

Polymorphisms affecting vitamin D-binding protein modify the relationship between serum vitamin D (25[OH]D₃) and food allergy

Jennifer J. Koplin, PhD,^{a,b} Noor H. A. Suaini, BSc (Hons),^{a,d} Peter Vuillermin, MBBS, FRACP, PhD,^{a,c} Justine A. Ellis, PhD,^{a,d} Mary Panjari, PhD,^a Anne-Louise Ponsonby, MBBS, FAFPHM, PhD,^{a,d} Rachel L. Peters, MPH,^{a,d} Melanie C. Matheson, PhD,^{a,b} David Martino, PhD,^{a,d} Thanh Dang, PhD,^a Nicholas J. Osborne, PhD,^{e*} Pamela Martin, PhD,^a Adrian Lowe, PhD,^{a,b} Lyle C. Gurrin, PhD,^{a,b} Mimi L. K. Tang, MD, PhD,^{a,d,f} Melissa Wake, MD, FRACP,^{a,d,g} Terry Dwyer, PhD,^{a,‡} John Hopper, PhD,^b Shyamali C. Dharmage, MBBS, MSc, MD, PhD,^{a,b} and Katrina J. Allen, MBBS, BMedSc, FRACP, PhD,^{a,d,f,h} for the HealthNuts Study *Parkville and Geelong, Australia, and Cornwall and Manchester, United Kingdom*

Background: There is evolving evidence that vitamin D insufficiency may contribute to food allergy, but findings vary between populations. Lower vitamin D-binding protein (DBP) levels increase the biological availability of serum vitamin D. Genetic polymorphisms explain almost 80% of the variation in binding protein levels.

From ^athe Murdoch Childrens Research Institute and ^bthe School of Population and Global Health, University of Melbourne, Parkville; ^cthe Child Health Research Unit, Barwon Health and Deakin University, Geelong, and ^dthe Department of Paediatrics, University of Melbourne, Parkville; ^ethe European Centre for Environment and Human Health, University of Exeter Medical School, Cornwall; ^fthe Department of Allergy and Immunology and ^gthe Centre for Community Child Health, Royal Children's Hospital, Parkville; and ^hthe School of Inflammation and Repair, University of Manchester, Manchester.

*Dr Osborne is currently affiliated with the Clinical Pharmacology and Toxicology Research Group, Discipline of Pharmacology, Sydney Medical School, University of Sydney, Australia.

‡Dr Dwyer is currently affiliated with the George Institute for Global Health, University of Oxford, United Kingdom.

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Corresponding author: Katrina J. Allen, MBBS, BMedSc, FRACP, PhD, Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Rd, Parkville, VIC 3052, Australia. E-mail: katie.allen@rch.org.au. 0091-6749/\$36.00

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Objective: We sought to investigate whether polymorphisms that lower the DBP could compensate for adverse effects of low serum vitamin D on food allergy risk.

Methods: From a population-based cohort study (n = 5276) we investigated the association between serum 25-hydroxyvitamin D₃ (25[OH]D₃) levels and food allergy at age 1 year (338 challenge-proven food-allergic and 269 control participants) and age 2 years (55 participants with persistent and 50 participants with resolved food allergy). 25(OH)D₃ levels were measured using liquid chromatography-tandem mass spectrometry and adjusted for season of blood draw. Analyses were stratified by genotype at rs7041 as a proxy marker of DBP levels (low, the GT/TT genotype; high, the GG genotype).

Results: Low serum 25(OH)D₃ level (≤50 nM/L) at age 1 years was associated with food allergy, particularly among infants with the GG genotype (odds ratio [OR], 6.0; 95% CI, 0.9-38.9) but not in those with GT/TT genotypes (OR, 0.7; 95% CI, 0.2-2.0; *P* interaction = .014). Maternal antenatal vitamin D supplementation was associated with less food allergy, particularly in infants with the GT/TT genotype (OR, 0.10; 95% CI, 0.03-0.41). Persistent vitamin D insufficiency increased the likelihood of persistent food allergy (OR, 12.6; 95% CI, 1.5-106.6), particularly in those with the GG genotype.

Conclusions: Polymorphisms associated with lower DBP level attenuated the association between low serum 25(OH)D₃ level and food allergy, consistent with greater vitamin D bioavailability in those with a lower DBP level. This increases the biological plausibility of a role for vitamin D in the development of food allergy. (*J Allergy Clin Immunol* 2015;■■■:■■■-■■■.)

Key words: Food hypersensitivity, vitamin D, food allergy, gene, polymorphism, vitamin D binding protein

Temporal, ecological, and epidemiologic evidence are all converging to suggest that vitamin D may play a causal role in allergic disease. Vitamin D insufficiency and food allergy have risen concurrently,^{1,2} and prescriptions of adrenaline autoinjectors and hydrolyzed formulas rise with distance from the equator (a proxy for lower ultraviolet exposure).^{3,4} Babies born in autumn and winter have both lower vitamin D stores and higher risk of food allergy.^{5,6}

We have recently shown a direct association between vitamin D insufficiency and challenge-proven food allergy (the criterion

Abbreviations used

DBP: Vitamin D binding protein

GC: Group-specific component

OR: Odds ratio

25(OH)D₃: 25-hydroxyvitamin D₃

standard for diagnosis) at age 12 months.⁷ This association is biologically plausible because vitamin D is critical to the healthy development of the immune system in early life. However, some other studies have not found such associations.^{8,9} These differences could be explained by chance and/or confounding, with vitamin D insufficiency a bystander in the development of food allergy. Alternatively, it could play a causal role, but one that differs in different populations, or at different ages. In addition, the temporality of the association between vitamin D insufficiency and food allergy has not been previously explored, particularly with regard to whether resolution of insufficiency results in an increased resolution of food allergy.

