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Gene–vitamin D interactions on food sensitization: a prospective birth cohort study

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Abstract

Background: It has been hypothesized that vitamin D deficiency (VDD) contributes to the development of food sensitization (FS) and then food allergy. However, the epidemiological evidence is conflicting. We aim to examine whether cord blood VDD is associated with FS and whether such association can be modified by genetic variants in a prospective birth cohort.

Methods: This study included 649 children who were enrolled at birth and followed from birth onward at the Boston Medical Center. We defined VDD as cord blood 25(OH)D $<$ 11 ng/ml, and FS as specific IgE $\geq$ 0.35 kUA/l to any of eight common food allergens in early childhood. We genotyped potentially functional single-nucleotide polymorphisms (SNPs) in 11 genes known to be involved in regulating IgE and 25(OH)D concentrations. Logistic regressions were used to test the effects of VDD on FS individually and jointly with SNPs.

Results: Among the 649 children, 44% had VDD and 37% had FS. When examined alone, VDD was not associated with FS. When examined jointly with SNPs, a significant interaction between *IL4* gene polymorphism (rs2243250) and VDD ($p_{interaction}$ = 0.003, p_{FDR} = 0.10) was found: VDD increased the risk of FS among children carrying CC/CT genotypes (OR = 1.79, 95%CI: 1.15–2.77). Similar but weaker interactions were observed for SNPs in *MS4A2* (rs512555), *FCER1G* (rs2070901), and *CYP24A1* (rs2762934). When all four SNPs were simultaneously considered, a strong gene–VDD interaction was evident ($p_{interaction} = 9 \times 10^{-6}$).

Conclusions: Our data demonstrate that VDD may increase the risk of FS among individuals with certain genotypes, providing evidence of gene–vitamin D interaction on FS.

Food allergy, a condition caused by an immunoglobulin (Ig) E-mediated hypersensitivity reaction to food, affects approximately 5% of children and 3–4% of adults (1) and is a growing clinical and public health problem in the USA and worldwide. The etiology underlying food allergy remains unclear, although several hypotheses have been suggested (2). The focus of the present report was to test the vitamin D hypothesis.

Serum 25 hydroxyvitamin D (25(OH)D) reflects the end result of both dietary intake and cutaneous synthesis after sun exposure and serves as the objective measure of an individual's vitamin D status (3). Approximately 1 billion people

worldwide have low vitamin D status (25(OH)D < 30 ng/ml) (4). In the USA, mean serum 25(OH)D concentrations dropped from 30 to 24 ng/ml during the last decade (5). Some studies conducted in the northern USA have also demonstrated that a high proportion of mothers and their infants have vitamin D deficiency (VDD) with the cutoff values of cord blood 25(OH)D concentration ranging from < 11 to < 15 ng/ml (6–9). Vitamin D deficiency or insufficiency has emerged as a rediscovered global public health problem.

Previous epidemiological studies have evaluated the associations between vitamin D status and allergic diseases and associated phenotypes (10–20), but the findings have been inconsistent. One reason for this discrepancy is the use of different methods of vitamin D assessment across studies. Some studies have used maternal/infant supplement intake, which is not an objective measure of serum 25(OH)D. With solar exposure serving as a major source of vitamin D (3), dietary/supplement intake alone cannot fully reflect an individual's vitamin D status. Another reason may be differences in study design as the majority are cross-sectional studies that are unable to determine temporal and causal association. Furthermore, the effect of vitamin D status on allergic diseases may only exist among a subgroup of subjects with particular genotypes. However, genetic susceptibility has yet to be considered in these studies. Finally, only a few vitamin D studies have focused on serum IgE levels (11, 16, 18, 20), an important intermediate phenotype of allergic diseases including food allergy. A recent large German birth cohort study demonstrated that early-onset and persistent FS were independent risk factors for physician-diagnosed food allergy at age six (21). Experimental evidence also strongly suggests that the hormonally active form of vitamin D, 1,25 dihydroxy-vitamin D ($1,25(OH)_2D$), could influence IgE production because of its regulatory effects on the immune system (22). We speculate that a careful evaluation of vitamin D status along with genetic susceptibility in relation to the development of FS in a prospective birth cohort study will provide new insight into the role of VDD in the development of FS and subsequent food allergy.

