

Evidence for a genetic interaction in allergy-related responsiveness to vitamin D deficiency

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Keywords

1958 British birth cohort; 25-hydroxyvitamin D; allergy; gene × environment interaction; total IgE.

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Abstract

Background: The hormonal form of vitamin D affects both adaptive and innate immune functions involved in the development of allergies. Certain genotypes have been seen to alter the association between vitamin D deficiency (VDD) and the risk of food sensitization in children.

Methods: We examined 27 functional single nucleotide polymorphisms (SNPs) in/near selected candidate genes for association with total immunoglobulin E (IgE) and effect modification by 25-hydroxyvitamin D in the 1958 British birth cohort (aged 45 years, $n = 4921$). A cut-off value of 50 nmol/L was used to define VDD.

Results: Four SNPs (in *FCER1A*, *IL13*, and *CYP24A1*) and three SNPs (in *IL4* and *CYP24A1*) were associated with total IgE and specific IgE, respectively, after correction for multiple testing. As in a previous study, *MS4A2* (rs512555, $P_{\text{interaction}} = 0.04$) and *IL4* (rs2243250, $P_{\text{interaction}} = 0.02$), and their composite score ($P_{\text{interaction}} = 0.009$) modified the association between VDD and allergy-related outcome. Vitamin D deficiency was associated with higher total IgE only in the carriers of the 'C' allele (*IL4*), which is present in 86% of white Europeans, while only 26% of Chinese and <20% of some African populations are carriers.

Conclusions: Our study on white European adults was consistent with a previous study on children from largely non-white ethnic groups, suggesting that *IL4* and *MS4A2* genotypes modify the association between VDD and allergy risk. The risk allele in *IL4* is present in nearly 90% of white Europeans, while less than a quarter are carriers in some other populations, highlighting the need to consider possible ethnic differences in allergy-related responsiveness to VDD.

The hormonal form of vitamin D exerts profound effects on inflammatory pathways and cells involved in the development of allergies (1). Epidemiological studies suggest that 25-hydroxyvitamin D [25(OH)D, an indicator for nutritional vitamin D status] may reduce the risk of immunological diseases such as type 1 diabetes (2) and multiple sclerosis (3). For allergic disease and asthma, the evidence is less consistent with both low and high 25(OH)D concentrations suggested to be associated with increased risk (1, 4–6). However,

at the molecular level, the active vitamin D metabolite, calcitriol, has been shown to suppress dendritic cell maturation and consecutive Th₁ cell development by blocking the IL-12 signal, which is an independent key mechanism for the development of allergy (5, 7–9). Also, studies in mice have shown that treatment with 1,25-dihydroxyvitamin D results in the reduced secretion of Th₁-type cytokines IL-2 and INF- γ and an increase in Th₂-type IL-4 (10). Furthermore, epidemiological studies have provided evidence for a link between early vitamin D supplementation and allergy development, where several vitamin D genes play a role (8).

The contribution of genetic factors involved in vitamin D metabolism to the development of allergic conditions comes from genetic studies investigating the association of vitamin D receptor polymorphisms with atopy and asthma (11, 12).

Abbreviations

25(OH)D 25-hydroxyvitamin D; GWAS genome-wide association study; HWE Hardy–Weinberg equilibrium; IgE Immunoglobulin E; SNP Single nucleotide polymorphism.

A study in 1064 individuals examined the association of 83 tagging polymorphisms (in 11 vitamin D-related genes) with atopy and asthma and found that a number of genes demonstrated modest levels of association with both the traits (13). In a recent study on 649 children, vitamin D deficiency (VDD) increased the risk of food sensitization only among individuals with certain genotypes, providing evidence for gene–vitamin D interaction on food sensitization (14). Using data from 9377 participants in the 1958 British birth cohort, we have previously reported evidence for a strong U-shaped association between 25(OH)D and total immunoglobulin E (IgE), with an association seen for polymorphism encoding the vitamin D activation enzyme, CYP27B1 (4).

Based on previous observations on gene–environment interactions in relation to food sensitization in children (14), we hypothesized that genetic variation may also affect vitamin D-related allergy responses in adults. Our study relies on an assumption that despite differences between the etiologies leading to food sensitization or elevations in systemic IgE levels, there may be shared influences on immune activation or modulation (5, 15). In line with this hypothesis, we tested whether three single nucleotide polymorphisms (SNPs) [(rs2243250 (*IL4*), rs512555 (*MS4A2*), and rs2762934 (*CYP24A1*)] that showed a significant interaction with VDD in the previous study on children (14) also modify the association between VDD and total IgE/specific IgE in adults. We also systematically examined 27 potentially functional SNPs in/near 12 genes that affect total IgE or 25(OH)D for their associations with total IgE and specific IgE.

isms (SNPs) [(rs2243250 (*IL4*), rs512555 (*MS4A2*), and rs2762934 (*CYP24A1*)] that showed a significant interaction with VDD in the previous study on children (14) also modify the association between VDD and total IgE/specific IgE in adults. We also systematically examined 27 potentially functional SNPs in/near 12 genes that affect total IgE or 25(OH)D for their associations with total IgE and specific IgE.

