APJAI
Asian Pacific Journal of
Allergy and Immunology

ORIGINAL ARTICLE

Genetic variations in Vitamin D Binding Protein (*VDBP*) impact vitamin D level and asthma susceptibility across the four ethnic populations

Miao-Hsi Hsieh,[1] Natalia Paramonova,[2] Brigita Gradauskiene (Sitkauskiene),[3] Ilva Trapina,[2] Ingrida Rumba-Rozenfelde,[4] Sonomjamts Munkhbayarlakh,[5] Samanta Plavina,[2] Nikolajs Sjakste,[2] Daina Bastyte,[3] Laura Tamasauskiene,[3] Ieva Stakaitiene,[6] Rasa Ugenskiene,[6] Pei-Chi Chen,[1,7] Jiu-Yao Wang,[1,8] Lawrence Shih-Hsin Wu[1,9]**

Abstract

Background: Asthma are associated with the vitamin D axis. Genetic variations of *VDBP*, notably rs7041 and rs4588, influence circulating vitamin D levels. However, data on their link to asthma are inconsistent, and ethnic differences remain unclear.

Objective: We explored how genetic variations in *VDBP* affect vitamin D levels and susceptibility to asthma across diverse ethnic populations.

Methods: In our cross-ethnic study, we analyzed vitamin D levels and *VDBP* polymorphisms (rs7041 and rs4588) in Taiwanese, Mongolian, Lithuanian, and Latvian populations. Our study included 363 asthmatic subjects and 481 non-asthma controls. We performed genotyping for rs7041 and rs4588 and assessed serum concentrations of 25-hydroxyvitamin D [25(OH)D], examining the associations between *VDBP* polymorphisms, vitamin D levels, and asthma.

Results: The study found significant differences in vitamin D levels among ethnic groups. Non-asthmatic individuals from Taiwan had higher concentrations, while asthma subjects in both Taiwanese and Lithuanian populations showed lower levels compared to their non-asthma counterparts (both p-value < 0.001). *VDBP* polymorphisms were associated with asthma in the Latvian population, with the rs7041 GG vs. GT+TT showing an odds ratio (OR) of 1.72 (95% confidence interval (CI): 1.10-2.69, p = 0.016) and the rs4588 CC vs. CA+AA showing an OR of 1.88 (95%CI: 1.24-2.84, p = 0.003). However, this association was not observed in other populations.

Conclusion: Our cross-ethnic study underscores the intricate relationship between *VDBP* genetic variations, vitamin D levels, and asthma vulnerability. The association of *VDBP* polymorphisms with asthma seems to differ among populations, emphasizing the importance of a nuanced comprehension of these connections.

Key words: vitamin D, vitamin D binding protein, genetic polymorphism, asthma, cross-ethnic comparison

Citation:
Hsieh, M. H., Paramonova, N., Gradauskiene (Sitkauskiene), B., Trapina, I., Rozenfelde, I. R., Munkhbayarlakh, S., Plavina, S., Sjakste, N., Bastyte, D., Tamasauskiene, L., Stakaitiene, I., Ugenskiene, R., Chen, P. C., Wang, J. Y., Wu, L. S. H. (0000). Genetic variations in Vitamin D Binding Protein (*VDBP*) impact vitamin D level and asthma susceptibility across the four ethnic populations. *Asian Pac J Allergy Immunol*, 00(0), 000-000. https://doi.org/10.12932/ap-240324-1825

Affiliations:
[1] Research Center of Allergy, Immunology, and Microbiome (A.I.M.), China Medical University Hospital, Taichung, Taiwan
[2] Laboratory of Genomics and Bioinformatics, Institute of Biology, University of Latvia, Riga, Latvia
[3] Department of Immunology and Allergology, Lithuanian University of Health Sciences, Kaunas, Lithuania
[4] Faculty of Medicine, University of Latvia, Riga, Latvia;
[5] Department of Pulmonology and Allergology, School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia
[6] Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania

Affiliations (Continued):
[7] Department of Nursing, National Tainan Junior College of Nursing, Tainan, Taiwan
[8] Department of Allergy and Immunology, China Medical University Children's Hospital, Taichung, Taiwan
[9] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan

Corresponding author:
1. Jiu-Yao Wang
E-mail: ??
2. Lawrence Shih-Hsin Wu
E-mail: ??

