Longitudinal trajectory of vitamin D status from birth to early childhood in the development of food sensitization

Xin Liu[1,2], Lester Arguelles[2], Ying Zhou[3], Guoying Wang[4], Qi Chen[1], Hui-Ju Tsai[1,5], Xiumei Hong[4], Rong Liu[1], Heather E. Price[6], Colleen Pearson[7], Stephanie Apollon[7], Natalie Cruz[7], Robert Schleimer[8], Craig B. Langman[6], Jacqueline A. Pongracic[9] and Xiaobin Wang[1,4,10]

BACKGROUND: Increasing evidence supports the immunomodulatory effect of vitamin D on allergic diseases. The combined role of prenatal and postnatal vitamin D status in the development of food sensitization (FS) and food allergy remains understudied.

METHODS: Plasma 25-hydroxyvitamin D (25(OH)D) levels of 460 children in the Boston Birth Cohort (BBC) were measured at birth and early childhood, and the subjects were genotyped for rs2243250 (C-590T) in the *IL4* gene. We defined FS as specific IgE levels of ≥0.35 kUA/l to any of eight common food allergens; we defined persistently low vitamin D status as cord blood 25(OH)D <11 ng/ml and postnatal 25(OH)D <30 ng/ml.

RESULTS: We observed a moderate correlation between cord blood 25(OH)D at birth and venous blood 25(OH)D measured at 2–3 y (r = 0.63), but a weak correlation at <1 y (r = 0.28). There was no association between low vitamin D status and FS at any single time point alone. However, in combination, persistence of low vitamin D status at birth and in early childhood increased the risk of FS (odds ratio (OR) = 2.03, 95% confidence interval (CI): 1.02–4.04), particularly among children carrying the C allele of rs2243250 (OR = 3.23, 95% CI: 1.37–7.60).

CONCLUSION: Prenatal and early postnatal vitamin D levels, along with individual genetic susceptibility, should be considered in assessing the role of vitamin D in the development of FS and food allergy.

Vitamin D has become increasingly recognized as an important regulator of immune responses (1). The vitamin D hypothesis, one of several hypotheses on the development of food allergy, was first suggested in 2007 (2), and the potential mechanisms were later proposed in detail by Vassallo and Camargo (3). Although several cross-sectional studies have been conducted to examine the associations between plasma 25-hydroxyvitamin D (25(OH)D) levels and allergic diseases and associated phenotypes (4–10), findings remain inconsistent. Previous studies (11–15) indicate that the immunomodulatory effects of vitamin D, including its contribution to the development of allergic diseases, begin *in utero*. To date, only three birth cohort studies have examined the effect of prenatal vitamin D exposure on allergic phenotypes using cord blood 25(OH)D concentrations, an objective measure of vitamin D status reflecting both dietary intake and sun exposure. Camargo *et al.* found that newborns in New Zealand with low cord blood 25(OH)D (<10 ng/ml) were at a higher risk for respiratory infection and childhood wheezing but not for incident asthma as compared with newborns with higher cord blood 25(OH)D (≥30 ng/ml) (16). In their Tucson cohort, Rothers *et al.* (17) reported that both higher (>40 ng/ml) and lower (<20 ng/ml) levels of vitamin D were associated with higher total IgE and detectable inhalant allergen-specific IgE; higher levels of vitamin D were also associated with positive allergy skin tests. Our study, conducted primarily in African-American children in the United States, was the first to show that genetic polymorphisms might modify the effects of vitamin D deficiency on the risk of food sensitization (FS) (18). However, findings regarding the combined effects of prenatal and postnatal vitamin D status on FS, two of the most critical periods for immune system development (19,20), are unclear.

In an earlier report, we examined a single time point gene–cord blood vitamin D interaction on FS in the Boston Birth Cohort (BBC) (16). This study further extends and strengthens our previous work by examining the risk of FS in relation to the longitudinal trajectory of vitamin D status from birth to early childhood in the same birth cohort. Herein, we examine the interaction of a promoter polymorphism (rs2243250: C-590T) in the *IL4* gene and the longitudinal trajectory of vitamin D status from birth to early childhood on the risk of FS. This particular gene variant was chosen on the basis of our most significant finding from the previous studies (18).