Materials and methods

Study population

Study participants are from the 1958 British birth cohort (1958BC) (Table 1), which initially included all births in England, Scotland, and Wales during first week in March 1958 ($n = 16\,751$) (16). Most recently, participants were contacted between September 2002 and April 2004, when the majority was aged 45 years [range 44 (31.1%)–46 (0.4%)]. The target population for the survey consisted of 11 971 individuals currently living in Britain. Seventy-eight percent ($n = 9349$) of the participants filled in a questionnaire, of whom 8302

Table 1 Characteristics of the participants in the 1958 British birth cohort ($n = 4921$)

	Number (%)	25(OH)D (nmol/L)		Immunoglobulin E (kU/l)	
		Geometric mean (95% CI)	25(OH)D ≤ 50 (%)	Geometric mean (95% CI)	Specific production (%)*
Sex					
Male	2464 (50.1)	52.9 (51.9, 53.8)	1013 (41.1)	35.6 (33.7, 37.7)	781 (31.8)
Female	2457 (49.9)	51.0 (50.0, 51.9)	1052 (42.8)	23.9 (22.6, 25.2)	532 (21.7)
P-value		0.001	0.161	<0.001	<0.001
Month in which blood sample was taken					
January	380 (7.7)	39.5 (37.7, 41.4)	258 (67.9)	31.5 (27.5, 36.2)	97 (25.5)
February	280 (5.7)	36.5 (34.6, 38.5)	203 (72.5)	30.1 (25.5, 35.6)	67 (24.0)
March	381 (7.7)	39.5 (37.7, 41.3)	252 (66.1)	24.9 (21.5, 28.9)	92 (24.2)
April	313 (6.4)	40.2 (38.4, 42.1)	207 (66.1)	27.0 (23.1, 31.5)	87 (27.9)
May	384 (7.8)	44.2 (42.2, 46.3)	225 (58.6)	27.3 (23.7, 31.6)	99 (25.8)
June	471 (9.6)	49.5 (47.7, 51.3)	213 (45.2)	28.0 (24.6, 31.7)	118 (25.1)
July	392 (7.9)	61.2 (59.0, 63.5)	89 (22.7)	30.0 (26.3, 34.1)	105 (26.8)
August	285 (5.8)	65.3 (62.7, 68.1)	58 (20.4)	35.0 (29.6, 41.3)	81 (28.4)
September	605 (12.3)	71.9 (69.8, 74.1)	85 (14.0)	32.6 (29.0, 36.7)	183 (30.4)
October	623 (12.7)	62.4 (60.4, 64.4)	158 (25.4)	26.5 (23.5, 29.8)	167 (26.8)
November	629 (12.8)	54.2 (52.5, 55.9)	241 (38.3)	30.4 (27.1, 34.0)	170 (27.0)
December	178 (3.6)	52.4 (49.1, 55.9)	76 (42.7)	28.9 (23.6, 35.5)	47 (26.4)
P-value		<0.001	<0.001	0.021	0.745
Region					
South England (including London)	1850 (37.6)	52.7 (51.6, 53.9)	748 (40.4)	28.6 (26.8, 30.5)	502 (27.2)
Middle England and Wales	1290 (26.2)	52.3 (51.1, 53.6)	516 (40.0)	29.4 (27.2, 31.8)	338 (26.2)
North England	1293 (26.3)	53.5 (52.1, 54.9)	525 (40.6)	29.8 (27.6, 32.2)	352 (27.2)
Scotland	488 (9.9)	44.1 (42.3, 46.0)	276 (56.6)	29.3 (25.7, 33.4)	121 (24.9)
P-value		<0.001	<0.001	0.834	0.441

P-values from likelihood ratio test, adjusted for sex, month of blood sample, and region, from linear regression for continuous outcomes and logistic regression for dichotomous outcomes.

*Number of individuals with at least one specific IgE (cat, grass, or dust) >0.35 kU/l.

(88.5%) also provided blood samples. The 1958BC is largely a white European population (98%), and despite some data attrition, it has been evaluated to be broadly representative of the surviving cohort (17).

