

Eczema phenotypes are associated with multiple vitamin D pathway genes in Chinese children

S. S. Wang¹, K. L. Hon¹, A. P. S. Kong², M. F. Tang¹, H. Y. Sy¹, J. C. N. Chan² & T. F. Leung¹

¹Department of Pediatrics, The Chinese University of Hong Kong, Prince of Wales Hospital; ²Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

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Correspondence

Ting F. Leung, MD, FRCPCH, FAACI,
Department of Pediatrics, 6/F, Lui Che Woo
Clinical Sciences Building, Prince of Wales
Hospital, Shatin, N.T., Hong Kong.
Tel.: (852) 2632 2981
Fax: (852) 2636 0020
E-mail: ttleung@cuhk.edu.hk

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Abstract

Background: Vitamin D is increasingly recognized to play crucial roles in cutaneous immunity, and vitamin D treatment improved eczema control in small clinical trials. Several vitamin D-related genes were associated with asthma, but there are no data for eczema.

Methods: Twenty-three single-nucleotide polymorphisms (SNPs) of five vitamin D-related genes (*CYP27A1*, *CYP2R1*, *CYP27B1*, *GC* and *VDR*) were genotyped in 1442 Chinese children with eczema and 1231 non-allergic controls. SNPs that followed Hardy–Weinberg equilibrium and yielded $\geq 95\%$ genotyping call-rate were included. Haplotypic associations and SNP–SNP interactions for eczema diagnosis and subphenotypes were analysed.

Results: Atopic eczema was associated with rs4674343 of *CYP27A1* (odds ratio 0.66, 95% confidence interval 0.53–0.83, $P = 0.0004$). Increased eosinophil percentage was associated with *CYP2R1* rs2060793A ($P = 0.001$) and rs1933064A ($P = 0.001$). Two *CYP2R1* haplotypes increased eczema risk whereas one *VDR* haplotype lowered eczema risk. *GC* rs7041 and *CYP2R1* rs7935792 interacted to modulate total IgE (cross-validation consistency 10/10, $P = 0.047$). Specifically, high-risk eczema patients had higher log-transformed total IgE than low-risk patients (2.76 ± 0.76 vs 2.60 ± 0.80 , $P = 0.002$).

Conclusion: A vitamin D-related SNP rs4674343 on *CYP27A1* was found to be protective against atopic eczema. *CYP2R1* and *VDR* haplotypes altered eczema susceptibility and eosinophil percentage, and *GC* and *CYP2R1* interacted to determine total IgE among eczema patients.

Despite the well-known effect on bone health, vitamin D pathway emerged as a new regulatory mechanism for immune responses (1). There are two sources of vitamin D, with the major one being the conversion of 7-dehydrocholesterol to provitamin D3 by UVB from sunlight exposure. Intake of vitamin D2 (ergocalciferol) or D3 from foods such as oily fish and cod liver oil is the other source. Inactive vitamin D is then metabolized to the major circulating form 25 (OH)D, with a long half-life of more than 2 weeks, by CYP2R1 in microsomes and CYP27A1 in liver that encode 25-hydroxylase. The activated form of vitamin D ($1\alpha,25$ (OH)₂D or calcitriol) is made following further hydroxylation step at C-1 position in the kidney by 1α -hydroxylase (CYP27B1), which is transported via circulation to the nuclei of target cells by the group-specific components of vitamin

D-binding protein (GC). The binding of $1\alpha,25$ (OH)₂D to vitamin D receptor (VDR), a ligand-activated transcription factor and nuclear hormone receptor, results in the transcription of vitamin D-responsive genes (2).

Vitamin D was reported to be involved in inflammatory pathways and development of allergies (3). VDR was found in most cells (T lymphocytes, neutrophils, macrophages, dendritic cells) of immune system. Vitamin D contributes to the increased production of cathelicidin and nitric oxide (4) in macrophages and inhibition of maturation and migration of dendritic cells. Vitamin D reduced the production of type 1 T helper cells by blocking IL-12 and induced type 2 T helper cell (Th2)-related cytokines IL-4, IL-5, and IL-10 (5). Based on these *in vitro* findings, higher vitamin D levels are expected in eczema cases. Nonetheless, serum 25-hydroxyvitamin D levels

showed inverse correlation with eczema severity (6), which might be due to reverse causation when patients with severe eczema were more likely to protect their skin from sun exposure. Thyssen et al. (7) reported that skin barrier abnormalities caused by filaggrin gene mutations were also associated with increased vitamin D levels. Association studies of vitamin D pathway genes will offer another approach to delineate the relationship between eczema and vitamin D.

Different research groups reported contradictory results of genetic variants encoding vitamin D pathway in the development of allergic conditions. *VDR* rs7975232 was associated with asthma in US children, but such result could not be replicated in a French Canadian family population. Nonetheless, the latter study reported six *VDR* single nucleotide polymorphisms (SNPs) to be weakly associated with asthma (8, 9). A recent genome-wide association study (GWAS) of circulating vitamin D level found that rs2282679 of *GC* and rs10741657 of *CYP2R1* were associated with 25(OH)D specifically among asthmatic children (10). Two case-control studies between asthma and genes along the vitamin D pathway were carried out in northern Chinese. Two common *GC* functional SNPs, rs4588 and rs7041, and *VDR* rs7975232 were found to be associated with asthma (11, 12), whereas *CYP2R1* showed no significant result. At present, there is only one report on possible relationship between eczema and vitamin D pathway genes. Heine and coworkers investigated the frequency of four common *VDR* polymorphisms in 265 adults with moderate-to-severe eczema and 265 healthy controls (13). They found an association between severe eczema and *VDR* rs1544410, rs7975232, and rs731236 as well as their haplotypes. This study investigated the association between eczema and SNPs of five genes (*CYP27A1*, *CYP2R1*, *CYP27B1*, *GC*, and *VDR*) along the vitamin D pathway in Hong Kong Chinese children.