Using a large, well-established US prospective birth cohort, we evaluated the relationship of cord blood 25(OH)D concentration with the development of FS in early childhood, with simultaneous consideration of individual genetic variants in 11 genes known to be involved in IgE synthesis (*IL4*, *IL13*, *IL4RA*, *IL13RA1*), regulation of IgE function through its receptor complex (*FCER1A*, *MS4A2*, *FCER1G*) (23, 24), and modulation of vitamin D metabolism (*CYP27B1*,*CYP24A1*, *VDR*, *GC*) (22, 25). We were particularly interested in whether individual genetic variations could modify the VDD–FS association, that is, if there are gene–VDD interactions on FS.

Materials and methods

Study population

The Boston Birth Cohort is an ongoing study that enrolls mother–infant pairs at birth and prospectively follows the infants at the Boston Medical Center (BMC). A detailed description of the initial recruitment (26) and follow-up (27) has been previously published.

We studied 649 children for whom we currently have available information on cord blood 25(OH)D concentration, specific IgE to common food allergens, and genotyping data of 11 candidate genes. The Institutional Review Board (IRB) at the Children's Memorial Hospital (CMH) in Chicago and at the BMC approved the study protocol.

Definition of food sensitization

Plasma-specific IgE (sIgE) for eight food allergens (milk, egg white, peanut, soy, shrimp, walnut, cod fish, and wheat) was measured using PhadiaImmunoCAP performed at Quest Diagnostics Nichols Institute, Chantilly, VA (CLIA 49D0221801). Food sensitization cases were defined as children with sIgE ≥ 0.35 kUA/l to any one of the above food allergens. Non–food-sensitized controls were defined as children without detectable sIgE (< 0.35 kUA/l).

Cord blood plasma 25(OH)D measurement

Cord blood plasma total 25(OH)D concentrations (sum of $25(OH)D_2$ and $25(OH)D_3$) were measured using HPLC–MS/MS assay in a well-established laboratory (28). As there is no universal criterion for defining VDD in cord blood, we used a cutoff value of 11 ng/ml, as specified for infants, neonates, and young children by the Institute of Medicine (IOM) (29).

Candidate gene and Single-Nucleotide Polymorphism (SNP) selection

The primary focus of this report was to test whether well-established genetic variants can modify VDD–FS relationships. To this end, we chose to examine 11 genes that are critical to the synthesis and regulation of plasma IgE and 25(OH)D. Specifically, we selected genes encoding interleukin-4 and interleukin-13 (*IL4* and *IL13*) and their receptors (*IL4R* and *IL13RA1*), given that IL-4 and IL-13 are the only cytokines known to induce the isotype class switching to IgE (23). We also selected genes encoding the IgE receptor complex (*FCER1A*, *MS4A2*, and *FCER1G*) because of recently significant findings for gene *FCER1A* from a genome-wide association study (GWAS) of total serum IgE (tIgE) levels (24) and a follow-up candidate gene study (30). Finally, we included the main genes encoding the molecules essential for 25(OH)D metabolism and regulation (*CYP27B1*, *CYP24A1*, *VDR*, *GC*) (22, 25).

To assure statistical power, we examined potentially functional SNPs with minor allele frequency (MAF) > 0.05 in African Americans or Yoruba populations. We selected potentially functional SNPs that were predicted by two bioinformatics tools: PupaSuite (http://pupasuite.bioinfo.cipf.es/) and FuncPred (http://manticore.niehs.nih.gov/snpfunc.htm). For example, we included SNPs that result in nonsynonymous amino acid changes, and SNPs located in predicted transcription factor binding sites, exonic splicing enhancers, exonic splicing silencers, microRNAs and their targets, and

DNA triplexes. After excluding SNPs with low Illumina design score (<0.60), a total of 40 SNPs were selected for testing gene–VDD interaction on FS.

Genotyping

Single-nucleotide polymorphisms were genotyped with the Illumina GoldenGate custom panel at the Genome Center, Washington University at St. Louis as detailed in our previous publication (31). Thirty-nine SNPs (Table S1) had a call rate >98.0% and were examined herein. We also genotyped 150 ancestry informative markers (AIMs), which are highly informative among three ancestral populations (African, European, and Asian). Individual ancestral proportion was estimated from 144 AIMs with a call rate >98.0% and then included as covariates in subsequent analyses (31).