Vitamin D can be obtained from dietary sources or endogenously synthesized in the epidermal layer of the skin after exposure to sunlight. Both forms of vitamin D are hydroxylated in the liver into 25-hydroxyvitamin D (25(OH)D), which is the main storage form and major circulating metabolite of vitamin D, with a plasma half-life of 2 to 3 weeks. This is considered to be the primary indicator of vitamin D status.¹⁰ Vitamin D-binding protein (DBP) is the major carrier of 25(OH)D in the circulation. 25(OH)D exists in serum in both free and DBP-bound forms. The criterion standard of measurement for 25(OH)D is liquid chromatography-tandem mass spectrometry, which measures total 25(OH)D, consisting of both free and DBP-bound forms.¹⁰

New knowledge about the biological functions of vitamin D and DBP reveals a plausible explanation for variations in vitamin D biology in different populations. There is increasing evidence that DBP-bound vitamin D is less available to target tissues (reduced bioavailability).¹¹ Higher levels of bound 25(OH)D are associated with indicators of decreased vitamin D function (decreased bone density and increased parathyroid hormone levels).¹² Lower DBP levels, by increasing the biological availability of serum vitamin D, may therefore counteract adverse effects of apparently low serum 25-hydroxyvitamin D₃ (25[OH]D₃).¹¹

There are 3 main phenotypic alleles of DBP, which differ both in the associated concentration of DBP in the serum and in their affinity for 25(OH)D. These are distinguished by genetic polymorphisms in the gene encoding vitamin D binding protein (group-specific component [GC]). Two single nucleotide polymorphisms, rs7041, and rs4588, explain almost 80% of the variation in levels of binding protein, with a greater change in the DBP level associated with variant alleles at rs7041.¹¹

The frequency of rs7041 polymorphisms varies greatly by race. For example, Asian populations have a 2.6-fold higher frequency than do Caucasian populations of the TT genotype at rs7041, which predicts a lower binding protein level,¹³ so that these populations might have more bioavailable vitamin D for any given serum 25(OH)D₃ level. This might obscure an association between low vitamin D level and food allergy, and fits with our previous findings in the HealthNuts study that vitamin D insufficiency strongly predicts food allergy in infants of Australian-born parents (most of whom are of Caucasian origin)

but not in those with parents born overseas (with a much higher Asian heritage).⁷

Using data from the population-based HealthNuts study, we therefore aimed to further characterize the plausibility of the vitamin D insufficiency food allergy risk association through investigating whether genotype-predicted DBP level modifies the relationship between vitamin D insufficiency and food allergy risk. In addition, we investigated the role of infant vitamin D status on the risk of food allergy resolution at age 2 years, and whether this differed by genotype-predicted DBP level. We also investigated whether genotype-predicted DBP level modifies the relationship between vitamin D supplementation and food allergy risk. We hypothesized that polymorphisms leading to a lower DBP level could increase the bioavailability of serum 25(OH)D₃ and that this could compensate for adverse effects of low serum 25(OH)D₃ level on food allergy risk.

METHODS

HealthNuts study population

HealthNuts is a large-scale, population-based cohort study undertaken to assess the prevalence and risk factors for allergic disease in early childhood.^{14,15} Briefly, by using a predetermined population-based sampling frame drawn from local government-led immunization clinics in Melbourne, Australia (population 4 million), infants were recruited while attending 1-year-old council-led immunization (September 2007 to August 2011). All infants aged between 11 and 15 months (inclusive) were eligible for recruitment (74% response rate).

Parents completed a questionnaire that included questions about maternal use of vitamin supplements during pregnancy and parental country of birth (used as a proxy for ethnicity). In a substudy of this cohort, more than 90% of the parents born in Australia reported Caucasian ethnicity and more than 95% of the parents born in Asia reported Asian ethnicity.⁷

Infants were skin prick tested to hen's egg, peanut, sesame, and either cow's milk (n = 2715) or shrimp (n = 2405) (ALK-Abelló, Madrid, Spain), with a positive control (histamine 10 mg/mL) and a negative control (saline) using single-tine lancets on the infant's back, and were examined for the presence of eczema (recorded as present or absent at 3 sites—back, face, and elbow creases—by research nurses who had been trained in recognition of eczema). All participants with a detectable wheal to 1 or more foods were invited to a Royal Children's Hospital-based clinic where staff administered repeat skin prick test and diagnostic oral food challenges, blinded to the infant's skin prick test wheal size and history of ingestion. We chose any detectable wheal size as our entry criterion to assess the food allergy status of participants to minimize the chances of missing cases of food allergy. Only those infants with both a positive food challenge by objective criteria¹⁶ and a wheal size of 2 mm or more on repeat skin prick test or a specific IgE level of 0.35 kU/L or more were deemed food allergic (see Fig E1 in this article's Online Repository at www.jacionline.org). A random sample of infants with negative skin prick test results was also invited to undergo a food challenge (negative controls for the clinic study). Skin prick testing at clinic attendance (6–8 weeks after the initial contact) used an extended panel of foods: egg, peanut, sesame, cow's milk, shrimp, cashew, hazelnut, almond, wheat, and soy (ALK-Abelló). Blood samples were obtained at clinic attendance for vitamin D and genotyping.