Patients and methods

Study population

This case-control study consisted of 1442 unrelated Chinese children with eczema and 1231 nonallergic controls who were recruited from several hospital-based and community studies conducted between January 2009 and December 2011 (14). Patients with eczema were younger than 18 years, and their eczema was physician-diagnosed according to the widely accepted criteria (15). All controls did not report any eczema, asthma, and allergic rhinitis by the Chinese International Study of Asthma and Allergies in Childhood questionnaire (16, 17), and they were recruited from (i) children attending a university teaching hospital for minor nonrespiratory and nonimmunologic complaints; and (ii) secondary schoolchildren who participated in an epidemiological study for obesity and diabetes (18). All subjects were free from any infectious symptoms for 4 weeks before study. Subjects and their parents gave informed written consent, and Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee approved this study.

Eczema subphenotypes

Plasma total IgE concentration was measured by microparticle immunoassay (IMx analyser; Abbott Laboratories, Abbott Park, IL, USA), which was logarithm-transformed before analysis (LogIgE). Peripheral blood eosinophils were enumerated using a Coulter STKS counter (Beckman Coulter, Miami, FL, USA), which was expressed as percentage of total leukocytes (eos%). Skin prick test (SPT) was performed with normal saline as negative control, histamine 10 mg/ml as positive control, and standardized extracts of locally prevalent allergens (ALK Abelló, Round Rock, TX, USA) (19, 20). Wheal ≥ 3 mm larger than negative control was considered positive. Plasma allergen-specific IgE levels to *Dermatophagoides pteronyssinus*, cat and cockroaches were measured by fluorescent enzyme immunoassay (AutoCAP, Phadia AB, Uppsala, Sweden), with ≥ 0.35 kIU/l being positive. Atopy was defined as $\geq$ one positive SPT or allergen-specific IgE.

SNP Selection and genotyping

Table S1 describes 24 SNPs genotyped in this study. Eleven SNPs were reported to have significant association with asthma or serum vitamin D level (2 for *VDR*, 6 for *GC*, and 3 for *CYP2R1*). For *CYP27A1*, *CYP2R1*, and *CYP27B1*, tagging SNPs were also selected based on genotypic data for Han Chinese in Beijing (CHB) in HapMap phase 3 database using TagSNP provided by National Institute of Environmental Health Sciences (NIEHS) (<http://snpinfo.niehs.nih.gov/snptag.htm>). The tagging strategy was applied for SNPs with MAF ≥ 0.05 and pairwise $r^2 \geq 0.8$ that were within 5–10 kb upstream and downstream of the five target genes. SNPs published to have significant associations with asthma or serum vitamin D level were forced to be included. Thirteen tagging SNPs were selected (5 for each of *CYP2R1* and *CYP27A1* and 3 for *CYP27B1*). Potential functions of SNPs were predicted using FuncPred (<http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm>) provided by NIEHS.

Rs6716642 of *CYP27A1* could not be genotyped due to failed probe design by both iPLEX and TaqMan assays. Thirteen and 10 SNPs were genotyped by iPLEX[®] Gold assay (Sequenom, San Diego, CA, USA) and TaqMan[®] SNP Genotyping assay (Applied Biosystems, Foster City, CA, USA), respectively (see Data S1 in the Supporting Information).

Statistical analysis

Results were expressed in percentage or mean and standard deviation. Allele frequencies were estimated by gene counting method. Hardy-Weinberg equilibrium (HWE) for SNPs among cases and controls was evaluated by chi-square exact test. Pairwise linkage disequilibrium (LD) coefficient was calculated for each SNP pair by HaploView software (Daly Lab, Cambridge, MA, USA) (21). Because of markedly skewed data distribution, peripheral blood eosinophils were dichotomized at $\geq 5\%$ of total leukocytes before analysis. The associations for single SNP with dichotomous variables were analysed by

logistic regression and with eczema subphenotypes by linear regression, adjusted for age, sex, and physician-diagnosed asthma as covariates. All comparisons were made two-tailed using R version 2.15.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The level of significance was set at 0.0022 (0.05/23) by Bonferroni correction to account for 23 SNPs being analysed. Haplotypes were determined by HaploView 4.2 according to LD coefficients. Haplotypic associations were performed using R package *haplo.stats*, version 1.6.3 (<http://www.R-project.org/>). Epistatic interactions between SNPs were analysed by generalized multifactor dimensionality reduction (GMDR) software, version 0.7 (14, 22, 23), the details of which are provided under Data S1 in the Supporting Information. One-way ANOVA and Kruskal–Wallis test with post hoc tests were then used to compare, respectively, LogIgE and eos% among different GMDR-assigned risk groups.

Results

Subjects

Table 1 summarizes characteristics of our subjects. Controls (13.7 ± 4.5 years) were older than eczema (11.1 ± 4.3 years) and had fewer males. Data for total IgE and eos% were available in 978 and 847 eczema patients and 699 and 654 controls, respectively, and 1152 (91.3%) eczema patients were atopic.