[1]Mary Ann and J. Milburn Smith Child Health Research Program, Department of Pediatrics, Northwestern University Feinberg School of Medicine and Ann & Robert H. Lurie Children's Hospital of Chicago Research Center, Chicago, Illinois; [2]Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois; [3]Biostatistics Research Core, Ann & Robert H. Lurie Children's Hospital of Chicago Research Center, Chicago, Illinois; [4]Center on the Early Life Origins of Disease, Department of Population, Family and Reproductive Health, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland; [5]Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan; [6]Division of Kidney Disease, Department of Pediatrics, Northwestern University Feinberg School of Medicine and Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois; [7]Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, Massachusetts; [8]Division of Allergy-Immunology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois; [9]Division of Allergy and Immunology, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois; [10]Division of General Pediatrics & Adolescent Medicine, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland. Correspondence: Xin Liu (xnliu@luriechildrens.org)

Received 8 August 2012; accepted 11 February 2013; advance online publication 14 August 2013. doi:10.1038/pr.2013.110

RESULTS

Approximately, one-third of the 460 children had detectable specific IgE (sIgE) to any food allergen by the age of 3 y and were defined as FS cases (Table 1). The FS children and those without detectable sIgE differed in regard to maternal race, age, maternal smoking, household income, infant gender, breastfeeding pattern, and African ancestral proportion ($P < 0.1$). On the whole, when looking at the studied children (Figure 1), total 25(OH)D concentration (ng/ml) in cord blood was quite low ($N = 460$, purple curve: 14.16 ± 7.90 ng/ml (mean ± SD)). Among children whose follow-up measurement was obtained within 1 y of age, vitamin D levels were dramatically increased ($N = 232$, black curve: 35.63 ± 11.43 ng/ml). Vitamin D levels measured at 1–2 y of age ($N = 163$, red curve: 33.60 ± 11.04 ng/ml) or 2–3 y of age ($N = 65$, green curve: 31.73 ± 8.40 ng/ml) were slightly lower than the values obtained within 1 y of age. Similarly, the proportions of children with low vitamin D status at birth (i.e., <11 ng/ml), <1, 1–2, and 2–3 y (i.e., <30 ng/ml) were 38, 29, 36, and 40%, respectively; and the correlation coefficients between cord blood 25(OH)D concentrations and 25(OH)D measures up to age 1, 1–2, and 2–3 y were 0.28, 0.39, and 0.63, respectively. Of note, doctor-diagnosed food allergy was reported in only 31 children. The mean (SD) plasma 25(OH)D concentrations at birth and at early childhood for these children in the FS group ($N = 21$) were 12.19 ng/ml (4.61) and 37.05 ng/ml (13.09), respectively, and 14.53 ng/ml (5.60) and 33.32 ng/ml (8.03) for those in non-FS group ($N = 10$).

Among the FS-associated variables, maternal race, infant African ancestry proportion, and household income were associated with the concentration of 25(OH)D in cord blood (Table 2), whereas only breastfeeding status was significantly associated with lower postnatal 25(OH)D levels, as compared with formula only (mean ± SD: 23.75 ± 14.07 vs. 36.15 ± 9.58 ng/ml, respectively). As such, our analyses have considered not only the possible confounding variables (i.e., ethnicity and household income) but also other FS-associated variables (i.e., infant sex, postnatal maternal smoking, and maternal age) in the regression model for testing the longitudinal effects of 25(OH)D on FS.

When we examined 25(OH)D concentrations across FS status, we found that FS cases had lower cord plasma 25(OH)D than nonsensitized controls (12.86 ± 5.91 vs. 14.87 ± 8.73 ng/ml, respectively; $P = 0.04$), but this difference was not apparent in the postnatal measures (34.24 ± 11.12 vs. 34.43 ± 10.93 ng/ml, respectively; $P = 0.86$) (Table 1). Individually, neither cord blood nor postnatal low vitamin D status was significantly associated with any FS (Table 3). However, children with persistently low vitamin D status had the highest risk of FS (odds ratio (OR) = 2.04, 95% confidence interval (CI): 1.02–4.04), as compared with those with sufficient vitamin D status at birth and follow-up. Similar association patterns between persistently low vitamin D and high risk of FS were seen among children with 25(OH)D measurements within 1 y of age and between 1 and 3 y of age, among children born in winter and nonwinter, and among children born preterm (<37 wk of gestation) and children born at

