal history of asthma	0.45	0.23	0.01	0.77	0.10
Paternal history of asthma	0.61	0.30	0.92	0.67	0.50
Maternal history of allergy	0.48	0.13	0.28	0.13	0.51
Paternal history of allergy	0.36	0.47	0.71	0.84	0.21
Child sex	0.67	0.87	0.98	0.47	0.48
Breastfeeding	0.02	0.16	0.23	0.44	0.20
Season of LMP	0.09	0.49	0.98	0.73	0.64

LMP: last menstrual period

Supplementary document

Methods

Maternal exposure – 25(OH)D levels

25(OH)D3 and 25(OH)D2 were determined using liquid chromatography - mass spectrometry. The methodology was previously described in (1). Briefly, 30 uL of plasma were deproteinized in microtiter plates using 120 uL acetonitrile containing 2H3-25(OH)D3 and 2H3-25(OH)D3 as internal standards. The supernatants were transferred to fresh plates and dried under a gentle flow of nitrogen. Subsequently, the samples were derivatized using 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) dissolved in acetonitrile. The derivatization reaction was quenched with quench solution and the samples were subjected to LC- MSMS analysis. Total 25(OH)D2 was calculated by combining 25(OH)D3 and 25(OH)D2 values.

After exclusion of samples with failed analysis (n=10), second and third cohort pregnancies (n=141), and twin pregnancies (n=26), we were left with 1,320 samples, of which 763 also had calculated prediction scores. The measurements were originally done for a case-control study on vitamin D and postpartum depression (PPD), which reported null findings (1), and therefore we included both cases and controls.

Interactions

Interactions by parental history of asthma and allergy, child sex, breastfeeding duration, and season of LMP were assessed using tertiles of vitamin D prediction score and evaluated using a score test. These were motivated by the findings of previous studies which had effect modification by parental history of allergic disease, child sex, and breastfeeding (2, 3). In a prior study, we observed an association between season of LMP and child asthma (4) and were interested in whether a potential season-associated risk may be modified by maternal vitamin D

prediction score status. Stratifications were carried out on significant interactions and those variables defined *a priori* to be of interest.

Results

Interactions

There were few interactions with vitamin D prediction score for any of the allergic disease outcome (**Supplementary Table 9**). Maternal history of asthma and breastfeeding displayed a significant interaction with vitamin D prediction score for *child ever admitted asthma* and *asthma at 18 months*, respectively. When stratified upon maternal history of asthma, no significant associations with vitamin D prediction score were found among offspring whose mother reported a history of asthma, though the sample size was small (N=2,960) and confidence intervals wide. Among those with no maternal history of asthma an increased risk of *child ever admitted asthma* was present both for <30 nmol/L (vs. 50-79.9 nmol/L) (1.98, 95% CI: 1.17, 3.35) and ≥100 (1.66, 95% CI: 1.01, 2.74). For breastfeeding, increased risks of *child asthma at 18 months* were observed both for women who reported shorter breastfeeding (<7 months) and had low prediction score (<30 vs. 50-79.9 nmol/L) (RR: 1.27, 95% CI: 0.94, 1.72), as well as for women with longer periods of breastfeeding (≥7 months) and high prediction score (≥100 vs. 50-79.9 nmol/L) (RR: 1.58, 95% CI: 1.05, 2.35).

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