Single SNP and haplotype analyses

Table S2 shows the 23 SNPs that were successfully genotyped in over 99% of samples. All of them were in HWE. Three

SNPs, namely rs11819875 (odds ratio [OR] 1.18, 95% confidence interval [CI] 1.03–1.36; $P = 0.014$) of *CYP2R1* and rs222020 (OR 1.27, 95% CI 1.00–1.60; $P = 0.047$) and rs2298849 (OR 1.37, 95% CI 1.08–1.72; $P = 0.008$) of *GC* showed a trend for eczema diagnosis (Table S3). Two *VDR* SNPs rs7975232 (OR 0.81, 95% CI 0.68–0.96; $P = 0.017$) and rs2239185 (OR 0.82, 95% CI 0.69–0.98; $P = 0.025$) were protective against eczema, but none of them survived Bonferroni correction. Rs4674343 of *CYP27A1* was protective against atopic eczema (OR 0.66, 95% CI 0.53–0.83; $P = 0.0004$). Besides, two SNPs of *CYP2R1* (rs2060793 and rs1553006) were associated with increased eos% among all subjects (Table 2). Among eczema patients, subjects homozygous for the minor A allele of rs2060793 had higher eos% than those with other genotypes (median: 8% vs 3%, $P = 0.002$). Similarly, those homozygous for A allele of rs1553006 had higher eos% than other subjects (median: 8% vs 6%, $P = 0.008$). None of the SNPs was associated with LogIgE (data not shown).

Figure S1 illustrates LD plots of SNPs from five target genes. *CYP2R1* and *GC* formed three and two haplotypes, respectively, and each of *CYP27A1* and *VDR* constructed one haplotype, but no haplotype was found for *CYP27B1*. Table 3 summarizes the haplotypic associations. Two *CYP2R1* haplotypes increased eczema risk, with GG of rs10766197_rs1553006 having OR 1.25 (95% CI 1.07–1.47) for eczema when compared with AG haplotype ($P = 0.006$). Eczema was also weakly associated with the other *CYP2R1* haplotype, AG of rs16930609_rs11819875 ($P = 0.043$), whereas AT haplotype of *VDR* protected against eczema ($P = 0.047$).

Gene–gene interactions for eczema traits

Table 4 shows the results of GMDR analysis for LogIgE. The model between *GC* rs7041 and *CYP2R1* rs7935792 yielded borderline significant results with testing accuracy 0.54, cross-validation consistency 10/10, and P -value 0.047 (see the Supporting Information), suggesting epistatic interaction between these SNPs for LogIgE. Figure 1A shows the predicted risk of all genotype combinations, whereas Fig. 1B illustrates box plots for the distribution of LogIgE according to GMDR-assigned risk groups among patients and controls. High-risk eczema patients had higher LogIgE than low-risk patients (2.76 ± 0.76 vs 2.60 ± 0.80 , $P = 0.002$), whereas LogIgE did not differ in relation to risk assignment among controls (1.89 ± 0.67 vs 1.83 ± 0.70 , $P = 0.609$). No significant SNP–SNP interaction was found for eczema diagnosis and eos%.

Discussion

This case–control association study investigated the relationship between eczema phenotypes and five candidate genes along the vitamin D pathway in southern Chinese children. A novel genetic variant rs4674343 of *CYP27A1* was found to lower the risk of atopic eczema. Two *CYP2R1* SNPs, rs2060793 and rs1553006, were associated with increased eos%. Two *CYP2R1* haplotypes were associated with eczema

Table 1 Population characteristics of eczema patients and controls

Parameter*	Eczema	Controls	<i>P</i>
<i>n</i>	1442	1231	
Age (year)†	11.1 ± 4.3	13.7 ± 4.5	<0.001
Male	52.2%	44.0%	<0.001
<i>n</i>	978	699	
LogIgE	2.67 ± 0.78	1.86 ± 0.69	<0.001
<i>n</i>	847	654	
Eosinophil percentage (%)	6 (3–10)	2 (1–3)	<0.001
Increased peripheral blood eosinophils‡	63.6%	15.1%	<0.001
<i>n</i>	1152	394	
Atopy§	91.3%	37.9%	<0.001

LogIgE, log-transformed plasma total IgE concentration.

*Results expressed as mean $\pm$ standard deviation, median (25–75% quartile), or percentage.

†Age ranges for cases and controls were 0.3–18.0 years and 0.4–20.9 years, respectively.

‡Defined as eosinophils $\geq 5\%$ of total leukocytes in peripheral blood.

§By either positive skin prick test or presence of allergen-specific IgE.

Table 2 Logistic regression analysis of the association between 23 SNPs and increased peripheral blood eosinophils ($n = 1501$)