Table 1. Major characteristics of 460 subjects in the Boston Birth Cohort

Variable	Food sensitization cases ($n = 162$) n (%)	Nonfood sensitization controls ($n = 298$) n (%)	P value
Maternal race			
Black	100 (62)	143 (48)	
White	4 (2)	23 (8)	
Hispanic	33 (20)	82 (28)	
Other	25 (15)	50 (17)	0.01
Maternal BMI (kg/m^2) (prepregnancy)			
<20	18 (11)	24 (8)	
20–24.9	57 (35)	115 (39)	
25–29.9	49 (30)	94 (32)	
≥30	38 (23)	63 (21)	0.64
Maternal age (y)			
<20	16 (10)	16 (5)	
20–24.9	30 (19)	89 (30)	
25–29.9	33 (20)	92 (31)	
30–34.9	45 (28)	54 (18)	
≥35	38 (23)	47 (16)	0.0004
Maternal education			
Middle school	44 (27)	92 (31)	
High school	66 (41)	111 (37)	
>High school	52 (32)	95 (32)	0.66
Maternal atopy	60 (37)	97 (33)	0.34
Maternal smoking during pregnancy	12 (7)	38 (13)	0.08
Infant sex (male)	97 (60)	145 (49)	0.02
Preterm (<37 GWs)	27 (17)	60 (20)	0.36
Birth season			
Winter (January to March)	37 (23)	61 (20)	
Spring (April to June)	40 (25)	84 (28)	
Summer (July to September)	35 (22)	72 (24)	
Fall (October to December)	50 (31)	81 (27)	0.67
Maternal smoking (postnatal)	20 (12)	55 (18)	0.09
Household income			
<$30,000	71 (44)	129 (43)	
≥$30,000	11 (7)	40 (13)	
Unknown	80 (49)	129 (43)	0.08
Breastfeeding			
Breastfeeding only	9 (6)	23 (8)	
Formula only	31 (19)	81 (27)	
Both	122 (75)	193 (65)	0.07
Food allergy	21 (13)	10 (3)	<0.0001
	Mean ± SD		
African ancestry proportion	0.68 ± 0.30	0.55 ± 0.34	0.0002
Cord blood 25(OH)D (ng/ml)	12.86 ± 5.91	14.87 ± 8.73	0.04
Follow-up blood 25(OH)D (ng/ml)	34.24 ± 11.12	34.43 ± 10.93	0.86

Due to rounding, percentages for certain variables do not add up to 100%.
25(OH)D, 25-hydroxyvitamin D; GW, gestational week.

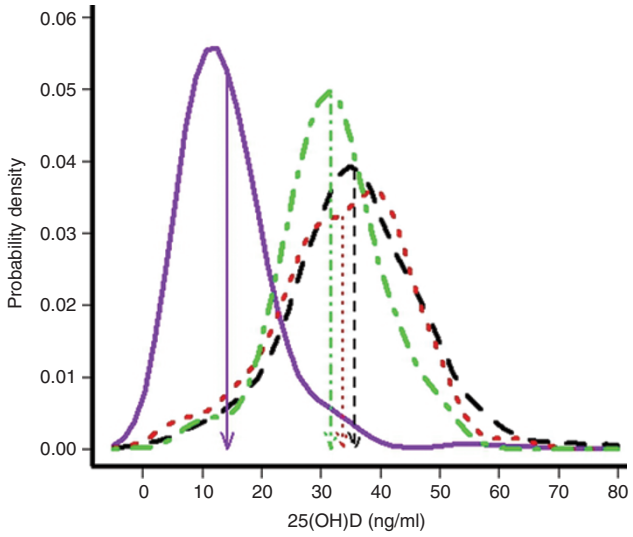

Figure 1. Distributions of plasma 25(OH)D at birth (cord blood) (solid purple line), <1 y (black dashed line), 1–2 y (red dotted line), and 2–3 y (green dashed-dot line) (means: vertical lines) among 460 subjects from the Boston Birth Cohort. 25(OH)D, 25-hydroxyvitamin D.

term (≥37 wk) (data not shown). Similar association patterns were also observed from weighted logistic regression analyses with two different weights assigned to preterm and term children according to the proportion of preterm cases in the study samples (19%) and those in the overall baseline sample (27%) (data not shown). Due to small sample size after stratification, the significant association between persistently low vitamin D and risk of FS was observed only in children with 25(OH)D measurements within 1 y of age (OR = 2.99, 95% CI: 1.05–8.52).