Longitudinal follow-up of egg-allergic infants

All infants with challenge-confirmed egg allergy at age 1 year recruited after February 2010¹⁷ were invited to undergo a baked egg challenge and to return at age 2 years for follow-up egg challenge. All follow-up food challenges were conducted irrespective of previous or current skin prick test results and severity of previous reactions. Overall, 189 infants completed follow-up food challenges at age 2 years (mean age, 26.9 ± 3.0 months) (see Fig E2 in this article's Online Repository at www.jacionline.org).

Measurement of exposure: Serum 25(OH)D₃

Serum 25(OH)D₃ level was measured using liquid chromatography-tandem mass spectrometry at the Royal Melbourne Institute of Technology. This laboratory has previously demonstrated high interbatch agreement for duplicate samples ($n = 39$ pairs; intraclass correlation, 0.89).¹⁸ To adjust for seasonal variation in vitamin D levels, we fitted a sinusoidal curve (with a period of 12 months) to data on 25(OH)D₃ levels using simple linear regression. We added the residuals from this fitted model to the population's average vitamin D level to produce a seasonally adjusted value of 25(OH)D₃.¹⁹

Vitamin D insufficiency was defined using cutoffs recommended by the US Institute of Medicine²⁰: 25(OH)D₃ level of 50 nmol/L or less; intermediate vitamin D: 51 to 74 nmol/L; high vitamin D: 75 nmol/L or more. This article builds on our earlier findings investigating the association between serum vitamin D and food allergy in this cohort.⁷ We have used the same cutoffs here to define insufficiency for consistency with this previous work. However, since publication of our earlier findings, a subsequent study suggested a potential link between high vitamin D levels and increased risk of food allergy⁹; therefore, we added an additional category of high vitamin D level (≥ 75 nmol/L).

Genotyping

DBP gene (*GC*) single nucleotide polymorphisms rs7041 and rs4588 were measured using Sequenom MassARRAY genotyping platform. There was no evidence of deviation from Hardy-Weinberg equilibrium ($P > .05$), and all samples had more than 90% genotype call rate.

Ethics

Ethical approval was obtained from the Office for Children Human Research Ethics Committee (reference no. CDF/07/492), Department of Human Services Human Research Ethics Committee (reference no. 10/07), and Royal Children's Hospital Human Research Ethics Committee (reference no. 27047). Parents/guardians of all participants provided written informed consent.

Statistical methods

We used a multivariable logistic regression model to estimate odds ratios (ORs) to quantify the association between serum vitamin D levels and the odds of food allergy at age 1 year and persistence of food allergy at age 2 years among food-allergic infants. The current use of infant formula (none, formula with continued breast-feeding, and formula alone) and the history of egg consumption (none, baked goods containing egg [eg, cakes and biscuits], cooked egg [eg, scrambled or soft boiled egg] on 1 occasion, and cooked egg on multiple occasions) were both selected a priori for inclusion in the final model because both are dietary sources of vitamin D and associated with the odds of infant food allergy.^{21,22} Other potential confounders were infant history of eczema (parent-reported eczema diagnosis in the infant at any time in the first 12 months of life); age (in months) at food challenge; infant's sex; duration of breast-feeding; infant consumption of fish (yes/no); number of siblings; socioeconomic status; pet ownership; maternal use of vitamin D supplements during pregnancy; maternal smoking during pregnancy; family history of allergy; filaggrin null mutations; season of birth; and ultraviolet radiation at the month of birth. These were retained in the regression model only if their inclusion caused a more than 10% change in the magnitude of the association between vitamin D status and the odds of food allergy.²³

To mitigate potential bias in estimated ORs due to lower participation fraction at the clinic for infants who were nonsensitized, we generated weights as the inverse of the probability of clinic attendance for all infants who were nonsensitized within groups defined by cross-classifying family history of food allergy, infant history of eczema, and parents' country of birth²⁴ (see Table E1 in this article's Online Repository at www.jacionline.org). These weights were used in the corresponding logistic regression models, with robust standard errors used to ensure that the precision of estimated ORs reflected the sample size.

Interactions between serum vitamin D level and the *GC* genotype at rs7041 and rs4588 (as a proxy marker of DBP levels) were examined using the Wald test. The T allele at rs7041 and the A allele at rs4588 are associated with lower

DBP levels, with the rs7041 variants having a greater effect on the DBP level.¹¹ As reported previously,¹¹ genotype was also associated with measured circulating vitamin D levels (data not shown). Because this analysis was restricted to an examination of the relationship between a given serum 25(OH)D₃ level and food allergy stratified by genotype, the effect of genotype on serum 25(OH)D₃ level was not considered further.