		Additive model				Dominant model				Recessive model			
Gene	SNP	OR	95% CI		P*	OR	95% CI		P*	OR	95% CI		P*
CYP2R1	rs7935792	0.77	0.53	1.12	0.174	0.69	0.45	1.06	0.090	1.20	0.39	3.77	0.750
	rs12794714	0.96	0.72	1.29	0.794	0.91	0.60	1.37	0.635	1.04	0.59	1.85	0.885
	rs2060793	1.37	1.01	1.87	0.043	1.17	0.78	1.76	0.439	3.04	1.54	6.03	0.001
	rs16930609	0.96	0.63	1.47	0.848	1.09	0.68	1.75	0.720	0.22	0.05	1.05	0.058
	rs11819875	0.85	0.62	1.16	0.300	0.76	0.51	1.14	0.182	0.99	0.50	1.97	0.973
	rs16930625	1.08	0.69	1.69	0.737	1.22	0.75	1.98	0.436	0.25	0.04	1.50	0.129
	rs10766197	0.83	0.62	1.11	0.202	0.84	0.56	1.28	0.421	0.67	0.38	1.18	0.165
	rs1553006	1.45	1.08	1.95	0.015	1.26	0.84	1.91	0.268	2.69	1.49	4.84	0.001
CYP27A1	rs17470271	0.86	0.45	1.64	0.647	0.87	0.44	1.72	0.681	0.57	0.02	13.61	0.725
	rs4674343	0.83	0.55	1.26	0.388	0.87	0.55	1.36	0.529	0.35	0.06	2.01	0.240
	rs645163	1.26	0.95	1.67	0.117	1.24	0.80	1.92	0.327	1.52	0.92	2.51	0.102
	rs13406831	0.68	0.41	1.13	0.136	0.71	0.41	1.23	0.218	0.17	0.02	1.62	0.123
CYP27B1	rs1048691	1.13	0.75	1.68	0.565	1.16	0.73	1.82	0.535	1.07	0.29	3.99	0.917
	rs4646538	1.16	0.58	2.30	0.682	1.05	0.51	2.16	0.890	22.91	0.47	1125.7	0.115
	rs4646536	1.19	0.91	1.56	0.210	0.12	0.74	1.70	0.599	1.54	0.93	2.56	0.092
GC	rs2282679	1.08	0.78	1.50	0.638	0.94	0.63	1.42	0.788	2.00	0.90	4.46	0.090
	rs4588	1.04	0.75	1.44	0.802	0.92	0.62	1.39	0.700	1.74	0.79	3.86	0.170
	rs7041	0.94	0.70	1.27	0.702	0.86	0.57	1.29	0.460	1.12	0.59	2.11	0.728
	rs222020	1.11	0.83	1.49	0.480	1.01	0.65	1.55	0.978	1.40	0.82	2.39	0.212
	rs1155563	0.96	0.71	1.31	0.816	0.83	0.56	1.25	0.377	1.39	0.71	2.71	0.341
	rs2298849	1.14	0.86	1.53	0.364	1.10	0.72	1.68	0.672	1.36	0.80	2.32	0.254
VDR	rs7975232	1.15	0.85	1.56	0.373	1.27	0.85	1.91	0.239	1.00	0.51	1.97	1.000
	rs2239185	1.10	0.81	1.50	0.531	1.18	0.79	1.77	0.415	1.00	0.50	2.02	0.998

CI, confidence interval; OR, odds ratio, SNP, single nucleotide polymorphism.

SNPs with *P*-value <0.05 are highlighted in bold.

*Adjusted for age, gender, and eczema status as covariates; level of significance at 0.0022 after Bonferroni correction.

Table 3 Summary of haplotypic associations with eczema diagnosis

Gene	Haplotype block	Haplotype	Frequency in eczema	Frequency in controls	OR	95% CI		P*
CYP2R1	rs7935792_rs12794714	AG	0.450	0.452	Ref.			
		AA	0.364	0.377	0.94	0.82	1.08	0.388
		CG	0.185	0.171	1.12	0.95	1.34	0.186
	rs16930609_rs11819875	AT	0.688	0.708	Ref.			
		AG	0.181	0.167	1.19	1.01	1.40	0.043
		CG	0.129	0.123	1.18	0.98	1.42	0.087
	rs10766197_rs1553006	AG	0.374	0.378	Ref.			
		GA	0.364	0.390	1.00	0.87	1.16	0.963
		GG	0.262	0.232	1.25	1.07	1.47	0.006
CYP27A1	rs645163_rs13406831	CT	0.487	0.493	Ref.			
		TT	0.429	0.421	1.00	0.88	1.13	0.957
		CC	0.083	0.084	0.98	0.77	1.24	0.838
GC	rs2282679_rs4588_rs7041	ACT	0.426	0.395	Ref.			
		ACG	0.312	0.327	0.95	0.82	1.09	0.441
		CAT	0.255	0.272	0.89	0.77	1.04	0.156
	rs222020_rs1155563	CT	0.433	0.412	Ref.			
		TC	0.321	0.342	0.91	0.78	1.05	0.187
VDR	rs7975232_rs2239185	TT	0.246	0.246	0.93	0.79	1.09	0.364
		CC	0.704	0.685	Ref.			
		AT	0.282	0.300	0.87	0.76	1.00	0.047
		AC	0.013	0.013	1.02	0.60	1.72	0.956

*Adjusted for age, gender, and asthma diagnosis as covariates.

Haplotypes with statistical significance are highlighted in bold.

Table 4 Gene–gene interaction analysis for LogIgE by generalized multifactor dimensionality reduction method

Model	TA	CVC	P*
rs2282679	0.50	9/10	0.186
rs7041_rs7935792	0.54	10/10	0.047
rs2298849_rs7041_rs7935792	0.52	4/10	0.002
rs4646536_rs7041_rs7935792_rs7975232	0.53	3/10	0.043

CVC, cross-validation consistency; TA, testing accuracy.

*Adjusted for age, gender, and asthma status as covariates and obtained from 5000 permutations.

susceptibility, whereas one *VDR* haplotype might protect against eczema. The two involved *VDR* SNPs were reported to increase asthma risk in previous studies (8, 9, 24). Two SNPs of *GC* and *CYP2R1* might interact to modulate total IgE levels among eczema patients.