Target sample for the current analyses arises from two genome-wide substudies, where a random sample of cohort members (white European ancestry only) was selected for use as a control population for genome-wide studies. Firstly, 3000 participants were randomly selected as part of the Wellcome Trust Case Control Consortium (WTCCC2) and genotyped on the Affymetrix 6.0 platform (18). Secondly, 2592 participants from the 1958BC were randomly selected as controls for a type 1 diabetes case-control (T1DGC) study and genotyped using the Illumina Infinium 550 K chip (19). From the 5233 participants with genome-wide data that passed quality control (QC) measures (2530 samples passing QC in T1DGC and 2703 after QC in WTCCC2), 94% ($n = 4921$) also had information on serum 25(OH)D concentrations and total IgE, qualifying for the current analyses. Written consent for the use of information in medical studies was obtained from the cohort members. The 45-year biomedical survey and genetic studies were approved by the South-East Multi-Centre Research Ethics Committee (ref: 01/1/44) and the joint UCL/UCLH Committees on the Ethics of Human Research (Ref: 08/H0714/40).

Measures

The 25(OH)D was measured using automated application of an enzyme-linked immunosorbent assay (IDS OCTEIA ELISA; IDS, Bolton, UK) and an analyser (BEP2000; Dade-Behring, Milton Keynes, UK) with sensitivity of 5.0 nmol/L, linearity ≤ 155 nmol/L, and intra-assay CV 5.5–7.2%. The 25(OH)D concentrations were standardized according to the mean of the values found by the Vitamin D External Quality Assurance Survey (DEQAS) (20). Total IgE was assayed using the HYTEC-automated enzyme immunoassay (21). Specific IgE to cat, grass, and dust were measured if total IgE was ≥ 30 kU/l. Specific IgE production was defined as at least one specific IgE > 0.35 kU/l. Information on specific IgE was missing in five individuals who had total IgE > 30 kU/l.

SNP selection and genotyping

Initially, 35 SNPs were chosen based on the study by Liu et al. (14). Seven SNPs were excluded from the analysis as they had a minor allele frequency (MAF) of $< 5\%$ in the population (Table 2). However, *MS4A2* (Fc epsilon receptor 1 beta-chain) SNP, rs512555, was included in our analysis (MAF = 0.02) because of previous evidence for effect modification (14). Genotype data were not available for *FCER1G* SNP rs2070901, which had also shown evidence for effect modification. In total, 27 SNPs were considered for the analysis.

Of the 27 SNPs, two SNPs, rs10877012 and rs4588, were genotyped using the Taqman platform (Applied Biosystems, Warrington, UK), while others were obtained from the

genome-wide platforms (18, 19). The overall call rate for the genotyping was $> 97\%$, and the genotype frequencies of all SNPs were in Hardy–Weinberg equilibrium ($P > 0.01$).

Statistical analysis

Variations in total IgE were evaluated by linear regression for continuous total IgE and by logistic regression for the dichotomous outcome of specific IgE production. Natural log transformation was applied to 25(OH)D and total IgE to calculate geometric means and to improve the approximation of normal distribution. All models were adjusted for sex, month of measurement, and region (Scotland or South, Middle or North of England). For analyses including SNPs, a further adjustment was made for the genotyping platform. All carriers of minor alleles were grouped together to improve the stability of the estimates. A cut-off value of 50 nmol/L was used to define VDD (22), and individuals with 25(OH)D > 135 nmol/L ($n = 33$) were excluded to reflect the nonlinear relationship between 25(OH)D and total IgE (4). Interactions between each SNP and VDD were assessed using the likelihood ratio test. A stratified analysis was performed when significant interactions were detected. A genetic risk score was constructed by combining the risk genotypes of SNPs that were found to interact with VDD (rs2443250 and rs512555), with risk genotypes defined as the groups in which VDD was associated with increasing total IgE levels. A chi-square goodness of fit test was used to assess deviation from Hardy–Weinberg equilibrium ($P > 0.01$ for all SNPs). All P -values were derived from two-sided tests. Multiple testing was corrected for all single SNP and exploratory interaction analyses using the false discovery rate method [$(\leq 0.05 \times 27 \text{ SNPs})/\text{rank}$]. All analyses were carried out using STATA, version 12 (23).

Results

The characteristics of the study participants are presented in the Table 1. Total IgE concentrations appeared higher in participants with VDD (< 50 nmol/L) compared to participants with 25(OH)D levels 50–135 nmol/L [coefficient (95% CI): 8.1% (−0.7%, 16.9%), $P = 0.07$, while no association was seen for specific IgE [odds ratio (95% CI): 1.07 (0.93, 1.24), $P = 0.33$] after adjusting for sex, region and month of blood sample.