Statistical analyses

We conducted a chi-square goodness-of-fit test to assess deviation from Hardy–Weinberg equilibrium (HWE) for SNPs on the autosomal chromosomes among nonsensitized African American children using the program Haploview (http://www.broadinstitute.org/haploview/haploview). Similar tests were performed for SNPs on the X chromosome but among nonsensitized African American girls only. Linkage disequilibrium (LD) between the SNPs of each gene was also calculated with Haploview. Three SNPs were removed from further analyses because of deviation from HWE ($P < 0.01$) or because they had an MAF <5% or were in high LD with other SNPs (Table S1).

We first evaluated the effect of VDD itself on the risk of FS to any food, and FS to the three most common food allergens (egg white, milk, and peanut: >10% of the studied samples). We used a logistic regression model with and without the adjustment of important covariates, which included the child's age and gender, maternal smoking after birth, breast-feeding, and ancestral proportion estimates. We also evaluated the interactions between VDD and each SNP on any FS by adding main effects and cross-product terms to the logistic regression after adjustment of the same covariates listed above. Statistical significance was tested via the likelihood ratio test comparing full and reduced models (with and without the interaction term). With consideration for the small number of minor allele homozygotes for some SNPs under additive coding, we grouped together subjects carrying a minor allele to assure statistical power and to obtain stable estimates. We corrected for multiple testing using the false discovery rate (FDR) method (PROC MULTTEST). All *P*-values were derived from two-sided tests, and all analyses were undertaken with SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA).

Results

Epidemiological characteristics of study children

Among the 649 children, 240 developed FS (37%). Compared with nonsensitized children, those sensitized to any food allergen were slightly older (years: 2.08 *vs* 1.77), were more likely to be male, have a mother who smoked, and were less likely to be non-black people and exclusively bottle-fed ($P < 0.05$) (Table 1). These significant covariates (excluding race) together with individual ancestral proportions were included in the subsequent regression analyses. Food sensitization was significantly associated with physician diagnosis of eczema and food allergy but not with wheezing. Cord blood plasma 25(OH)D concentrations (ng/ml) appeared to be lower for children with any FS than for nonsensitized controls (12.52 *vs* 13.83, $P = 0.14$ based on *t*-test of log-transformed 25(OH)D). When cord blood plasma 25(OH)D was dichotomized using the IOM cutoff of 11 ng/ml, 286 children (44%) had VDD (48% *vs* 42% for FS and control children, respectively; $P = 0.09$).

Association between vitamin D deficiency and food sensitization

There were no significant associations between VDD and FS to any food, or FS to egg white, milk, and peanut (Table 2). Vitamin D deficiency only slightly increased the risk of FS to any food with the ORs of 1.16 (95%CI: 0.83–1.63) and 1.32 (95%CI: 0.95–1.81), with and without the adjustment of the covariates, respectively.

Gene–vitamin D deficiency interactions on food sensitization

To assure statistical power for the tests of gene–vitamin D interactions, we focused on FS to any food for the interaction tests. We presented the genetic associations of 36 SNPs with FS in Table S1. None of the associations were significant after correcting for multiple testing. Table 3 presents the significant gene–VDD interactions on FS based on a nominal significance level of 0.05. Of note, one SNP, rs2243250 (C-590T) in the gene *IL4,* remained marginally significant with an FDR correction ($p_{interaction} = 0.003$, $p_{FDR} = 0.1$). Specifically, VDD increased the risk of FS among those carrying the rs2243250 C allele (OR = 1.79, 95%CI: 1.15–2.77), and VDD decreased the risk of FS among children with the rs2243250 TT genotype, but this was not statistically significant (OR = 0.60, 95%CI: 0.34–1.06).

Similar interaction patterns were observed for the SNPs in *MS4A2* (rs512555), *FCER1G* (rs2070901), and *CYP24A1* (rs2762934) (Table 3). Given that qualitative interactions were found for all four SNPs in Table 3, we tested the interaction between VDD and the combined genotypes. We first defined risk genotypes as the groups in which VDD increased the risk of FS (right panel of Table 3) and then regrouped the subjects according to the number of such genotypes. The proportion of FS increased with an increasing number of risk genotypes among subjects having VDD. The opposite trend was observed for those having cord blood 25(OH)D ≥ 11 ng/ml (Fig. 1). The test for the combined SNP–VDD interaction on FS was highly significant ($p_{interaction} = 9 \times 10^{-6}$).