Finally, we observed an interaction effect between rs2243250 and persistently low vitamin D status on the risk of FS ($P_{\text{interaction}}$ = 0.02). Among children carrying the C allele of rs2243250 (~65% of the study subjects), persistently low vitamin D status was associated with a more than threefold increased risk of FS (OR = 3.23, 95% CI: 1.37–7.60) as compared with those with sufficient vitamin D at both time points. Of note, when low vitamin D at birth was followed by sufficient vitamin D in early childhood, children carrying the C allele were not at an increased risk for FS (OR = 1.26, 95% CI: 0.65–2.43). However, a decreased risk of FS was observed for those carrying the TT genotype (OR = 0.32, 95% CI: 0.12–0.82) as compared with the reference group (**Table 3**).

DISCUSSION

To our knowledge, this is the first study to examine the effects of longitudinal trajectory vitamin D status, by measurement of plasma 25(OH)D concentrations from birth to early childhood, on the development of FS. We found that persistently low vitamin D status from birth to early childhood was associated with FS in the BBC. Our findings suggest that both prenatal and early postnatal vitamin D levels appear to play an important role in the development of FS, especially among those with specific genotypes.

Table 2. Distribution of pre- and postnatal plasma 25(OH)D concentration by major characteristics of 460 subjects in the Boston Birth Cohort

	Cord blood 25(OH)D (ng/ml)	Postnatal 25(OH)D (ng/ml)
Variable	Mean (SD)	Mean (SD)
Maternal race**		
Black	12.13 (5.84)	33.69 (11.00)
White	21.26 (10.55)	38.89 (12.39)
Hispanic	16.34 (9.62)	34.69 (10.13)
Other	14.86 (7.38)	34.40 (11.43)
Maternal BMI (kg/m^2) (prepregnancy)*		
<20	14.69 (7.98)	34.85 (10.01)
20–24.9	15.01 (8.48)	34.60 (11.52)
25–29.9	14.64 (8.28)	34.82 (11.34)
≥30	11.87 (5.68)	32.90 (9.90)
Maternal age (y)		
<20	12.60 (5.66)	32.59 (12.19)
20–24.9	14.14 (8.59)	34.97 (10.46)
25–29.9	13.91 (7.49)	33.28 (11.25)
30–34.9	14.45 (8.98)	36.22 (10.91)
≥35	14.82 (6.90)	33.59 (10.80)
Maternal education		
Middle school	14.19 (7.74)	34.99 (10.75)
High school	13.48 (8.13)	33.99 (10.73)
>High school	14.95 (7.75)	34.22 (11.53)
Maternal atopy		
No	14.43 (7.88)	34.94 (11.30)
Yes	13.40 (7.30)	33.22 (10.31)
Maternal smoking during pregnancy		
No	13.90 (7.56)	34.36 (11.24)
Yes	16.29 (10.12)	34.38 (8.68)
Infant sex		
Male	13.59 (7.16)	34.96 (11.50)
Female	14.79 (8.63)	33.69 (10.36)
Preterm (<37 GWs)*		
No	13.63 (7.20)	34.17 (11.37)
Yes	16.44 (10.13)	35.19 (9.16)
Birth season*		
Winter (January to March)	13.05 (8.29)	35.36 (9.95)
Spring (April to June)	14.08 (7.75)	32.80 (11.31)
Summer (July to September)	15.37 (7.90)	34.17 (10.14)
Fall (October to December)	14.09 (7.71)	35.25 (11.97)
Maternal smoking (postnatal)		
No	NA	34.13 (11.40)
Yes		35.55 (8.51)
Household income*		
<$30,000	12.95 (6.94)	33.69 (11.38)
≥$30,000	14.89 (7.23)	34.98 (11.05)
Unknown	15.14 (8.76)	34.86 (10.58)
Breastfeeding**		
Breastfeeding only	NA	23.75 (14.07)
Formula only		36.15 (9.58)
Both		34.81 (10.56)
	Pearson correlation coefficient (*P* value)	
African ancestry proportion	−0.27 (<0.0001)	−0.07 (0.11)
Cord blood 25(OH)D (ng/ml)	1	0.07 (0.15)
Follow-up blood 25(OH)D (ng/ml)	0.07 (0.15)	1

25(OH)D, 25-hydroxyvitamin D; GW, gestational week, NA, not available.