Abbreviations:

ERCHS	European Community Respiratory Health Survey
Gc	group-specific component
Gc1f	Gc1 fast
Gc1s	Gc1 slow
HWE	Hardy-Weinberg equilibrium
ISAAC	International Study of Asthma and Allergies in Childhood
PSMA3	Proteasome subunit, alpha-type, 3
PSMA6	Proteasome subunit, alpha-type, 6
PSMC6	Proteosome 26S subunit, ATPase, 6
PCR	polymerase chain reaction
SNP	single nucleotide polymorphism
VDBP	vitamin D binding protein

Introduction

Pulmonary disorders, such as bronchial asthma, have been intricately linked to the vitamin D axis, a complex interplay involving vitamin D, vitamin D binding protein (*VDBP*), and the vitamin D receptor.[1,2] *VDBP*, a serum protein, exhibits a high affinity for circulating vitamin D and possesses immunomodulatory functions crucial in various lung diseases, including chronic obstructive pulmonary disease, tuberculosis, and asthma.[3,4,5] Its role in functions such as macrophage activation and neutrophil chemotaxis makes *VDBP* a pivotal component of the Vitamin D axis.[3,6]

The GC (group-specific component) gene, located at 4q11-13, gives rise to *VDBP*, with prominent genetic variants, namely rs7041 and rs4588, exerting influence on approximately eighty percent of *VDBP* level variation.[7] These single nucleotide polymorphisms (SNPs) have been linked to circulating vitamin D levels or function[8,9] and display distinct allele frequencies across diverse ethnic groups.[10]

Explorations into *VDBP* polymorphisms and their association with bronchial asthma have been conducted in various populations. Noteworthy associations have been reported in adult and pediatric Chinese in patients with a significant association between *VDBP* polymorphism and bronchial asthma.[11,12] Conversely, in Egyptian children and adolescents, protective effects were observed for rs4588 CA and AA genotypes, while the rs7041 GG genotype exhibited an increased frequency in asthma patients.[13] The Kurdish population revealed a correlation between the rs7041 GG genotype and an elevated risk of asthma progression.[14] However, studies in Hong Kong Chinese children found no significant association between *VDBP* polymorphism and asthma.[15] These investigations often focused solely on *VDBP* gene polymorphisms without considering potential environmental or non-genetic influences.

The Baltic Sea region in Europe, Mongolia in Central Asia, and Taiwan in east Asia are situated at varying latitudes in the northern hemisphere, present diverse ethnic backgrounds and distinct geographical conditions, including variations in sunshine exposure, temperature fluctuations, and dietary practices. Earlier, a comparison was made regarding the prevalence of allergic asthma among children in the populations of Lithuania, Latvia, and Taiwan.[16] In a population genetic study, diversity in the *PSMA6* (rs2277460, rs1048990), *PSMC6* (rs2295826, rs2295827), and *PSMA3* (rs2348071) genes was observed among these three populations.[17] Subsequently, these genetic variants were recognized to be correlated with childhood asthma in both Latvian and Taiwanese populations.[18] Moreover, Vitamin D plasma concentration and vitamin D receptor genetic variants were found to confer risk of asthma in Taiwanese and Mongolian population.[19] Hence, conducting a comparative analysis, encompassing factors such as vitamin D serum levels and genetic variations in *VDBP* linked to heightened asthma susceptibility, assumes great significance. This research aims to contribute insights into the role of vitamin D in preventing or potentially treating bronchial asthma within distinct ethnic populations across diverse geographical locations.

Materials and Methods

Study Population and Clinical Evaluation

Informed consent, obtained through a comprehensive respiratory questionnaire modeled after the British Medical Society and aligned with the European Community Respiratory Health Survey (ERCHS)[20] and ISAAC,[21] was mandatory for all participants or their guardians.

Asthma diagnosis criteria comprised a history of wheezing, shortness of breath during or without concurrent respiratory infections, chronic coughing for over a month, and a bronchodilator test confirming a 15% increase in FEV_1. Non-asthma controls met stringent criteria ensuring the absence of asthma history and diagnosis. Additional evaluations included skin prick tests for allergen identification of sensitization to allergen, differential blood counts, total serum IgE levels, and IgE levels specific to house dust and mixed pollen, analyzed using the Unicap system (Pharmacia, Diagnostic, Sweden) or immunoblot method (Euroline, EUROIMMUN, Germany). A positive skin prick test was defined as the presence of ≥ 1 reaction with a wheal diameter ≥ 3 mm. Total serum IgE levels were measured using solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics). In the Lithuanian study subjects' measurements of total IgE level in serum were performed by the enzyme-linked immunosorbent assay (ELISA) commercial kit (IBL International, Hamburg, Germany). Limit of detection was 0.8 IU/ml. Non-allergic subjects were identified by a total serum IgE < 100 IU and negative skin prick test results.