Further adjustment of maternal atopy or FS-associated phenotypes (having either eczema or food allergy) in the multivariate logistic models did not alter our findings (data not

Table 1 Epidemiological characteristics of 649 BMC subjects by food sensitization

Variables†	Food sensitization (*N* = 240) *N* (%)	Non–food sensitization (*N* = 409) *N* (%)
Maternal race*		
Black people	151 (63)	221 (54)
White people	8 (3)	31 (8)
Hispanic	48 (20)	100 (24)
Others	33 (14)	55 (13)
Maternal BMI (kg/m^2) (pre-pregnancy)		
<20	26 (11)	35 (9)
20–25	81 (34)	154 (38)
25–30	78 (33)	129 (32)
≥30	55 (23)	88 (22)
Gestational diabetes	14 (6)	13 (3)
Household income		
<$30 000	106 (44)	174 (43)
≥$30 000	27 (11)	64 (16)
Unknown	107 (45)	171 (42)
Maternal atopy	89 (37)	138 (34)
Paternal atopy	46 (20)	66 (17)
Infant sex (male)*	135 (56)	194 (47)
Preterm (<37 GWs)	41 (17)	75 (18)
Birth season		
Winter (December–March)	81 (34)	123 (30)
Spring (April–June)	57 (24)	109 (27)
Summer (July–September)	54 (23)	103 (25)
Fall (October–November)	48 (20)	71 (17)
Maternal smoking (postnatal)*	31 (13)	80 (20)
Breast-feeding*		
Breast-feeding only	14 (6)	27 (7)
Formula only	45 (19)	116 (29)
Both	181 (75)	264 (65)
Vitamin D deficiency (25(OH)D < 11 ng/ml)	116 (48)	170 (42)
Wheezing	34 (14)	46 (11)
Eczema*	121 (50)	156 (38)
Food allergy*	13 (6)	9 (2)
	Mean ± SD	
Age (years)*	2.08 ± 1.88	1.77 ± 1.65
Cord blood 25(OH)D (ng/ml)**	12.52 ± 6.41	13.83 ± 8.18

BMC, Boston Medical Center; FS, food sensitization.
**P*-values of the associations between the variables and FS ≤0.05.
***P* = 0.14 based on t-test of log-transformed 25(OH)D.
†Number of missing data for birth season and BMI is 3; numbers of missing data for maternal smoking (postnatal), breast-feeding, maternal and paternal atopy, and food allergy are 1, 2, 4, 22, and 22, respectively.

shown). In addition, we performed race-specific analyses (black people *vs* non-black people) and found that gene–VDD interactions on FS appeared to be more pronounced in black people than in non-black people (Table S2), although the general pattern is similar.

Discussion

This is the first study to evaluate the effect of low cord blood vitamin D and gene–VDD interactions on the development of FS during early childhood using a prospective birth cohort design. Consistent with previous studies, VDD was widespread and the incidence of FS was high in this urban US birth cohort. We observed that VDD alone, as assessed in cord blood, was not associated with FS. However, when individual genetic susceptibility was also considered, VDD increased the risk of FS among children with certain high-risk genotypes. Our study may partially explain the conflicting results on VDD and allergic phenotypes in previous epidemiological studies. More importantly, it underscores the need to consider individual genetic susceptibility in assessing the effect of VDD on allergic diseases. Our findings, if confirmed, could ultimately help to assess individual risk in terms of both VDD and genotypes and could be used to design personalized health care that would maximize the benefits and minimize the risk.

Our findings on gene–VDD interactions may stimulate future laboratory investigations to better understand the molecular basis of VDD on FS. Previous studies have indicated that vitamin D could increase IgE production by shifting the Th1/Th2 balance toward Th2 dominance or decrease IgE production through the inhibition of B-cell proliferation/differentiation and induction of Treg cells and their suppressive capacity (22). The central role of IL-4 in IgE regulation has also been well recognized. The T allele of promoter polymorphism rs2243250 (C-590T) has been demonstrated to increase IL-4 promoter activity and thus increase IgE production (32). Moreover, this SNP has been repeatedly reported to be associated with asthma and serum IgE in different populations (33). A negative association between rs2243250 and FS in 649 BMC subjects (predominantly black people) was consistent with a previous report (33), although it was not statistically significant (Table S1). As such, mechanistic study underlying the observed *IL4*–VDD interaction on FS should be seriously considered in the near future.