We examined whether the DBP genotype also modified the association between maternal or infant vitamin D supplementation (reported by questionnaire at recruitment) and food allergy at age 1 year using multivariable logistic regression models with interaction terms for genotype and supplementation.

RESULTS

Characteristics of participating infants

Overall, 607 infants, 338 food-allergic and 269 nonallergic, were included in the analysis at age 1 year (Fig E1) and 105 infants, 55 with persistent egg allergy and 50 with resolved egg allergy, were included in the analysis at age 2 years (Fig E2). Characteristics of participating infants are presented in Table I. The GG genotype, with the highest predicted DBP level, was most common (28.5%) in infants with both parents born in Australia (see Table E2 in this article's Online Repository at www.jacionline.org).

Modification of serum 25(OH)D₃ level/food allergy association at age 1 year by predicted DBP level

As we have previously reported, there was evidence of an increased risk of food allergy with concurrent vitamin D insufficiency at age 1 year among infants of Australian-born parents (OR, 2.0; 95% CI, 1.0-4.3) but not among those with parents born outside Australia (OR, 0.9; 95% CI, 0.3-2.6).

The association between vitamin D insufficiency and food allergy was also modified by rs7041 genotype (Table II). There was no evidence of an association between vitamin D insufficiency and food allergy in those with the GT or TT genotype at rs7041, irrespective of the genotype at rs4588. There was some evidence to support an association between a lower serum vitamin D level (deficiency, levels ≤ 25 nM/L) and food allergy in this group (OR, 4.4; 95% CI, 1.0-18.8; $n = 16$). There was modest evidence of an association between vitamin D insufficiency and food allergy in infants with the GG genotype at rs7041 (OR, 6.0; 95% CI, 0.9-38.9), although CIs were wide. High vitamin D levels (≥ 75 nM/L) were also associated with an increase in food allergy in infants with the GG genotype at rs7041 (OR, 4.0; 95% CI, 1.3-12.9). Only 4 infants with the GG genotype had vitamin D deficiency (levels ≤ 25 nM/L); the OR for food allergy in the deficient group was 2.8 (95% CI, 0.2-33.9).

Although statistical power to assess differential effects between peanut and egg allergy stratified by genotype was limited, within each genotype group we tended to see a similar association between vitamin D insufficiency and different types of food allergy: egg allergy: OR 7.5 ($P = .029$) for the GG genotype and OR 0.6 ($P = 0.43$) for the GT/TT genotype; peanut allergy: OR 4.0 ($P = .24$) for the GG genotype and OR 1.3 ($P = .58$) for the GT/TT genotype.

Modification of vitamin D supplementation/food allergy association by predicted DBP level

Among infants with the GT/TT genotype at rs7041 (low predicted DBP level), maternal use of vitamin D supplements

TABLE I. Characteristics of HealthNuts study infants by food allergy outcome

Characteristic	Included in age 1 y analysis (%) [*]		Included in egg allergy follow-up (%) [*]	
	Nonallergic at age 1 y (n = 269)	Food allergic at age 1 y (n = 338)	Egg tolerant at age 2 y (n = 50)	Egg allergic at age 2 y (n = 55)
Family characteristic				
Family history of allergy [†]	77.1	76.0	85.7	72.4
Family history of eczema	37.4	39.9	46.4	44.8
Family history of food allergy	19.5	13.6	19.6	15.5
Maternal smoking during pregnancy	3.1	2.6	1.8	0.0
Maternal multivitamin use in pregnancy [‡]	78.6	76.8	72.2	74.1
Maternal vitamin D use in pregnancy	9.3	4.7	7.4	3.5
Any siblings	55.2	43.8	42.9	43.1
Infant characteristic				
Age at assessment (mo), mean $\pm$ SD	14.6 $\pm$ 1.3	14.4 $\pm$ 1.4	27.9 $\pm$ 3.8	27.3 $\pm$ 4.0
Sex: male	47.0	58.0	44.6	65.5
Current eczema (at 12 mo)	28.2	48.0	46.0	59.7
History of eczema diagnosis (by age 12 mo)	29.5	61.8	45.3	79.0
Ever breast-fed	95.6	95.4	96.4	94.8
Ever used infant formula	72.6	77.9	88.0	75.9
Vitamin D supplement in infancy [§]	14.9	8.8	9.1	8.3
Serum vitamin D ₃ level (nM/L)				
≤ 25	1.2	5.9	2.1	8.2
26-50	20.5	15.8	34.9	38.7
50-74	40.6	35.8	45.7	34.7
≥ 75	37.8	42.5	17.4	18.4
Seasonally adjusted vitamin D ₃ level (nM/L)				
≤ 25	2.0	4.0	2.1	2.0
26-50	17.6	17.0	28.3	40.9
50-74	47.8	40.7	56.5	44.9
≥ 75	32.7	38.3	13.0	12.2
Both parents born in Australia	64.7	52.2	55.4	53.6
rs7041 genotype				
GG	26.8	24.9	28.0	23.6
GT	49.8	48.8	40.0	47.3
TT	23.4	26.3	32.0	29.1
rs4588 genotype				
CC	46.1	50.3	50.0	50.9
CA	42.8	39.9	38.0	40.0
AA	11.2	9.8	12.0	9.1
Season of blood draw				
Summer	20.1	21.5	25.5	13.2
Autumn	29.4	29.2	23.5	30.2
Winter	17.8	22.8	25.5	22.6
Spring	32.8	26.6	25.5	34.0

^{*}Restricted to those infants with both vitamin D results available and food allergy status known at age 1 or 2 years (see Figs E1 and E2).