All Taiwanese subjects were Han-Taiwanese residing in Taiwan, while study subjects of Mongolia, Latvia and Lithuania were all enrolled from an outpatient department in their own countries, including asthmatic children and adults, with clinical assessments mirroring the Taiwanese study group.

Ethics approval and consent to participate

Approval for this study was obtained from the Ethical and Clinical Trial Committee of National Cheng-Kung University Hospital in Tainan, Taiwan, and from the School of Medicine at Mongolian National University of Medical Sciences in Ulaanbaatar, Mongolia (IRB No: A-BR-106-021; collaborative project). The research conducted in Latvian and Lithuanian populations adhered to the principles of the Declaration of Helsinki, with approval the study protocol by the Central Medical Ethics Committee of Latvia (IRB No: 01-29.1.2/4798) and Lithuania (IRB No: BE-2-74), respectively.

Measurement of 25-hydroxyvitamin D [25(OH)D] Concentration

For participants originating from Taiwan and Mongolia, the serum concentration of 25(OH)D was evaluated using a sandwich enzyme immunoassay (EIA) employing a commercial kit (catalog number AC-57SF1; Immunodiagnostic Systems, Fountain Hills, AZ, USA). In the Lithuanian research cohort, 25(OH)D serum levels were determined via ELISA using a commercial kit (BioVendor, Brno, Czech Republic) with a detection limit set at 2.81 ng/ml. In a Latvian study group encompassing both patients and healthy individuals, serum 25(OH)D levels were assessed utilizing an enzyme-linked immunosorbent assay (25-OH-vitamin D ELISA, IBL International GmbH, TECAN).

DNA Preparation

Genomic DNA extraction from nucleated blood samples of all study subjects from participating countries using the QIAamp DNA blood kit (QIAGEN, Valencia, CA, USA/Hilden, Germany) or similar kit for genomic DNA extraction (Thermo Fisher Scientific) following the manufacturer's instructions. Extracted genomic DNA underwent agarose gel electrophoresis, spectrophotometry quantification, and subsequent storage at −80°C until use.

SNP Genotyping

In the Lithuanian group the selected genetic polymorphisms were analyzed using predesigned and readily available TaqMan SNP Genotyping Assays probes (Thermo Fisher Scientific, CA, USA) using a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, MA, USA). The standard genotyping PCR program was used: 95°C for 10 min (polymerase activation), 95°C for 15 s (denaturation), and 60°C for 1 min (annealing and extension). The genotyping was following the manufacturer's instructions.

In Latvian study group the SNP rs4588 C>A (chr4:71752606) and rs7041 G>T (chr4:71752617) were genotyped by restriction fragment length polymorphism (RFLP) analysis. Since these polymorphisms are 11 base pairs apart, a single primer pair was used to amplify the PCR product. Oligonucleotide primers were designed using an online Primer-BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/): forward, 5′ GCCTGTGTTCACAGACTC TTTTG 3′, and reverse, 5′ GGACTTCCAATTCAGCAGCGA 3′. The PCR products (634 bp) were digested in a total volume of 10 µl using StyI and HaeIII (5 U/µl, Thermo Scientific, USA) restriction enzymes, respectively. The quality and quantity of DNA were determined using agarose gel electrophoresis and spectrophotometry. For quality control, 16 randomly selected samples were genotyped in duplicate in different experiments with a genotyping concordance of 100%. Genotyping data were also verified by direct sequencing of the corresponding DNA fragments in both directions. An Applied Biosystems 3130 ×l genetic analyzer was used for this purpose.

In Taiwan and Mongolian populations, selected SNPs underwent genotyping utilizing the high-throughput 384-microtiter plate MassARRAY™ System, SEQUENOM®. The genotyping process involved amplification of DNA containing the SNP site, followed by the homogenous MassEXTEND™ (hME) assay. This assay utilized label-free primer extension chemistry to generate allele-specific diagnostic products, each with a unique molecular weight distinguishable through matrix-assisted laser desorption ionization time-of-flight mass spectrometry. SNPs with a call rate below 90% were excluded from statistical analysis.

Statistical Analysis

The quality of genotype data underwent assessment through tests for Hardy-Weinberg equilibrium (HWE) proportions and Mendelian inheritance consistency. The χ^2 test identified associations between allergic asthma and each SNP. Logistic regression analyses were conducted to detect associations between allergic asthma status and the interaction of *VDBP* SNP and serum vitamin D concentration in case–control samples. In the logistic regression models, the SNP genotype was coded as 0 and 1, representing whether the risk genotype was carried or not by the individual.