A genetic variant, rs2427837 in gene *FCER1A*, which was a hit in a recent GWAS of total serum IgE levels (24), did not show a significant interaction with VDD for FS. Instead, we observed marginally significant interactions between VDD and SNPs in genes encoding the beta and gamma chains of the IgE high-affinity receptor. The test for the interaction between VDD and the combined genotypes of rs2243250 in *IL4*, rs512555 in *MS4A2*, rs2070901 in *FCER1G*, and rs2762934 in *CYP24A1* appeared to be more substantial than an individual test, indicating that these four functional SNPs may jointly interact with VDD to influence the risk of FS, although the underlying biological mechanisms are unclear. This flip-flop pattern of association (Table 3 and Fig. 1) is commonly observed in testing G × E interactions in asthma according to a recent review by Ober et al.(34). It underscores the need to examine VDD and multiple genetic polymorphisms simultaneously in order to elucidate the complex interplay between gene and VDD on FS. Our findings need

Table 2 Associations between cord blood 25(OH)D and food sensitization in 649 BMC subjects

CB 25(OH)D (ng/ml)	Cases/ controls	Crude OR (95%CI)	Crude *P*-values	Adjusted OR (95%CI)*	Adjusted *P*-values*
Any food sensitization					
≥11	124/239	Reference		Reference	
<11	116/170	1.32 (0.95–1.81)	0.09	1.16 (0.83–1.63)	0.38
Egg sensitization					
≥11	86/239	Reference		Reference	
<11	55/170	0.90 (0.61–1.33)	0.59	0.84 (0.56–1.27)	0.41
Milk sensitization					
≥11	72/239	Reference		Reference	
<11	68/170	1.33 (0.90–1.95)	0.15	1.15 (0.76–1.73)	0.52
Peanut sensitization					
≥11	43/124	Reference		Reference	
<11	38/170	1.24 (0.77–2.01)	0.37	1.06 (0.64–1.75)	0.84

BMC, Boston Medical Center; CB 25(OH)D, cord blood 25(OH)D.
*All the OR estimates were adjusted for age, sex, maternal smoking after birth, breast-feeding, and ancestry proportion.

to be replicated, and underlying biological mechanisms need to be explored.

Our study is limited in several ways. The major limitations are the relatively modest sample size for testing the interaction effects on FS and the lack of replication. Our findings need to be confirmed in larger and independent studies. Also, this study only focused on 11 well-known candidate genes involved in IgE and 25(OH)D synthesis and regulation. Other genes that have been demonstrated to be associated with total serum IgE or IgE-mediated phenotypes or regulated by 1,25(OH)D, for example, *HLA-DRB1*, *STAT6*, and *IL10* (25, 35), warrant further study. In addition, this study is the first attempt to examine the relationships between vitamin D, genes, and FS, with the adjustment for individual ancestral proportion. However, the confounding effect of population stratification could not be entirely excluded because of heterogeneous ancestral background in the Boston Birth Cohort. Finally, although cord blood 25(OH)D < 11 ng/ml has been suggested as a deficiency cutoff for neonates by the IOM, other cutoffs [15 (8) and 12 ng/ml (9)] have been used earlier. Our findings remained similar when we used a cutoff of 12 ng/ml (data not shown), but because close to half of our

Table 3 Gene–vitamin D deficiency interactions on any food sensitization in 649 BMC subjects

	Cases/controls	OR (95%CI)	*P*-value	Cases/controls	OR (95%CI)	*P*-value	$p_{interaction}$
IL4	rs2243250 = TT			rs2243250 = CC/CT			
CB 25(OH)D (ng/ml)							
≥11	63/69	Reference		61/168	Reference		
<11	39/61	0.60 (0.34–1.06)	0.08	77/107	1.79 (1.15–2.77)	0.01	0.003*
MS4A2	rs512555 = AA/AG			rs512555 = GG			
CB 25(OH)D (ng/ml)							
≥11	30/29	Reference		94/210	Reference		
<11	18/32	0.40 (0.17–0.98)	0.04	98/138	1.43 (0.99–2.08)	0.06	0.009
FCER1G	rs2070901 = GG			rs2070901 = TT/TG			
CB 25(OH)D (ng/ml)							
≥11	49/84	Reference		75/154	Reference		
<11	38/68	0.69 (0.38–1.25)	0.23	78/102	1.52 (1.00–2.33)	0.05	0.04
CYP24A1	rs2762934 = GG			rs2762934 = AA/AG			
CB 25(OH)D (ng/ml)							
≥11	86/157	Reference		38/82	Reference		
<11	75/131	0.88 (0.58–1.32)	0.53	41/38	2.29 (1.22–4.32)	0.01	0.02