*$P \le 0.05$, **$P < 0.0001$, nonparametric tests of plasma 25(OH)D by the variables. Significance symbols apply only to cord blood 25(OH)D concentration except for breastfeeding.

Table 3. Associations between plasma total 25(OH)D and food sensitization in the Boston Birth Cohort, stratified by *IL4* promoter polymorphism (rs2243250: C-590T)

25(OH)D (ng/ml)		Whole sample			rs2243250 = CC/CT			rs2243250 = TT			
Cord blood	Postnatal	Case/control[a]	OR (95% CI)[b]	*P* value	Case/control[a]	OR (95% CI)[b]	*P* value	Case/control[a]	OR (95% CI)[b]	*P* value	P_{int}[c]
≥11		93/193	Reference		45/138	Reference		48/55	Reference		
<11		69/105	1.28 (0.84–1.95)	0.26	51/67	2.04 (1.18–3.54)	0.01	18/38	0.46 (0.21–0.98)	0.04	0.003
	≥30	106/203	Reference		63/141	Reference		43/62	Reference		
	<30	56/95	1.10 (0.71–1.70)	0.66	33/64	1.06 (0.60–1.87)	0.84	23/31	1.21 (0.56– 2.59)	0.63	0.62
≥11	≥30	64/121	Reference		34/89	Reference		30/32	Reference		
≥11	<30	29/72	0.73 (0.42–1.29)	0.28	11/49	0.52 (0.23–1.18)	0.12	18/23	0.74 (0.29– 1.90)	0.53	
<11	≥30	42/82	0.90 (0.54–1.51)	0.69	29/52	1.26 (0.65–2.43)	0.49	13/30	0.32 (0.12– 0.82)	0.02	
<11	<30	27/23	2.03 (1.02–4.04)	0.04	22/15	3.23 (1.37–7.60)	0.007	5/8	0.80 (0.21– 3.04)	0.74	0.02

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio.

[a]Case and control refer to food sensitization cases and nonfood sensitization controls. [b]All OR estimates were adjusted for a child's sex and ancestry proportion, breastfeeding, postnatal maternal smoking, household income, and maternal age. [c]*P* value for interaction.

The majority of the vitamin D requirement for most people in the United States can be met through sun exposure and fortified food or vitamin D nutritional supplements. Toddlers' patterns of physical activity (i.e., sun exposure) and dietary habits (i.e., supplementation) are more similar to those of their mothers, whereas infants obtain their vitamin D mainly through fortified formula. This could explain the higher correlations observed between cord and postnatal plasma 25(OH)D concentrations among those older than 2 y of age ($r = 0.63$) as compared with the same measures at the age of 1–2 y ($r = 0.39$) and 6–12 mo ($r = 0.28$). We also found high proportions of children with low vitamin D status (i.e., <30 ng/ml) during the first year of life, at 1–2 y, and at 2–3 y (i.e., 29, 36, and 40%, respectively). Given that <10% of our subjects were exclusively breastfed (Table 1), these infants and toddlers should have had the highest intake of vitamin D via fortified formulas, which, in the United States, all contain at least 400 IU/l of vitamin D (21). As such, it is possible that some of the children in this study did not consume 1,000 ml vitamin D-fortified formula per day and were not fed additional vitamin D supplements to meet the recommended intake of 400 IU/day for infants, children, and adolescents (22). Another explanation is that because these 460 children were predominantly black (>50%), they tend to be more likely to have vitamin D insufficiency (10).