We investigated the association of the 27 SNPs with total IgE and specific IgE and found that only four SNPs [rs2427837 (*FCER1A*), $P < 0.001$; rs20541 (*IL13*), $P = 0.001$; rs848 (*IL13*), $P = 0.001$; and rs2248359 (*CYP24A1*), $P = 0.05$] remained associated with total IgE and 3 SNPs [rs2243250 (*IL4*), $P = 0.05$, rs2070874 (*IL4*), $P = 0.05$; and rs2248359 (*CYP24A1*), $P = 0.04$] with specific IgE, after correction for multiple testing (Table 2).

Of the three SNPs [(rs2243250 (*IL4*), rs512555 (*MS4A2*), and rs2762934 (*CYP24A1*)] that showed an interaction with VDD in an earlier study on children (14), two also modified the association between VDD and total IgE concentrations in our study (*IL4*, rs2243250, $P_{\text{interaction}} = 0.02$; *MS4A2*,

Table 2 Association of the potentially functional single nucleotide polymorphisms (SNPs) with total immunoglobulin E (IgE) levels and specific IgE production in the 1958 British birth cohort

Genes/SNPs	Chromosome	MAF	SNP–total IgE association (n = 4921)*		SNP–specific IgE association (n = 4916)*	
			Coefficient (95% CI) [†]	P-value [‡]	Odds ratio (95% CI)	P-value [‡]
IgE synthesis and IgE receptor complex						
<i>FCER1A</i> (IgE fc receptor subunit alpha)						
rs2427837	1	0.30	−26.2 (−34.2, −18.2)	<0.001 (<0.001)	0.83 (0.73, 0.95)	0.006 (0.08)
<i>FCER1G</i> (IgE fc receptor subunit gamma)						
rs11587213	1	0.17	3.9 (−4.7, 12.5)	0.39	1.06 (0.92, 1.22)	0.40
rs11421	1	0.15	1.9 (−7.1, 10.8)	0.68	1.00 (0.87, 1.15)	0.99
<i>IL-4</i> (interleukin 4)						
rs2243248	5	0.06	−6.8 (−18.9, 5.3)	0.27	0.79 (0.65, 0.97)	0.026 (0.14)
rs2243250	5	0.13	8.9 (−0.4, 18.2)	0.06 (0.18)	1.22 (1.06, 1.42)	0.007 (0.05)
rs2070874	5	0.13	9.0 (−0.2, 18.3)	0.06 (0.19)	1.23 (1.06, 1.42)	0.006 (0.05)
<i>IL-13</i> (interleukin 13)						
rs1295687	5	0.06	12.6 (−4.7, 29.9)	0.16	0.88 (0.73, 1.07)	0.19
rs2069744 [§]	5	0.00	—	—	—	—
rs20541	5	0.18	17.2 (8.7, 25.6)	<0.001 (0.001)	1.16 (1.02, 1.33)	0.03 (0.13)
rs848	5	0.17	17.5 (9.0, 26.0)	<0.001 (0.001)	1.16 (1.01, 1.33)	0.04 (0.14)
<i>MS4A2</i> (<i>Fc epsilon</i> receptor 1 beta-chain)						
rs512555	11	0.02	2.3 (−16.9, 21.5)	0.83	1.13 (0.84, 1.53)	0.418
<i>IL4R</i> (interleukin 4 receptor alpha-chain)						
rs2057768	16	0.30	9.6 (1.6, 17.5)	0.02 (0.09)	0.97 (0.85, 1.10)	0.59
rs3024633	16	0.08	4.1 (−7.0, 15.2)	0.46	1.08 (0.91, 1.29)	0.39
rs1805010	16	0.44	−0.4 (−13.1, 12.3)	0.96	0.83 (0.68, 1.01)	0.06 (0.18)
rs1805016 [§]	16	0.04	—	—	—	—
rs2074570 [§]	16	0.03	—	—	—	—
rs3024682 [§]	16	0.00	—	—	—	—
Vitamin D metabolic pathway						
<i>Near DHCR7</i> (7-dehydrocholesterol reductase)						
rs12785878	11	0.21	−4.9 (−13.1, 3.3)	0.23	0.99 (0.86, 1.12)	0.83
<i>Near CYP2R1</i> (cytochrome P450, family 2, subfamily R, polypeptide 1)						
rs10741657	11	0.39	−10.6 (−19.0, −2.3)	0.01 (0.07)	0.9 (0.79, 1.03)	0.12
<i>VDR</i> (vitamin D (1,25-dihydroxyvitamin D3) receptor)						
rs2853563 [§]	12	0.02	—	—	—	—
rs7954412 [§]	12	0.00	—	—	—	—
rs3858733 [§]	12	0.02	—	—	—	—
rs11168293	12	0.35	−3.5 (−11.6, 4.5)	0.38	0.92 (0.81, 1.04)	0.19
<i>CYP27B1</i> (cytochrome P450, family 27, subfamily B, polypeptide 1)						
rs8176351 [§]	12	0.00	—	—	—	—
rs10877012	12	0.32	2.5 (−5.7, 10.7)	0.55	1.05 (0.92, 1.19)	0.49
<i>CYP24A1</i> (cytochrome P450, family 24, subfamily A, polypeptide 1)						
rs6013897	20	0.19	4.2 (−4.1, 12.5)	0.32	1.08 (0.95, 1.24)	0.24
rs4809957	20	0.21	−8.6 (−20.0, 2.7)	0.14	1.16 (0.88, 1.53)	0.29
rs2762934	20	0.18	6.8 (−1.7, 15.2)	0.11	1.10 (0.96, 1.26)	0.17
rs2296239	20	0.21	−8.2 (−16.4, −0.0)	0.05 (0.19)	0.91 (0.79, 1.04)	0.15
rs2296241	20	0.45	3.4 (−5.6, 12.5)	0.47	0.97 (0.84, 1.13)	0.72
rs2248359	20	0.36	−11.7 (−20.2, −3.2)	0.007 (0.05)	0.80 (0.70, 0.92)	0.002 (0.04)
<i>GC</i> (group-specific component – vitamin D binding protein)						
rs2282679	4	0.29	0.5 (−8.4, 7.4)	0.91	0.89 (0.78, 1.01)	0.06
rs4588	4	0.26	−0.9 (−9.3, 7.5)	0.84	0.87 (0.76, 0.99)	0.04 (0.15)
rs1155563	4	0.26	1.3 (−7.1, 9.8)	0.76	0.93 (0.81, 1.07)	0.31
rs3733359	4	0.06	4.3 (−8.4, 16.7)	0.50	1.12 (0.92, 1.22)	0.40