[†]Asthma, allergic rhinitis, eczema, or food allergy.

[‡]The most commonly used multivitamin supplements taken during pregnancy in this cohort were Blackmores and Elevit pregnancy supplements containing 6.3 μ g and 12.5 μ g of vitamin D₃ per capsule, respectively.

[§]Collected in a substudy of infants attending clinic. Data available for 101 and 137 infants without and with food allergy, respectively, at age 1 year; 22 and 24 infants without and with egg allergy, respectively, at age 2 years.

||Seasonally adjusted measure of vitamin D.

during pregnancy was associated with a reduced risk of food allergy (OR, 0.1; 95% CI, 0.03-0.41; Table III). This association appeared somewhat stronger than for infants with the GG genotype (OR, 0.34; 95% CI, 0.04-2.79; *P* interaction = .05). Vitamin D supplementation directly to infants was uncommon (12.6%); thus, there was insufficient power to investigate the relationship between infant vitamin D supplementation and food allergy in detail. There was little evidence of an association between current infant formula use (which contains added vitamin D) and food allergy, and this was not modified by genotype (Table III).

Modification of serum 25(OH)D₃ level/food allergy association at age 2 years by predicted DBP level

Among infants who were egg allergic at age 1 year, those who were insufficient at both ages 1 and 2 years were most likely to have persistent egg allergy, with a lower risk of persistent egg allergy in those whose insufficiency had resolved before age 2 years (Table IV). Vitamin D insufficiency at only 1 time point alone (either age 1 or 2 years) was not associated with persistent egg allergy. There was some evidence that vitamin D insufficiency at 2 years was more strongly associated with persistent

TABLE II. Association between vitamin D and food allergy according to genotype-predicted binding protein level

Genotype-predicted DBP level	Serum 25(OH)D ₃	N	% Food allergic	aOR (95% CI)*	P interaction†
Model 1					
Higher predicted DBP level	≤50	17	52.9	6.0 (0.9- 38.9)	
Genotype: rs7041 GG; rs4588 CC‡	51-74	56	37.5	1.0	
	≥75	62	64.5	4.0 (1.3-12.9)	
Lower predicted DBP level	≤50	86	53.5	0.7 (0.2-2.0)	.014
Genotype: rs7041 GT/TT; rs4588 any	51-74	175	52.0	1.0	
	≥75	114	54.4	1.1 (0.3-3.5)	.16
Model 2					
rs7041 GT and rs4588 CA‡	≤50	31	45.2	0.4 (0.05-2.9)	
Intermediate-high predicted DBP level	51-74	69	47.8	1.0	
	≥75	42	52.4	2.3 (0.3-18.0)	
rs7041 GT and rs4588 CC	≤50	17	76.5	2.1 (0.4-10.8)	
Intermediate predicted DBP level	51-74	44	50.0	1.0	
	≥75	42	54.8	1.0 (0.2-4.7)	
rs7041 TT and rs4588 AA	≤50	17	47.1	2.3 (0.3-15.1)	
Intermediate-low predicted DBP level	51-74	28	46.4	1.0	
	≥75	6	66.7	2.7 (0.2-34.9)	
rs7041 TT and rs4588 CA/CC‡	≤50	21	52.4	0.6 (0.1-3.0)	
Lowest predicted DBP level	51-74	34	67.7	1.0	
	≥75	24	54.2	0.06 (0.01-0.3)	

aOR, Adjusted odds ratio.

*Analyses were adjusted for infants' consumption of egg and formula use and parents' country of birth and used a seasonally adjusted measure of serum 25(OH)D₃. To mitigate bias in estimated ORs owing to the low attendance fraction at the clinic for infants who were nonsensitized, the final model also included the use of *sampling weights*, defined as the inverse of the probability of clinic attendance for all infants who were nonsensitized within groups defined by cross-classifying family history of food allergy, infant history of eczema, and parents' country of birth (see Table E1). Only infants with available data on all covariates were included in these analyses.

†The interaction term between vitamin D category and genotype at rs7041 (as a proxy marker of DBP levels) was calculated from adjusted models using the Wald test.

‡All infants with rs7041 GG genotype also had rs4588 CC genotype. All infants with rs7041 GT genotype had rs4588 CA or CC genotypes. For rs7041 TT genotype, infants with rs4588 CA and CC genotypes were combined because of small numbers in the CC group (n = 16).

egg allergy among infants with the GG genotype at rs7041 (Table V), although numbers in this analysis were small.

DISCUSSION

Using data from a population-based cohort study, our findings present the first evidence that genetic polymorphisms affecting vitamin D metabolism could play a role in the development of food allergy by altering the bioavailability of serum 25(OH)D₃. In addition, there was evidence that vitamin D deficiency (≤25nM/L) increased the risk of food allergy even in the low DBP level population, suggesting that different cutoffs to define low vitamin D level might be warranted for populations with different genotype-predicted DBP levels. Second, and in an entirely novel finding, infant vitamin D levels also appear to be important in predicting resolution of food allergy and may also be modified by genotype-predicted DBP levels, providing support for the role of vitamin D in regulating immune health in disease resolution as well as disease development.