Insufficient vitamin D status is undesirable from many viewpoints, especially because of its impact on bone health and immune function. We previously reported the qualitative interactions between vitamin D deficiency, assessed from cord blood and a genetic variant in the gene *IL4* (rs2243250), and FS in the same birth cohort (18). The current study included two-thirds of the samples included in the previous report; samples here were included only if 25(OH)D was measured by the age of 3 y. The findings from this extended study emphasize the important role of postnatal vitamin D status in the development of FS. Children who had very low vitamin D status at birth but had sufficient vitamin D during their early life had no risk or a lower risk of FS, whereas children who were exposed to persistently low vitamin D both pre- and postnatally had the highest risk of FS (Table 3). These findings were not materially changed when stratified by birth season or preterm status. Several studies have shown an association between season of birth and risk of food allergy (23–25). A significant association was not seen for FS in this study (Table 1). Furthermore, our findings remained similar after controlling for season of birth or stratification by season of birth, indicating that birth season is unlikely to mediate the associations between persistently low vitamin D and risk of FS. In addition, the current study sample is a small subset of the parental birth cohort and, in particular, it includes many fewer preterm cases than exist among the 6,255 children currently in the database (**Supplementary Table S1** online). Nevertheless, the lower percentage of preterm births in this study did not appear to substantially affect the observed associations—based on the similar association patterns from preterm-stratified analyses and also from weighted logistic regression analyses. Of note, our findings from the stratified analyses suggested stronger associations between low vitamin D status and high risk of FS among children born preterm, which needs to be further explored in a larger sample. Furthermore, we observed significant interaction effects between the *IL4* gene polymorphism and persistently low vitamin D status on FS in this smaller-sized study sample. Among subjects carrying the C allele of rs2243250, persistently low vitamin D status dramatically increased the risk of FS, whereas sufficient vitamin D status during early childhood attenuated the risk of perinatal vitamin D deficiency on FS to null. Among those carrying the TT genotype, postnatal sufficient vitamin D status even showed a decreased risk of FS. It should be noted that four single-nucleotide polymorphisms showed significant interaction effects with vitamin D deficiency at birth on FS in our previous report (18). For this subset study, we have presented only findings for the *IL4* promoter polymorphism, given that rs2243250 has been commonly studied and has already been shown to have the most

significant gene–vitamin D deficiency interaction on FS (18). The other three single-nucleotide polymorphisms (*MS4A2* (rs512555), *FCER1G* (rs2070901), and *CYP24A1* (rs2762934)) showed similar interaction patterns as rs2243250, but only one (rs512555) reached the nominal significance level of 0.05 because of the reduced sample size.

Our findings should be interpreted with caution due to the relatively small sample size and should be duplicated in larger cohorts in the future. The postnatal samples and measurements were not taken at the same time. However, the results remained the same when we reanalyzed the data stratified by follow-up age (i.e., <1 and 2–3 y of age). There is no gold standard for how to define low vitamin D status at birth and in early childhood. Therefore, we chose the cutoffs of 11 and 30 ng/ml for cord and postnatal 25(OH)D measures, respectively, not only based on the suggestion by the Institute of Medicine for newborns (26) and the Endocrine Society Clinical Practice Guidelines on Vitamin D Deficiency for both children and adults (27,28), respectively, but also based on the distributions of the study subjects (**Table 1**). Note that ~3% of non-FS children were reported to have doctor-diagnosed food allergy. In this regard, it is possible that FS to relatively rare food allergens might be missed here but also that these non-FS children were misreported by their parents. However, the results remained similar after excluding these 10 subjects (data not shown). Finally, this study had plasma 25(OH)D measurements at only two time points, which may not comprehensively reflect a longitudinal pattern during early childhood. Nevertheless, our data are valuable to the field given that there is a lack of longitudinal data on vitamin D and allergic outcomes in early childhood.

The biological mechanisms underpinning the associations between persistently low vitamin D and the development of FS and then food allergy include excessive exposure to abundant food allergens caused by increased gastrointestinal barrier permeability and decreased immune tolerance. This so-called "multiple hit" model was recently proposed by Vassallo and Camargo (3). Due to the small number of food allergy cases (N = 31), this study is limited to FS; future studies should examine food allergy as a primary outcome. Future laboratory studies also should be seriously considered to help better understand the molecular basis underlying the joint influence on the risk of FS of the immunomodulatory effect of vitamin D and the regulatory effect of the *IL4* gene on IgE production. If these findings are replicated in other independent studies, then more attention to vitamin D nutrition should be given to very young children in the toddler age range, and particularly to those with very low cord blood vitamin D values and specific genotypes. Overall, this study underscores the need to simultaneously consider both cord blood and postnatal vitamin D levels, along with genetic susceptibility, in the development of FS and food allergy. The influence of vitamin D insufficiency in this process may be underestimated by a static, single value, which emphasizes that longer-term exposure to a vitamin D deficient state might have profound health consequences in a specific genetic environment.