MAF, minor allele frequency.

All models were adjusted for sex, month of measurement, region, and genotyping platform.

*N for analysis varies according to the call rate of each SNP.

†Coefficients presented as percentage change in the outcome.

‡False discovery rate-adjusted P-value in brackets, where appropriate.

§Excluded from analysis because of MAF < 0.05.

Table 3 Interaction between SNPs and vitamin D deficiency (VDD) (<50 vs 50–135 nmol/L) on total IgE and specific IgE production

Interaction between SNPs and VDD on total IgE levels using linear regression analysis (<i>n</i> = 4888)						
Genes	Genotypes	25(OH)D nmol/L	<i>N</i> *	Coefficient (95% CI) [†]	<i>P</i> -value from LRT	Interaction <i>P</i> -value
<i>IL4</i>	rs2243250 = CC	50–135	2127	Reference	0.01	0.02
		<50	1550	12.8 (2.7, 22.8)		
	rs2243250 = CT/TT	50–135	671	Reference	0.68	
		<50	503	–3.9 (–22.6, 14.8)		
<i>MS4A2</i>	rs512555 = CC	50–135	2697	Reference	0.16	0.04
		<50	1972	6.4 (–2.5, 15.4)		
	rs512555 = CT/TT	50–135	126	Reference	0.04	
		<50	91	52.6 (0.1, 105.1)		
<i>CYP24A1</i>	rs2762934 = GG	50–135	1875	Reference	0.56	0.24
		<50	1372	3.2 (–7.7, 14.1)		
	rs2762934 = GA/AA	50–135	921	Reference	0.01	
		<50	677	19.4 (4.1, 34.6)		
Interaction between SNPs and VDD on specific IgE using logistic regression analysis (<i>n</i> = 4883)						
Genes	Genotypes	25(OH)D nmol/L	Specific IgE cases [‡] /controls [*]	OR (95% CI)	<i>P</i> -value from LRT	Interaction <i>P</i> -value
<i>IL4</i>	rs2243250 = CC	50–135	529/1595	Reference	0.06	0.15
		<50	404/1144	1.17 (0.99, 1.38)		
	rs2243250 = CT/TT	50–135	209/462	Reference	0.51	
		<50	141/362	0.91 (0.68, 1.21)		
<i>MS4A2</i>	rs512555 = CC	50–135	717/1977	Reference	0.45	0.19
		<50	517/1453	1.06 (0.92, 1.22)		
	rs512555 = CT/TT	50–135	34/92	Reference	0.13	
		<50	30/61	1.76 (0.84, 3.66)		
<i>CYP24A1</i>	rs2762934 = GG	50–135	497/1376	Reference	0.99	0.19
		<50	347/1024	1.00 (0.83, 1.19)		
	rs2762934 = GA/AA	50–135	252/668	Reference	0.08	
		<50	198/478	1.25 (0.98, 1.66)		

LRT, likelihood ratio test.