Plausible biological mechanisms by which vitamin D status could play a role in risk of food allergy include through vitamin D's capacity to induce expression of IL-10-secreting regulatory T cells, which may play a role in induction or maintenance of immune tolerance to foods. Vitamin D also plays a role in induction of innate epithelial defenses (such as cathelicidins) and regulation of tight junction proteins; thus, low vitamin D levels could increase the risk of food allergy through compromised intestinal barrier function or vitamin D-mediated alteration of gastrointestinal microbiota composition.^{2,25}

Strengths of this study include the population base of the study and the use of carefully phenotyped participants with challenge-proven food allergy measured at an early point in immune

development at age 1 year as well as at age 2 years. Unlike the 2 previous studies investigating the effects of genotype on the association between serum 25(OH)D₃ levels and sensitization, we measured confirmed food allergy, and measured rs7041 as well as rs4588. Thus, our results are congruent with but extend those of Vimalaswaran et al²⁶ and Liu et al²⁷; both reported no effect modification for total IgE level based on rs4588 (similar to our findings), and neither measured DBP levels.

Limitations include the fact that we could not further investigate the role of infant vitamin D supplementation because this is uncommon in Australian infants. Small numbers in some subgroups resulted in wide CIs; hypotheses generated from these observations need to be confirmed in other studies, although this will be challenging because HealthNuts is one of only a few large-scale population cohorts studying measured food allergy. Information on maternal use of vitamin supplements was collected retrospectively so could be subject to recall bias; however, limited public awareness of a potential link between vitamin D and food allergy at the time of recruitment makes recall bias based on infant food allergy status less likely. Recall error (nondifferential by food allergy status) would tend to attenuate any true underlying associations; thus, recall error is unlikely to explain the observed association between maternal vitamin D supplementation and infant food allergy. A further limitation is the lack of measured DBP levels, which is an important area for future research.

Our findings on rs7041 may explain population differences in the association between serum 25(OH)D₃ and allergic disease and contribute to explaining why some previous studies found a protective association between either low serum 25(OH)D₃ level or vitamin D supplementation and food sensitization or allergy whereas others did not.^{7-9,28,29} For example, low serum 25(OH)D₃ level was common but showed no association with atopy in

TABLE III. Relationship between infant vitamin D supplementation and food allergy according to genotype-predicted binding protein level

Dietary vitamin D intake	OR (95% CI)*	P value	aOR (95% CI)†	P value
Vitamin supplements during pregnancy				
Among infants with GG at rs7041				
No supplements (n = 16)	1.0		1.0	
Multivitamin (n = 108)	0.59 (0.17-2.04)	.40	0.50 (0.15-1.65)	.25
Additional vitamin D (n = 9)	0.40 (0.07-2.43)	.32	0.34 (0.04-2.79)	.32
Among infants with GT/TT at rs7041				
No supplements (n = 53)	1.0		1.0	
Multivitamin (n = 292)	0.61 (0.26-1.43)	.25	0.64 (0.24-1.66)	.36
Additional vitamin D (n = 28)	0.18 (0.06-0.55)	.003	0.10 (0.03-0.41)	.001
P interaction = .05‡				
Current use of infant formula				
Among infants with GG at rs7041				
None (n = 38)	1.0		1.0	
Formula + breast-feeding (n = 16)	0.96 (0.17-5.58)	.96	1.10 (0.21-5.46)	.93
Formula alone (n = 84)	0.40 (0.10-1.59)	.19	0.66 (0.21-2.03)	.47
Among infants with GT/TT at rs7041				
None (n = 89)	1.0		1.0	
Formula + breast-feeding (n = 60)	2.03 (0.91-4.50)	.083	1.48 (0.62-3.54)	.38
Formula alone (n = 235)	2.29 (1.13-4.66)	.022	1.86 (0.85-4.10)	.12
P interaction = .24‡				
Infant vitamin D supplements§				
Among infants with GG at rs7041				
No (n = 41)	1.0			
Yes (n = 3)	ND			
Among infants with GT/TT at rs7041				
No (n = 134)	1.0		1.0	
Yes (n = 19)	0.36 (0.10-1.28)	.12	0.38 (0.09-1.67)	.20

aOR, Adjusted odds ratio; ND, not determined.

*Unadjusted model including sampling weights.

†Adjusted for current use of infant formula or vitamin D supplements during pregnancy and serum 25(OH)D₃ levels.

‡The interaction term was calculated using the Wald test by assuming a linear association between categories of vitamin D supplementation (none, multivitamin, additional vitamin D) or formula use (none, formula + breast-feeding, formula alone) and the outcome of food allergy.

§Collected for a subcohort of infants.

||No infants in this group who had used vitamin D supplements were food allergic. P interaction could not be calculated.