METHODS

The study sample for the analyses included 460 children, a subset of the BBC, which is an ongoing birth cohort study that so far has recruited ~7,800 mother–infant pairs at birth from 1998 to 2012. Since 2004, the infants of the BBC who sought pediatric care at the Boston Medical Center and their mothers who gave informed consent have been followed prospectively for postnatal outcomes, including the development of FS, food allergy, and other allergic phenotypes. This study focused on children who (i) had plasma total 25(OH)D measured at two time points, one at birth and the other in early childhood ranging from ~6 to 36 mo, and (ii) had available genotype data for rs2243250 (C-590T) in the *IL4* gene, a potentially functional single-nucleotide polymorphism in the promoter region (29). As compared with the 6,255 mother–infant pairs enrolled in the BBC, from which this study sample was drawn, this sample included a much lower proportion of preterm infants (19 vs. 27%) (**Supplementary Table S1** online). Although we found statistically significant differences between these 460 children and the parent cohort in terms of maternal race, age, BMI, and birth season, the magnitude of the difference was relatively small, and its statistical significance was probably driven by the large sample size. Furthermore, this study sample had more than 99% overlap with the children included in our previous report, which examined only plasma total 25(OH)D measured at birth (18). A detailed description of the recruitment (30) and follow-up (31), FS definition, plasma 25(OH)D measures, and genotyping methods has been published (18). The study protocol was approved by the individual institutional review boards of Ann & Robert H. Lurie Children's Hospital of Chicago, Boston Medical Center, and Johns Hopkins University.

Consistent with our previous publications from this cohort (18,32,33), we defined FS cases as children who had allergen-specific IgE ≥0.35 kUA/l to any of eight common food allergens (i.e., egg white, milk, peanut, walnut, soy, shrimp, cod fish, and wheat). Plasma 25(OH)D was measured using a high-performance liquid chromatography–tandem mass spectrometry assay. Genotyping was conducted using the Illumina Golden Gate Assay (Illumina, San Diego, CA). We grouped the children according to the longitudinal trajectory of 25(OH)D based on (i) low vitamin D status at birth (<11 ng/ml) as suggested by the Institute of Medicine (26) or (ii) low postnatal vitamin D status (<30 ng/ml) according to the Endocrine Society clinical practice guidelines on vitamin D insufficiency (27,28). Persistently low vitamin D status was defined as having low levels at both time points.

Multiple logistic regression was used to test the association between persistently low vitamin D and FS after adjustment for maternal age (<20, 20–24.9, 25–29.9, 30–34.9, ≥35 y), postnatal exposure to maternal smoking, household income, child's gender, history of breastfeeding, and ancestry proportion estimated based on 144 ancestry-informative markers as previously detailed (18). We also conducted the above regression analyses stratified by the genotypes of rs2243250 and then examined the statistical significance for the interaction between low vitamin D status and rs2243250. All of the analyses were performed using SAS software (v. 9.2) (SAS Institute, Cary, NC) and R software (http://www.r-project.org/).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

ACKNOWLEDGMENTS

We thank all of the participants in this study and Tami R. Bartell for English editing.

STATEMENT OF FINANCIAL SUPPORT

This study was supported in part by March of Dimes Perinatal Epidemiological Research Initiative grants (USA) (20-FY02-56, 21-FY07-605), National Institutes of Health (NIH, Bethesda, MD) grants (R21 ES011666, R01 HD041702, R21AI079872, R21AI088609, U01AI090727, and R21AI087888), and the Food Allergy Initiative (USA). X.L. and L.A. are supported by the NIH/National Center for Research Resources, through the Clinical and Translational Science Awards Program, Northwestern University KL2RR025740. C.B.L. is supported in part by NIH grants (DK084634, DK066174, and DK083908). H.-J.T. is supported in part by a National Science Council grant (Taiwan) (NSC 101-2314-B-400-009-MY2).

Disclosure: None of the authors have a conflict of interest pertaining to this work.

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