All models were adjusted for sex, month of measurement, region, and genotyping platform.

**N* for analysis varies according to the call rate of each SNP.

†Coefficients presented as percentage change in the outcome.

‡Cases defined as those with at least one specific IgE (cat, grass, or dust) >0.35 kU/l.

rs512555, $P_{\text{interaction}} = 0.04$), while no significant associations were seen for specific IgE (Table 3). Vitamin D deficiency was associated with higher total IgE only in the carriers of the 'T' allele in *MS4A2* [coefficient (95% CI): 52.6% (0.1%, 105.1%), $P = 0.04$] and 'C' allele in *IL4* [coefficient (95% CI): 12.8% (2.7%, 22.8%), $P = 0.01$] (Table 3). In the HapMap (24), 86% of Caucasian populations carry the 'C' allele in *IL4* (87% current study), while only 20% of the Yoruban and 26%/27% Chinese/Japanese are 'C' allele carriers (Table 4).

There was a strong interaction between the genetic risk score (comprising *IL4* and *MS4A2*) and VDD on total IgE levels ($P_{\text{interaction}} = 0.009$), such that the elevated total IgE concentrations associated with VDD were most pronounced in the carriers of the two risk alleles ('T' allele in *MS4A2* and 'C' allele in *IL4*) (Fig. 1). Further exploratory analyses provided no evidence for interaction between the remaining 24 SNPs and VDD on total IgE ($P > 0.16$ for all comparisons).

Table 4 Allele frequencies of the interleukin-4 (*IL4*) SNP rs2243250 in HapMap populations (HapMap Data Rel 28/phase II + III August 10, on NCBI B36 assembly, dbSNP b126)

HapMap population	'C' allele frequency of the <i>IL4</i> SNP rs2243250 (%)
Gujarathi Indians, Houston, Texas	89
Tuscans, Italy	87
European ancestry, Utah	86
Mexican ancestry, Los Angeles, California	60
Maasai, Kinyawa, Kenya	46
African ancestry, Southwest USA	44
Japanese, Tokyo, Japan	27
Han Chinese, Beijing, China	26
Chinese, Metropolitan Denver, Colorado	25
Yoruban in Ibadan, Nigeria	20
Luhya in Webuye, Kenya	18

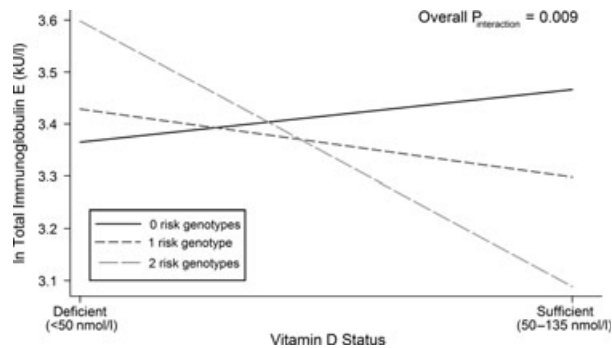


Figure 1 Interactive effect between the combined risk genotypes of the genes *IL4* (rs2243250) and *MS4A2* (rs512555) and vitamin D deficiency in the 1958 British birth cohort. Risk genotypes refer to the groups in which vitamin D status was associated with increasing total immunoglobulin E levels.

Discussion

In this study, we have examined the association of 27 potentially functional SNPs (14) with total IgE and specific IgE in the 1958BC and found that after accounting for multiple testing, four SNPs were significantly associated with total IgE levels and three SNPs with specific IgE. Interestingly, one of the genes implicated with both outcomes (*CYP24A1*) has a primary effect on vitamin D metabolism (25) rather than on immunological factors more closely related to allergy risk. Our study also confirmed that modification by other immune-related genetic factors is likely to affect the influence of vitamin D status on allergy risk. A previous study had shown that VDD increases the risk of food sensitization among children, depending on their *IL4* and *MS4A2* genotypes (14). Our findings for total IgE suggest that such effect modification is implicative of a gene–environment interaction that is also relevant to adults.