BMC, Boston Medical Center; FDR, false discovery rate; *IL4*, interleukin 4; *MS4A2*, Membrane-spanning 4-domains subfamily A member 2/ IgE Fc receptor subunit beta; *FCER1G*, IgE Fc receptor subunit gamma; *CYP24A1*, cytochrome P450, family 24, subfamily A, polypeptide 1; CB 25(OH)D, cord blood 25(OH)D.
*FDR-corrected $P = 0.10$. All the OR estimates were adjusted for age, sex, maternal smoking after birth, breast-feeding, and ancestry proportion.

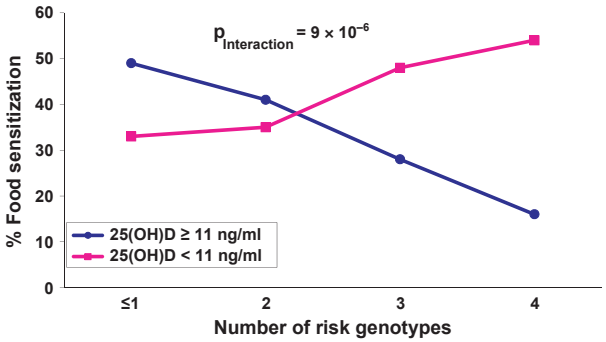

Figure 1 Interactive effect between the combined risk genotypes of the genes *IL4* (rs2243250), *MS4A2* (rs512555), *FCER1G* (rs2070901), and *CYP24A1* (rs2762934) and cord blood 25(OH)D on food sensitization in Boston Birth Cohort. Risk genotypes refer to the groups in which vitamin D deficiency increased risk of food sensitization. They are CC/CT, GG, TT/TG and AA/AG for rs2243250, rs512555, rs2070901, and rs2762934, respectively.

study subjects had cord blood 25(OH)D < 11 ng/ml, the sample size was not enough to test the interactions at a 15 ng/ml cutoff, and analyses among those with high vitamin D concentrations (25(OH)D > 54 ng/ml (16)) could not be conducted. Interactions between log-transformed 25(OH)D concentrations and SNPs on FS were not explored because the assumption of linearity between 25(OH)D and FS in each genotype group may not be correct.

Our study has several strengths. This is a prospective birth cohort, which could overcome many drawbacks related to cross-sectional or retrospective studies. Objective and clinically relevant biomarkers for assessing vitamin D status in cord blood were used. Finally, this study expands previous studies by incorporating individual genotypes in the assessment of VDD–FS relationships.

In summary, we found that low cord blood vitamin D levels significantly increased the risk of FS among children carrying certain genotypes in a prospective urban US birth cohort. Our study also raises the possibility that the simultaneous consideration of both VDD and individual genotypes may improve our ability to identify newborns who may be at high risk of developing FS and subsequent food allergy. If further confirmed in independent studies, our findings could lead to the design of targeted, cost-effective, clinical and public health interventions with the goal of preventing or reducing the risk of FS and subsequent food allergy.

Author contributions

XL has the primary responsibility for this manuscript. XL, GW, XH, DW, HT, SZ, LA, RK, HW, RL, YZ, CP, KO, RS, PH, JP, HEP, CL, and XW all played a role in the conception, design, acquisition and analysis of data, and interpretation of results. XL drafted the manuscript. HT, LA, RK, RS, PH, JP, CL, and XW involved in the revision of the article. GW, XH, SZ, HW, RL, CP, KO, and HEP involved in laboratory and clinical data collection. All authors have read and approved the final manuscript.

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Conflict of interest

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

Supporting Information

Additional Supporting Information may be found in the online version of this article found at: http://www.wileyonlinelibrary.com.

Table S1. Summary of 39 potentially functional SNPs in 11 candidate genes.

Table S2. Gene–vitamin D deficiency interactions on any food sensitization in 649 BMC subjects: stratified by maternal race.

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