Four human cytochrome P450s were able to convert vitamin D to its circulating form, but *CYP2R1* and *CYP27A1* are the most important ones (25). Being the only mitochondrial enzyme exhibiting 25-hydroxylase activity, *CYP27A1* was also studied for its 27-hydroxylation of sterol intermediates in bile acid synthesis. *CYP27A1* SNPs have not been reported to be associated with eczema, asthma, or atopy. Our study was thus the first to suggest possible protective effect of *CYP27A1* rs4674343 for atopic eczema (OR 0.66; Table S4). *CYP2R1* was identified as a key microsomal 25-hydroxylase for vitamin D in 2003 (26). Discordant association between *CYP2R1* and asthma was found between Caucasian and Chinese populations. *CYP2R1* SNPs had modest association with asthma (27) and total IgE (24) in Caucasian studies, but *CYP2R1* was not associated with asthma in Chinese (12). Our study first reported two SNPs (rs2060793 and rs1553006) of this gene to be associated with eos% among eczema children. Rs1553006 also formed a haplotype with rs107661697 to increase eczema risk. Another *CYP2R1* haplotype from rs16930609 and rs11819875 also conferred eczema susceptibility. *CYP2R1* functions to produce stable vitamin D, and this enzyme showed inverse correlation with eczema severity (6).

GC was best known by its two functional SNPs rs4588 and rs7041 (28). These SNPs expressed three different *GC* globulin isoforms, GC1-F, GC1-S, and GC2 (29), which varied at positions 416 and 420 to alter the affinity (GC1F > GC1S > GC2) in carrying vitamin D metabolites to target cells (30). GC2 genotype was associated with less chronic obstructive pulmonary disease in White people, whereas GC1F increased this disease among Asians (31). Another SNP rs2282679 was found in GWAS to be associated with vitamin D level (32) and insufficiency (33) in Europeans. The latest GWAS in asthmatic children of Hispanic ancestry showed that *GC* affected circulating vitamin D levels (10). Our study could not detect any major effect of *GC* on eczema phenotypes, with the exception that rs222020 and rs2298849 were marginally associated with increased risk of atopic eczema and *GC* interacted with *CYP2R1* to modulate total IgE among eczema patients.

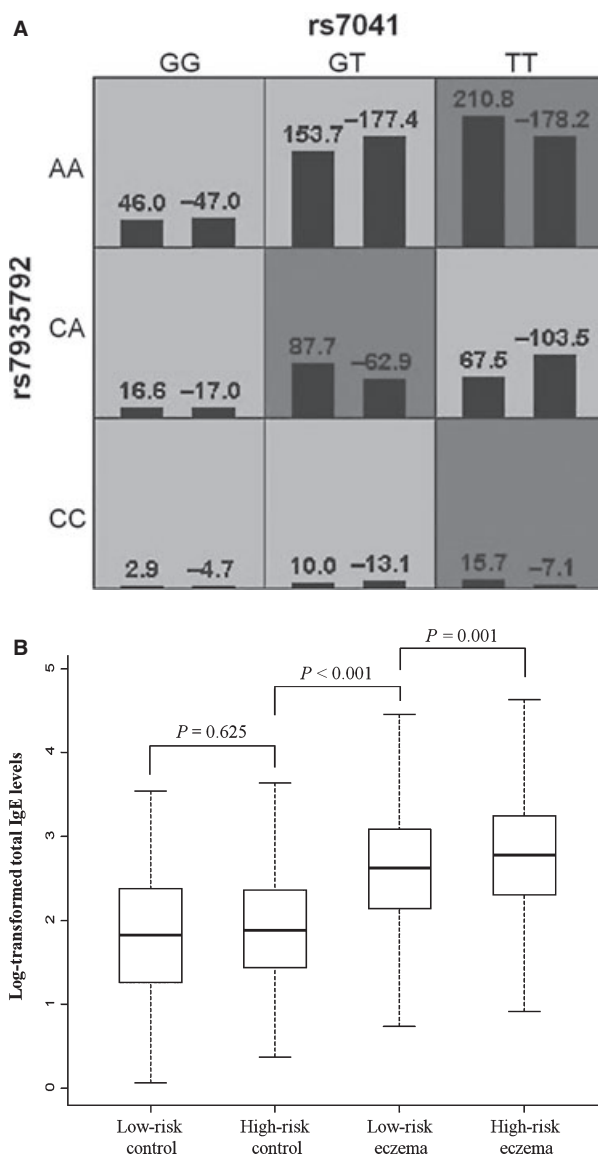


Figure 1 Generalized multifactor dimensionality reduction (GMDR) analysis for LogIgE among our eczema patients and controls. (A) Risk groups of the best two-locus model for this subphenotype, with the numbers in brackets being subject counts of specific groups. Light gray squares are low-risk groups, whereas dark gray squares are high-risk groups. Left bar inside each square denotes the score of the control group processing respective genotypes, whereas right bar denotes eczema group. (B) The distribution of LogIgE among different GMDR-assigned risk groups. LogIgE level was significantly different between high- and low-risk eczema groups, but such difference was not found between control groups.

*VDR*s rs7975232 and rs2239185 were the first variants along the vitamin D pathway reported to be associated with asthma. The former was also replicated in a Chinese case–control study (11). The effect of *VDR* on eczema differed from its role for asthma, and the two SNPs showed modest protective effect.

The AT haplotype was less prevalent in eczema patients (0.282) than controls (0.3). This discrepancy could be explained by interethnic difference between Asians and Caucasians of European ancestry, and AT was the major haplotype in the latter population (NCBI dbSNP at <http://www.ncbi.nlm.nih.gov/projects/snp/>). Our results found rs7975232C and rs2239185C to be risk alleles for allergies. Future studies should delineate the mechanisms whereby these intronic SNPs altered vitamin D binding or receptor function.

A number of studies suggested that targeting vitamin D metabolism and signaling might be an effective treatment for eczema. $1\alpha,25(\text{OH})_2\text{D}$ increased *in vitro* cathelicidin expression and antimicrobial activity in keratinocytes (34). Serum $25(\text{OH})\text{D}$ levels were significantly higher in children with mild eczema than those with moderate or severe eczema (6). Several small clinical trials reported daily vitamin D supplementation of 1000–6000 IU to be beneficial in eczema (35–37). Larger randomized controlled trials are needed to confirm the therapeutic benefit of vitamin D on eczema.