Results

In total, DNA samples were gathered from 178 individuals of Taiwanese origin, 90 from Mongolia, 175 from Lithuania, and 401 from Latvia. Among these, 51 individuals with bronchial asthma were studied in the Taiwanese group, 65 in the Mongolian group, 98 in the Lithuanian group, and 149 in the Latvian group. The demographic characteristics of enrolled samples were listed in **Table 1**.

SNPs rs7041 and rs4588 of the *VDBP* gene were conducted genotyping to enrolled samples. The genotype distribution and comparison between asthma and non-asthma subjects were listed in **Table 2**. In the 2 × 3 contingency table analysis by χ^2 test, only rs4588 showed significance (p = 0.006) in Latvian group.

We conducted additional analysis on the genotype data using a dominant/recessive model. Within the Lithuanian population, the comparison of rs4588 AA vs. CA+CC through a 2 × 2 χ^2 test resulted in a p-value of 0.045. After adjusting for sex and age through logistic regression, the p-value remained significant at 0.044, with an odds ratio (OR) of 3.50 (95% confidence interval: 1.03, 11.84). Although a nominal significance was detected, the findings did not achieve statistical significance following multiple comparison correction (Bonferroni correction, with a significance level of $p < 0.025$, adjusted from $p < 0.05/2$).

Table 1. Demographic characteristics of study subjects.

Population	N (%)/Mean (SD)		*p*
	Asthma	Non-Asthma	
Asian			
Taiwanese (n = 178)	51	127	
Sex (male)	36 (71%)	15 (12%)	< 0.001
Age	7.76 (4.087)	28.16 (9.196)	< 0.001
Mongolian (n = 90)	65	25	
Sex (male)	39 (60%)	14 (56%)	0.730
Age	12.00 (10.434)	27.75 (13.159)	< 0.001
Caucasian			
Lithuanian (n = 175)	98	77	
Sex (male)	32 (33%)	28 (36%)	0.072
Age	42.68 (13.557)	35.08 (12.414)	< 0.001
Latvian (n = 401)	149	252	
Sex (male)	95 (64.181)	104 (41.012)	< 0.001
Age	11.72 (9.77)	48.52 (9.07)	< 0.001

Table 2. Genotyping Distribution of rs7041/rs4588, Vitamin D Levels, and Their Association with Asthma in a Multicultural Study Cohort

	Taiwanese		Mongolia		Lithuania		Latvia	
	AS	NAS	AS	NAS	AS	NAS	AS	NAS
rs7041* (Asp416Glu)								
GG	24 (47%)	60 (48%)	4 (6%)	1 (4%)	17 (17%)	9 (11%)	72 (48%)	97 (39%)
GT	23 (45%)	47 (38%)	33 (51%)	17 (68%)	43 (47%)	43 (57%)	63 (42%)	119 (47%)
TT	4 (8%)	17 (14%)	28 (43%)	7 (28%)	31 (34%)	24 (32%)	14 (9%)	36 (14%)
P value		0.468		0.338		0.364		0.053
rs4588* (Thr420Lys)								
CC	29 (57%)	72 (58%)	32 (49%)	17 (68%)	46 (49%)	39 (52%)	47 (32%)	58 (23%)
CA	19 (37%)	47 (38%)	31 (63%)	8 (32%)	34 (36%)	32 (43%)	73 (49%)	124 (50%)
AA	3 (6%)	5 (4%)	2 (3%)	0	14 (15%)	4 (5%)	28 (19%)	66 (27%)
P value		0.868		0.227		0.128		0.006
Vitamin D**	26.44 (13.22)	69.05 (29.33)	14.96 (5.91)	-	17.29 (6.87)	24.24 (7.19)	26.69 (10.74)	24.68 (7.68)
P value		< 0.001				< 0.001		0.26

AS: asthma; NAS: Non-asthma; *The genotype was presented by count (%); **The vitamin D concentration is presented as the mean (SD) of vitamin D concentration.

Table 3. The association of genetic variants of *VDBP* and Vitamin D concentration.

	Taiwan mean (SD)	Lithuania mean (SD)	Latvia mean (SD)
rs7041 (Asp416Glu)			
GG	51.85 (28.93)	18.57 (6.36)	29.22 (11.57)
GT	58.59 (34.96)	21.74 (7.66)	25.76 (9.49)
TT	70.71 (32.90)	19.87 (8.55)	24.54 (8.63)
P value (ANOVA)	0.047	0.132	0.153
rs4588 (Thr420Lys)			
CC	56.22 (33.35)	20.79 (8.40)	26.91 (10.97)
CA	58.43 (31.94)	20.47 (7.54)	24.83 (8.51)
AA (GC2/GC2)	50.78 (23.31)	20.18 (7.81)	27.68 (10.51)
P value (ANOVA)	0.790	0.943	0.409

In the Latvian population, the comparison of rs7041 GG vs. GT+TT yielded a *p*-value of 0.016, with an OR of 1.72 (95% confidence interval: 1.10-2.69). For rs4588 CC vs. CA+AA, the *p*-value was 0.003, and the corresponding odds ratio was 1.88 (95% confidence interval: 1.24-2.84). No significant results were found in Taiwanese and Mongolian groups.