TABLE IV. Change in vitamin D levels over the first 2 years of life and persistence of egg allergy at age 2 years

Age 1 y vitamin D	Age 2 y vitamin D	Vitamin D levels over first 2 y of life	N	% With persistent egg allergy	OR (95% CI)*	P value
Among all infants						
Sufficient	Sufficient	Sufficient	56	48.2	1.0	
Insufficient	Sufficient	Resolved insufficiency	11	54.6	1.3 (0.4-5.0)	.64
Sufficient	Insufficient	New insufficiency	23	47.8	0.7 (0.3-2.1)	.58
Insufficient	Insufficient	Persistent insufficiency	11	90.9	12.6 (1.5- 106.6)	.020

*Adjusted for parents' country of birth.

an Asian population of children aged 5 to 18 years,⁸ unlike US children and adults in a 60% white population.²⁸ In addition, our results could explain our previous finding that infants with parents born outside Australia showed no association between vitamin D level and food allergy, whereas low vitamin D level was a risk factor for food allergy among infants with parents born in Australia.⁷ Infants with parents from Asia were more likely to have genotypes with low predicted DBP level, which appeared to compensate for vitamin D levels in the insufficient range.

One recent small study found that high maternal and cord blood vitamin D levels were associated with a higher risk for food sensitization and allergy in the first 2 years of life.⁹ We also found some evidence of a higher risk of food allergy in those with higher

vitamin D levels among those with high predicted DBP levels but not among those with low predicted DBP levels, potentially supporting a U-shaped association, providing evidence that future intervention trials with vitamin D should measure the potential impact of high as well as low vitamin D levels and take the DBP genotype into account. Surprisingly, high vitamin D levels (as well as low levels) were associated with the risk of food allergy only in those with high predicted DBP levels, who might be expected to have less vitamin D bioavailability.

In conclusion, we found evidence that genotype modifies the association between vitamin D insufficiency and food allergy. These findings have implications for both prevention and treatment of food allergy. Reference ranges to define low levels of

TABLE V. Relationship between serum 25(OH)D₃ level at age 2 years and persistence of egg allergy by age 2 years

Serum 25(OH)D ₃ levels (nM/L) at age 2 y*	OR for persistent egg allergy (95% CI)
Among all infants (n = 105)	
≤50 (n = 36)	1.7 (0.7-4.1)
51-74 (n = 57)	1.0
≥75 (n = 12)	1.1 (0.3-3.9)
Among infants with GG at rs7041 (n = 27)	
≤50 (n = 7)	5.5 (0.8-38.7)†
51-74 (n = 16)	1.0
≥75 (n = 4)	6.6 (0.5-80.2)‡
Among infants with GT/TT at rs7041 (n = 78)	
≤50 (n = 29)	1.2 (0.5-3.2)
51-74 (n = 41)	1.0
≥75 (n = 8)	0.5 (0.1-2.5)

*Seasonally adjusted measure of serum 25(OH)D₃.†P = .090 for interaction between serum 25(OH)D₃ insufficiency and genotype with the outcome of persistent egg allergy.‡P = .18 for interaction between high serum 25(OH)D₃ level and genotype with the outcome of persistent egg allergy.

serum 25(OH)D₃ with a detrimental biological effect (including on food allergy risk) may need to take into account differences in the DBP level, including racial variation, and treatment dosages might also need tailoring. Finally, further studies should determine whether correction of vitamin D insufficiency could aid in the development of tolerance including during oral immunotherapy to foods.

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Clinical implication: Reference ranges to define low levels of serum 25(OH)D₃ with a detrimental biological effect, including on food allergy risk, may need to take into account differences in the DBP level, including racial variation.

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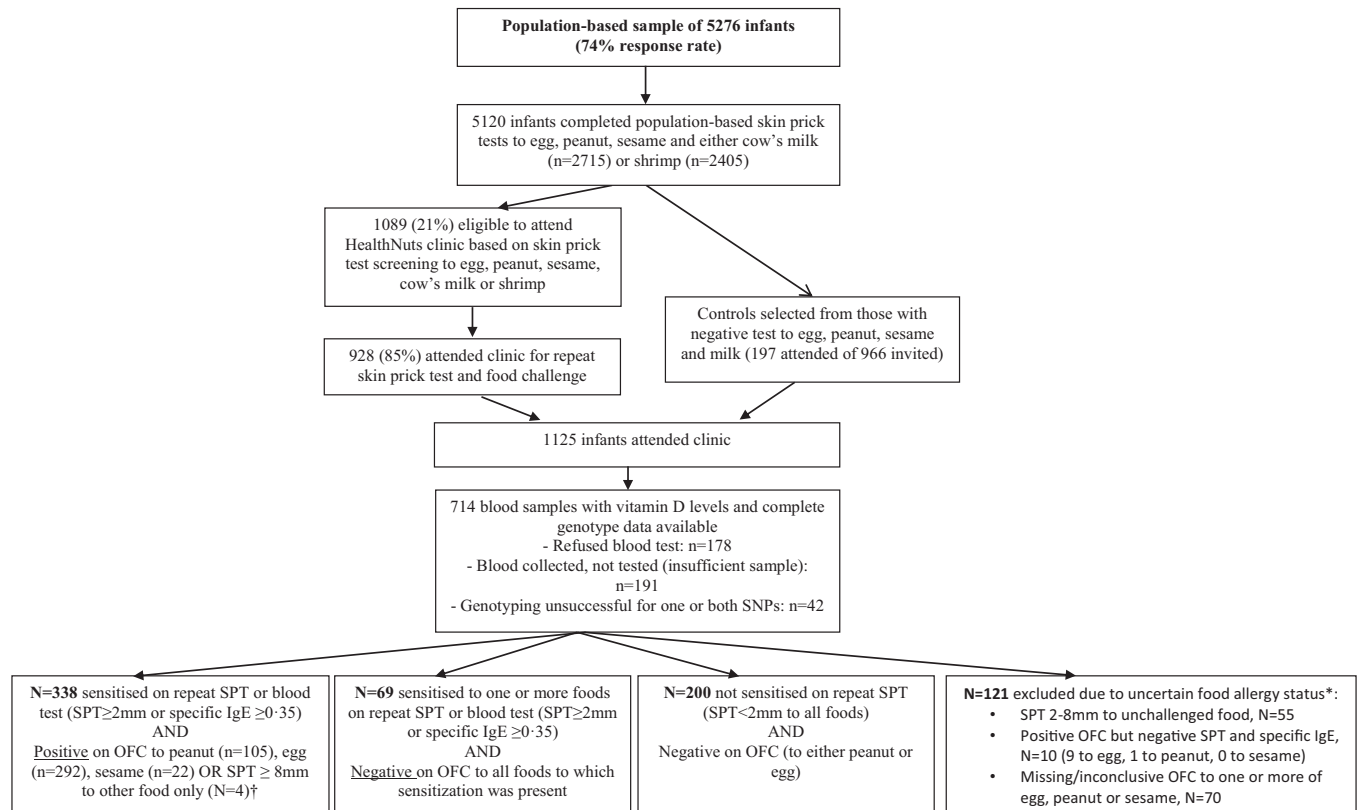