The production of active vitamin D requires the concerted actions of many genes. Apart from our five selected genes, *CYP24A1* is the other vitamin D pathway gene that inactivates vitamin D by 24-hydroxylation of the active form. We did not select this gene because it is not involved in vitamin D activation. Two Caucasian studies analysed possible associations between asthma and many genes encoding vitamin D metabolism and signaling (24, 27), which did not detect consistent association for *CYP24A1*.

This study has several limitations. Most patients were recruited from community studies that did not include the assessment of eczema severity. Only 418 cases had data for severity of their eczema. This study did not have sufficient power to analyse any effect of studied SNPs on eczema severity. Another drawback relates to the lack of vitamin D measurements in our subjects, which could serve as positive control to evaluate whether our studied SNPs alter serum vitamin D levels.

In conclusion, this case-control study investigating genetic association between childhood eczema and vitamin D pathway genes found rs4674343 in *CYP27A1* to be associated with atopic eczema in southern Chinese. Peripheral blood eosinophil percentage was modulated by rs2060793 and rs1553006 of *CYP2R1* as well as their haplotypes. *CYP2R1* and *VDR* haplotypes altered eczema susceptibility, and *GC* rs7041 and *CYP2R1* rs7935792 interacted to modulate total IgE among eczema patients. Replication of our findings in other ethnic populations is needed to confirm these vitamin D pathway genes as major eczema candidates.

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Author contributions

Dr Ting F. Leung developed the idea, designed research protocol, and obtained funding for the study; Ms Susan S. Wang assessed subjects, collected study data, and performed genotyping experiments; Drs Kam L. Hon, Alice P. S. Kong, and Juliana C. N. Chan recruited and managed study participants; Mr Man F. Tang and Dr Hing Y. Sy collected study data and assisted genotyping experiments; Ms Susan S. Wang and Drs Hing Y. Sy and Ting F. Leung analysed data and interpreted study findings; Dr Ting F. Leung wrote the original draft of this manuscript; and all authors approved the final version for submission.

Conflicts of interest

The authors state no conflicts of interest.

Supporting Information

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

Data S1. Patients and methods (genotyping method and GMDR analysis).

Table S1. Selection criteria, function prediction, and genotyping method of 24 target SNPs of five genes along the vitamin D pathway.

Table S2. Genotyping efficiency of 23 SNPs and their respective Hardy–Weinberg equilibrium (HWE) among the controls.

Table S3. Logistic regression analysis of the association between 23 SNPs and eczema diagnosis.

Table S4. Case-control comparisons of minor allele frequencies of the studied SNPs.

Figure S1. Haplotype blocks of the five selected genes.

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SUPPLEMENTARY INFORMATION

Eczema phenotypes are associated with multiple vitamin D pathway genes in Chinese children

S. S. Wang¹, K. L. Hon¹, A. P. S. Kong², M. F. Tang¹, H. Y. Sy¹, J. C. N. Chan² & T. F. Leung¹

Departments of ¹Pediatrics and ²Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

Patients and methods

SNP genotyping

Genomic DNA from peripheral blood leukocytes was extracted by QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). Rs6716642 of *CYP27A1* could not be genotyped due to failed probe design by both iPLEX and TaqMan assays (Table S1). Thirteen SNPs were genotyped by multiplex PCR using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MassARRAY[®] system; Sequenom, San Diego, CA) (22). All multiplex primers were designed by Human GenoTyping Tools (Sequenom), based on which single bases were extended using iPLEX[®] Gold assay. PCR condition was 94°C for 2 min and 45 cycles of 94°C for 30 sec then 56°C for 30 sec followed by 72°C for 1 min. The mass spectrum and genotyping data were analyzed by MassARRAY Typer 3.4 software (Sequenom). Ten SNPs were genotyped by TaqMan[®] SNP Genotyping assays (Applied Biosystems, Foster City, CA) using ABI-7900HT thermocycler. The thermal cycles were 95°C for 10 min followed by 40 repeats of 95°C for 15 sec and 60°C for 1 min. Both genotyping methods were performed on 384-well plates containing 380 unknown samples and four no-template controls.

Analysis for epistatic interactions

Interactions among SNPs of our five studied vitamin D pathway genes were analyzed by generalized multifactor dimensionality reduction (GMDR) software version 0.7, which permits adjustment for discrete and quantitative covariates and is applicable to both continuous and dichotomous phenotypes. Briefly, GMDR computes the maximum-likelihood estimates and the score values of all individuals under the null hypothesis. The cumulative score value is calculated within each multifactor cell, which will be classified either as high-risk if the average score meets or exceeds a pre-assigned threshold of 0 or as low-risk if the

score is less than 0. An exhaustive search was performed to test for all possible one- to five-locus interactions using 10-fold cross validation. In evaluation of the models, a number of outcome parameters including testing accuracy (TA), cross-validation consistency (CVC) and statistical significance of the model are considered. TA measuring how accurate individuals are classified with respect to their case/control status is used to pick the best model. A TA score of 0.5 indicates that the model predicts no better than chance whereas 1.0 indicates perfect prediction. In general, a TA score ≥ 0.55 for a model is considered satisfactory. CVC evaluates the consistency with which the selected interaction is classified as the best model among all possible combinations. The statistical significance of a model is determined by comparing average prediction error from the observed data with the distribution of average prediction errors under the null hypothesis of no association derived empirically from 5000 permutations. The null hypothesis was rejected when the permuted P value was 0.05 or lower.