It is important to note that while our study was conducted in an adult British white population, the study by Liu et al. (14) was performed in children comprising approximately 57% Blacks, 22% Hispanics, 6% Whites, and 15% others. Differences in the ethnic mix between the studies are likely to explain the difference in the proportion carrying the ‘C’ allele of the *IL4* SNP rs2243250 between the two studies [87% in the 1958BC vs 37% in the Liu et al. (14)]. This difference in allele distribution is in accordance with large differences in allele frequencies between populations, as reported in the recent HapMap (24) (Table 4), which has been shown to be a reliable tool in making inferences based on the comparison of allele frequencies and genetic association data across populations (26). Despite these differences in the frequency of risk allele carriers, the association between VDD and increased allergy risk only for ‘C’ allele carriers appears to be consistent across diverse populations (14). However, as most individuals in white European groups, or indeed individuals of Indian origin, carry the risk allele, VDD is likely to be a more important determinant for allergy risk for them than for individuals from some other ethnic groups.

Similar large variation in allele frequencies was not seen for the minor ‘T’ allele of the *MS4A2* SNP, rs512555, which was relatively rare across populations (<20% across all) (24). However, in our study, VDD was associated with higher total IgE among the carriers of *MS4A2* ‘T’ allele, while in the earlier study, VDD had a protective effect (14). This could suggest possible variation also in the alleles conferring the risk between populations or perhaps variation arising from differences in the etiology of allergy-related outcomes. A recent study in the Boston Birth Cohort ($n = 1104$) has also highlighted the importance of ethnic differences in relation to the genetic risk of developing food sensitization, which could possibly be attributed to the vitamin D status (27).

Our findings also confirm the results from the recent genome-wide association study for plasma total IgE levels (28), with *FCER1A* (IgE fc receptor subunit alpha) and *IL13* (interleukin 13) SNPs having significant associations after accounting for multiple testing. Our findings on *CYP24A1*, a known vitamin D-related candidate gene affecting the clearance of 25(OH)D and other vitamin D metabolites (29), are extremely intriguing and could provide further evidence for a causal role of vitamin D status in determining total IgE concentrations (25). *IL4* is a well-known candidate gene for total IgE (30, 31), and the locus for asthma and atopy has been linked to chromosome 5q31, the region where *IL4* gene has been mapped (32). Previous linkage studies have shown that gene predisposing to atopy is localized on chromosome 11q13 (33, 34), the region where *MS4A2* is located. Hence, our finding in the 1958BC further confirms the role of *IL4* and *MS4A2* in contributing to the risk of allergy, in part by altering the risk associated with VDD. Unfortunately, genotype data were not available for the *FCER1G* SNP rs2070901 from our study; hence, we were unable to investigate the related interaction also identified by Liu et al. (14).

The lack and difficulty of replication is a major drawback to obtaining evidence on gene–environment interaction affecting allergy risk but also more generally (35). One explanation is in the large sample size requirements, and even with our study which is the largest to date, we did not have sufficient power to detect interactions in relation to specific IgE as an outcome. Furthermore, previous evidence for genetic interaction by VDD arose from a study investigating determinants for food sensitization, and although it is possible that VDD could affect both food sensitization and systemic IgE levels through a shared pathway related to immune activation or modulation (5, 15), also differences in the etiology could explain the lack of association with specific IgE to inhalant allergens as well as the observed opposing effects of VDD in the carriers of ‘T’ allele of the *MS4A2* SNP rs512555 (14).

In summary, our findings confirm the association of *FCER1A* and *IL13* SNPs with total IgE levels and the previously reported interactions of the *IL4* and *MS4A2* SNPs with VDD on allergy risk. Together with a previous study (14) and known variation in allele frequencies across populations (24), these data highlight the need to consider possible ethnic differences in the allergy-related responsiveness to VDD.

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Author contributions

KSV wrote the first draft and contributed to the study hypothesis, literature search, and data interpretation. AC carried out all analyses and contributed to drafting and interpretation. EH had the study idea and formed the hypothesis, obtained funding, supervised the study and contributed to the interpretation, drafting and critical revision.

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Conflict of interest

We declare that we have no conflicts of interest.