FIG E1. Participation in assessments at age 1 year. *OFC*, Oral food challenge; *SNP*, single nucleotide protein; *SPT*, skin prick test. *Note that some infants fell into more than 1 of these categories. Of these infants, 44 had at least 1 negative challenge to a food to which they were sensitized; however, their full status was unknown because they did not undergo a challenge to all the foods to which they were sensitized. †A total of 73 infants were allergic to more than 1 food.

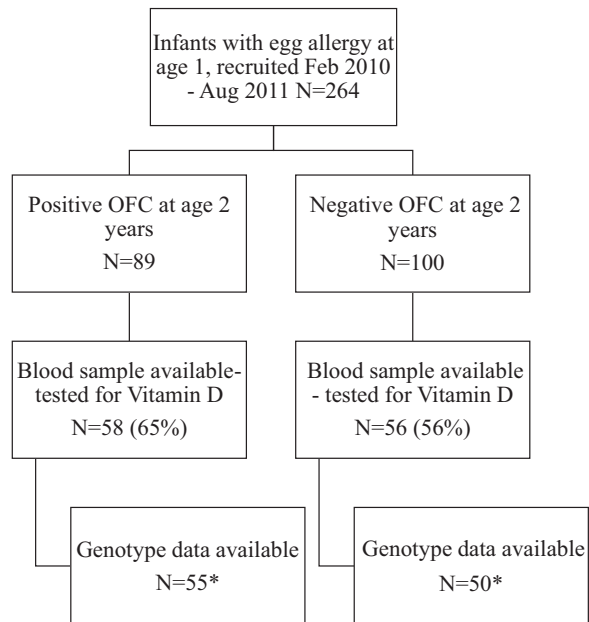


FIG E2. Participation in egg allergy longitudinal study. *OFC*, Oral food challenge. *Note: 101 of these 105 infants had vitamin D levels measured at both ages 1 and 2 years and were therefore also included in the age 1 year analysis.

TABLE E1. Sampling weights used in logistic regression models

Group	Family history of food allergy	Infant history of eczema	Parents' country of birth	Weight (inverse probability of clinic attendance)
1	No	No	Australia	21
2	No	Yes	Australia	15
3	No	No	Other	43
4	No	Yes	Other	26
5	Yes	No	Australia	12
6	Yes	No	Other	24
7	Yes	Yes	Australia	5
8	Yes	Yes	Other	2

TABLE E2. Distribution of DBP genotype/phenotypes by parental country of birth

Genotype			Predicted binding protein level	Parents' country of birth (%)		
rs7041	rs4588	Both Australia (n = 442)		One/both Asia (n = 129)	Other (n = 238)	
Binding protein level predicted by rs7041 genotype						
GG	Any	Highest	28.5	5.4	28.6	
GT	Any	Intermediate	53.9	43.4	45.4	
TT	Any	Lowest	17.7	51.2	26.1	
				<i>P</i> < .001*	<i>P</i> = .024*	
Binding protein level predicted by rs7041 and rs4588 genotype						
GG	CC	Lower	96.8	100	97.1	
	CA	Higher	3.2	0	2.9	
				<i>P</i> = .89	<i>P</i> = .48	
GT	CC	Lower	40.8	51.8	35.2	
	CA	Intermediate	59.2	48.2	63.9	
	AA	Higher	0.0	0.0	0.9	
				<i>P</i> = .13	<i>P</i> = .22	
TT	CC	Lower	12.8	28.8	12.9	
	CA	Intermediate	42.3	50.0	40.3	
	AA	Higher	44.9	21.2	46.8	
				<i>P</i> = .004	<i>P</i> = .97	

* P value from χ^2 test compared with both parents born in Australia as baseline group.