Table S1 Selection criteria, function prediction and genotyping method of 24 target SNPs of five genes along vitamin D pathway

Gene	Locus	SNP	GRCh37.p5*	Selection criteria [†]	SNP type	Genotyping Method
<i>CYP2R1</i>	11p15.2	rs10766197	14921880	Associated with [25(OH)D ₃]	Intergenic	TaqMan assay
		rs2060793	14915310	Associated with [25(OH)D ₃]	5'UTR, predicted TFBS	TaqMan assay
		rs12794714	14913575	Associated with [25(OH)D ₃]	Exon, Silent S [Ser]	Sequenom multiplex
		rs7935792	14909461	± 10kb tagging SNPs	Intron	Sequenom multiplex
		rs1553006	14922950	± 10kb tagging SNPs	Intergenic	TaqMan assay
		rs16930625	14918308	± 10kb tagging SNPs	5'UTR, predicted TFBS	TaqMan assay
		rs11819875	14917297	± 10kb tagging SNPs	5'UTR, predicted TFBS	Sequenom multiplex
		rs16930609	14915908	± 10kb tagging SNPs	5'UTR, predicted TFBS	Sequenom multiplex
		rs4674343	219660346	± 5kb tagging SNPs	Intron	Sequenom multiplex
		rs17470271	219651995	± 5kb tagging SNPs	Intron	TaqMan assay
<i>CYP27A1</i>	2q35	rs6716642	219651819	± 5kb tagging SNPs	Intron	Failed primer design
		rs13406831	219684024	± 5kb tagging SNPs	Intergenic	TaqMan assay
		rs645163	219682257	± 5kb tagging SNPs	Intergenic	TaqMan assay
		rs1048691	58152948	± 5kb tagging SNPs	Predicted miRNA	TaqMan assay
<i>CYP27B1</i>	12q13.1-13.3	rs4646538	58155038	± 5kb tagging SNPs	Intergenic	TaqMan assay
		rs4646536	58157988	± 5kb tagging SNPs	Intron	TaqMan assay
<i>GC</i>	4q13.3	rs2282679	72608383	Associated with [25(OH)D ₃]	Intron	Sequenom multiplex

		rs4588	72618323	Associated with asthma	Exon, Missense T [Thr] → K [Lys]	Sequenom multiplex
		rs7041	72618334	Associated with asthma	Exon, Missense D [Asp] → E [Glu]	Sequenom multiplex
		rs222020	72636272	Associated with [25(OH)D ₃]	Intron	Sequenom multiplex
		rs1155563	72643488	Associated with [25(OH)D ₃]	Intron	Sequenom multiplex
		rs2298849	72648851	Associated with [25(OH)D ₃]	Intron	Sequenom multiplex
VDR	12q13.1	rs7975232	48238837	Associated with asthma	Intron	Sequenom multiplex
		rs2239185	48244559	Associated with asthma	Intron	Sequenom multiplex

SNP, single-nucleotide polymorphism; TFBS, transcription factor binding site; UTR, untranslated region.

* Denotes publication number of respective SNP from HapMap database version 37.

† For *CYP27A1*, *CYP2R1* and *CYP27B1*, tagging strategy for selecting SNPs was targeted to within 5-kb for *CYP27A1* and *CYP27B1* and within 10-kb for *CYP2R1*. Larger flanking region (10-kb) was chosen for the smaller gene *CYP2R1* (14.2 kb) and smaller flanking region for the larger gene *CYP27A1* (32.9 kb). Five-kilobase flanking region was applied to *CYP27B1* (4.9 kb) because of its close proximity to *METTL1* in the upstream region and *MARCH9* in the downstream region.

Table S2 Genotyping efficiency of 23 SNPs and their respective Hardy-Weinberg equilibrium (HWE) among the controls

Gene	SNP	Major/minor allele	Genotyping efficiency	P-values for HWE among control
<i>CYP2R1</i>	rs7935792	A/C	99.97%	0.943
	rs12794714	G/A	99.71%	0.733
	rs2060793	G/A	99.68%	1.000
	rs16930609	A/C	100.0%	0.971
	rs11819875	T/G	99.97%	0.990
	rs16930625	A/G	99.90%	0.709
	rs10766197	G/A	99.71%	0.766
	rs1553006	G/A	99.61%	0.477
	rs17470271	A/T	99.42%	0.998
	rs4674343	A/G	100.0%	0.232
<i>CYP27A1</i>	rs645163	C/T	99.74%	0.925
	rs13406831	T/C	99.87%	0.925
	rs2282679	A/C	99.90%	0.834
<i>CYP27B1</i>	rs1048691	C/T	98.27%	0.806
	rs4646538	C/G	98.24%	0.752
	rs4646536	G/A	97.12%	0.174
	rs4588	C/A	99.90%	0.909
<i>GC</i>	rs7041	T/G	99.81%	0.365
	rs222020	T/C	99.87%	0.192
	rs1155563	T/C	99.84%	0.547
	rs2298849	T/C	99.94%	0.104
<i>VDR</i>	rs7975232	C/A	99.64%	1.000
	rs2239185	G/A	99.90%	0.885

Table S3 Logistic regression analysis[†] of the association between 23 SNPs and eczema diagnosis