References

- Reinholz M, Ruzicka T, Schaubert J. Vitamin D and its role in allergic disease. *Clin Exp Allergy* 2011; DOI:10.1111/j.1365-2222.2011.03923.x.
- Hyppönen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 2001;**358**:1500–1503.
- Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 2006;**296**:2832–2838.
- Hyppönen E, Berry DJ, Wjst M, Power C. Serum 25-hydroxyvitamin D and IgE – a significant but nonlinear relationship. *Allergy* 2009;**64**:613–620.
- Benson AA, Toh JA, Vernon N, Jariwala SP. The role of vitamin D in the immunopathogenesis of allergic skin diseases. *Allergy* 2012;**67**:296–301.
- Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and respiratory infections: the vitamin D hypothesis. *Allergy* 2012;**67**:10–17.
- Annesi-Maesano I. Perinatal events, vitamin D, and the development of allergy. *Pediatr Res* 2002;**52**:3–5.
- Wjst M. The vitamin D slant on allergy. *Pediatr Allergy Immunol* 2006;**17**:477–483.
- Meyts I, Hellings PW, Hens G, Vanaudenaerde BM, Verbinen B, Heremans H et al. IL-12 contributes to allergen-induced airway inflammation in experimental asthma. *J Immunol* 2006;**177**:6460–6470.
- Matheu V, Back O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. *J Allergy Clin Immunol* 2003;**112**:585–592.
- Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E et al. Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med* 2004;**170**:967–973.
- Raby BA, Lazarus R, Silverman EK, Lake S, Lange C, Wjst M et al. Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. *Am J Respir Crit Care Med* 2004;**170**:1057–1065.
- Bosse Y, Lemire M, Poon AH, Daley D, He JQ, Sandford A et al. Asthma and genes encoding components of the vitamin D pathway. *Respir Res* 2009;**10**:98.
- Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S et al. Gene-vitamin D interactions on food sensitization: a prospective birth cohort study. *Allergy* 2011;**66**:1442–1448.
- Beck LA, Leung DY. Allergen sensitization through the skin induces systemic allergic responses. *J Allergy Clin Immunol* 2000;**106** (5 Suppl):S258–S263.
- Power C, Elliott J. Cohort profile: 1958 British birth cohort (National Child Development Study). *Int J Epidemiol* 2006;**35**:34–41.
- Atherton K, Fuller E, Shepherd P, Strachan DP, Power C. Loss and representativeness in a biomedical survey at age 45 years: 1958 British birth cohort. *J Epidemiol Community Health* 2008;**62**:216–223.
- Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;**447**:661–678.
- Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet* 2009;**41**:703–707.
- Hyppönen E, Turner S, Cumberland P, Power C, Gibb I. Serum 25-hydroxyvitamin D measurement in a large population survey with statistical harmonization of assay variation to an international standard. *J Clin Endocrinol Metab* 2007;**92**:4615–4622.
- Nolte H, DuBuske LM. Performance characteristics of a new automated enzyme immunoassay for the measurement of allergen-specific IgE. Summary of the probability outcomes comparing results of allergen skin testing to results obtained with the HYTEC system and CAP system. *Ann Allergy Asthma Immunol* 1997;**79**:27–34.
- Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academies Press (US), 2011.
- StataCorp LP. *StataCorp: Stata Statistical Software. Release 11 Version ed*. College Station, TX: StataCorp LP., 2009.
- HapMap Data Release 28/phase II + III August 10, on NCBI B36 assembly, dbSNP b126 In.
- Berry D, Hyppönen E. Determinants of vitamin D status: focus on genetic variations. *Curr Opin Nephrol Hypertens* 2011;**20**:331–336.
- Manolio TA, Brooks LD, Collins FS. A HapMap harvest of insights into the genetics of common disease. *J Clin Invest* 2008;**118**:1590–1605.
- Kumar R, Tsai HJ, Hong X, Liu X, Wang G, Pearson C et al. Race, ancestry, and development of food-allergen sensitization in early childhood. *Pediatrics* 2011;**128**:e821–e829.

28. Granada M, Wilk JB, Tuzova M, Strachan DP, Weidinger S, Albrecht E et al. A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. *J Allergy Clin Immunol* 2012;**129**:840–845.
29. Jones G, Prosser DE, Kaufmann M. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys* 2012;**523**:9–18.
30. Basehore MJ, Howard TD, Lange LA, Moore WC, Hawkins GA, Marshik PL et al. A comprehensive evaluation of IL4 variants in ethnically diverse populations: association of total serum IgE levels and asthma in white subjects. *J Allergy Clin Immunol* 2004;**114**:80–87.
31. Haller G, Torgerson DG, Ober C, Thompson EE. Sequencing the IL4 locus in African Americans implicates rare noncoding variants in asthma susceptibility. *J Allergy Clin Immunol* 2009;**124**:1204–1209.e1209.
32. Doull IJ, Lawrence S, Watson M, Begishvili T, Beasley RW, Lampe F et al. Allelic association of gene markers on chromosomes 5q and 11q with atopy and bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 1996;**153**(4 Pt 1):1280–1284.
33. Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989;**1**:1292–1295.
34. Young RP, Sharp PA, Lynch JR, Faux JA, Lathrop GM, Cookson WO et al. Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J Med Genet* 1992;**29**:236–238.
35. Hunter DJ. Gene-environment interactions in human diseases. *Nat Rev Genet* 2005;**6**:287–298.