Gene	SNP	Additive model				Dominant model				Recessive model			
		OR	95% CI		P	OR	95% CI		P	OR	95% CI		P
<i>CYP2R1</i>	rs7935792	1.15	0.98	1.36	0.083	1.16	0.96	1.40	0.117	1.33	0.82	2.16	0.243
	rs12794714	0.91	0.80	1.04	0.155	0.93	0.77	1.11	0.400	0.81	0.62	1.05	0.104
	rs2060793	0.91	0.80	1.04	0.171	0.93	0.78	1.11	0.407	0.78	0.58	1.05	0.103
	rs16930609	1.13	0.94	1.36	0.193	1.13	0.92	1.38	0.262	1.41	0.75	2.68	0.291
	rs11819875	1.18	1.03	1.36	0.014	1.21	1.02	1.45	0.033	1.32	0.98	1.79	0.068
	rs16930625	1.21	1.00	1.46	0.054	1.22	0.98	1.51	0.074	1.53	0.77	3.05	0.229
	rs10766197	0.91	0.80	1.04	0.155	0.91	0.76	1.09	0.329	0.83	0.64	1.07	0.149
	rs1553006	0.91	0.80	1.04	0.171	0.90	0.75	1.08	0.256	0.87	0.67	1.12	0.275
<i>CYP27A1</i>	rs17470271	0.96	0.72	1.27	0.755	0.93	0.69	1.25	0.630	1.85	0.40	8.63	0.434
	rs4674343	0.80	0.67	0.96	0.018	0.74	0.61	0.91	0.003	1.34	0.71	2.52	0.370
	rs645163	1.00	0.88	1.13	0.962	0.98	0.81	1.18	0.807	1.02	0.82	1.28	0.835
	rs13406831	0.95	0.76	1.19	0.671	0.96	0.75	1.22	0.720	0.79	0.26	2.36	0.673
	rs1048691	1.05	0.88	1.25	0.600	1.06	0.87	1.29	0.572	1.02	0.54	1.93	0.953
<i>CYP27B1</i>	rs4646538	0.94	0.70	1.27	0.694	0.95	0.69	1.30	0.733	0.69	0.11	4.29	0.693
	rs4646536	1.06	0.95	1.20	0.306	1.05	0.88	1.26	0.582	1.15	0.92	1.43	0.218
	rs2282679	0.91	0.79	1.05	0.212	0.93	0.78	1.11	0.418	0.77	0.55	1.09	0.144
	rs4588	0.92	0.80	1.06	0.255	0.93	0.78	1.12	0.449	0.80	0.56	1.12	0.195
<i>GC</i>	rs7041	0.98	0.86	1.12	0.782	1.03	0.87	1.23	0.729	0.85	0.64	1.13	0.258
	rs222020	1.09	0.96	1.24	0.182	1.04	0.86	1.25	0.714	1.27	1.00	1.60	0.047
	rs1155563	0.93	0.81	1.06	0.275	0.88	0.73	1.05	0.145	1.00	0.75	1.33	0.999
	rs2298849	1.05	0.93	1.19	0.445	0.91	0.76	1.10	0.326	1.37	1.08	1.72	0.008
<i>VDR</i>	rs7975232	0.88	0.77	1.01	0.063	0.81	0.68	0.96	0.017	0.98	0.73	1.32	0.903
	rs2239185	0.87	0.76	1.00	0.044	0.82	0.69	0.98	0.025	0.90	0.66	1.22	0.498

[†] Adjusted for age, sex and asthma status as covariates.

SNP with statistically significant results are highlighted in bold.

Table S4 Case-control comparisons of minor allele frequencies of the studied SNPs

Gene	SNP	CHS [*]	CHB [†]	Asian [‡]	Control	Eczema	<i>P</i>
<i>CYP2R1</i>	rs7935792	0.185	0.155	0.156	0.172	0.186	0.378
	rs12794714	0.335	0.345	0.364	0.378	0.364	0.566
	rs2060793	0.295	0.340	0.334	0.321	0.302	0.314
	rs16930609	0.185	0.160	0.147	0.125	0.131	0.767
	rs11819875	0.365	0.304	0.297	0.290	0.311	0.247
	rs16930625	0.155	0.129	0.140	0.108	0.119	0.362
	rs10766197	0.320	0.340	0.348	0.378	0.374	0.953
	rs1553006	0.365	0.387	0.379	0.390	0.364	0.121
<i>CYP27A1</i>	rs17470271	0.035	0.062	0.063	0.049	0.049	0.877
	rs4674343	0.195	0.160	0.185	0.156	0.134	0.027
	rs645163	0.395	0.407	0.364	0.422	0.430	0.840
	rs13406831	0.085	0.077	0.084	0.086	0.085	0.957
	rs2282679	0.315	0.294	0.283	0.274	0.257	0.395
<i>CYP27B1</i>	rs1048691	0.180	0.191	0.171	0.146	0.155	0.362
	rs4646538	0.055	0.057	0.047	0.045	0.044	0.799
	rs4646536	0.350	0.361	0.313	0.388	0.388	0.721
<i>GC</i>	rs4588	0.315	0.289	0.283	0.277	0.260	0.397
	rs7041	0.325	0.278	0.287	0.328	0.313	0.506
	rs222020	0.360	0.397	0.365	0.412	0.433	0.157
	rs1155563	0.400	0.371	0.388	0.342	0.320	0.076
	rs2298849	0.355	0.356	0.341	0.411	0.421	0.017
<i>VDR</i>	rs7975232	0.245	0.289	0.288	0.313	0.295	0.086
	rs2239185	0.230	0.289	0.280	0.301	0.283	0.203

* CHS, southern Chinese in Hunan Province from 1000 Genomes database, $n = 100$. [†] CHB, Chinese in Beijing from 1000 Genomes database, $n = 97$. [‡] Asian, the summation of Southern Chinese, Chinese in Beijing and Japanese in Tokyo from 1000 Genomes database, $n = 286$.

P-values less than 0.05 were highlighted in bold.

Figure S1 Haplotype blocks of the five selected genes