The comparison of vitamin D blood levels between asthma and non-asthma subjects revealed that in Taiwanese and Lithuanian populations, asthma subjects exhibited lower vitamin D levels than their non-asthma counterparts (**Table 2**). However, this trend was not observed in the Latvian population. In the Mongolian population vitamin D levels were determined only in asthma patients.

The relationship between blood vitamin D levels and the genotypes of rs7041 and rs4588 was assessed using Analysis of Variance (ANOVA). A statistically significant association was observed solely in the Taiwanese population for the rs7041 genotype (**Table 3**). Subsequent post hoc analysis revealed that the GG genotype group was significantly associated with the lowest vitamin D levels ($p = 0.017$).

Discussion

Our investigation into the relationship between *VDBP* genetic variants, vitamin D levels, and bronchial asthma across diverse ethnic populations yielded several noteworthy findings. Notably, we observed significant differences in vitamin D levels among ethnic groups, with non-asthmatic individuals in Taiwan displaying notably higher vitamin D concentrations than counterparts in Mongolia, Lithuania, and Latvia. Surprisingly, *VDBP* polymorphisms were associated with asthma in the Latvian populations but not in the Lithuanian, Taiwanese and Mongolian populations.

The association between *VDBP* polymorphisms and asthma has been explored in various populations[11-14,22-25] yielding conflicting results in previous studies.[26] Our investigation extends this inquiry to diverse ethnic groups, including Taiwanese, Mongolian, Lithuanian, and Latvian individuals. The observed differences in vitamin D levels and *VDBP* polymorphisms underscore the need for a cross-ethnic perspective in understanding the role of these genetic variants in asthma pathogenesis. Previous studies have found that community-dwelling black Americans, compared to whites, exhibit lower levels of total 25-hydroxyvitamin D and vitamin D-binding protein, leading to similar concentrations of estimated bioavailable 25-hydroxyvitamin D.[7] This illustrates differences in vitamin D levels across ethnicities. One important finding is the significant disparity in vitamin D levels among the ethnic groups, particularly in Taiwan, where non-asthmatic individuals exhibited significantly higher average vitamin D concentrations compared to the other populations. This observation aligns with the existing literature on the influence of geographical and environmental factors on vitamin D synthesis, emphasizing the importance of considering regional variations in vitamin D exposure. The differences in vitamin D levels observed in various study groups may be attributed to environmental, geographical and genetic factors. Geographical locations significantly influence the amount of sunlight exposure individuals receive. Consequently, individuals residing in different regions may exhibit distinct Vitamin D levels due to variations in sunlight availability or dietary habits.[7] The dietary habits in Latvia, Lithuania and Taiwan or Mongolia differ significantly due to cultural and geographic factors. These distinctions can influence the intake of vitamin D through food sources, which, in turn, may impact the vitamin D levels in the respective populations. Moreover, there is a possibility that the *VDBP* gene interacts with other genes in the Vitamin D metabolic pathway and may influence the impact vitamin D level.

Contrary to our expectations, *VDBP* polymorphisms did not show a consistent association with asthma across all populations. While associations were identified only in Latvia, no significant correlation was found in Lithuania, Taiwanese and Mongolian populations. This discrepancy underscores the complexity of the relationship between genetic factors and asthma susceptibility and suggests that the impact of *VDBP* polymorphisms on asthma may be modulated by vitamin D level and population-specific factors.

It is crucial to note that the *VDBP* variants studied (Gc1F, Gc1S, and Gc2) exhibit distinct functional characteristics. Gc2 corresponds to the rs4588 C allele, while Gc1 corresponds to the A allele. Gc1 can further be differentiated into Gc1F and Gc1S based on the rs7041 allele. The Gc1F and Gc1S variants reportedly have a higher affinity for 25(OH)D than the Gc2 variant.[28] This distinction suggests a potential mechanism wherein the Gc1F and Gc1S variants may lead to a more efficient delivery of 25(OH)D to target tissues. Conversely, the Gc2 variant is associated with decreased circulating concentrations of 25(OH)D, 1,25(OH)2D, and DBP.[29,30] These functional nuances contribute to the complexity of the relationship between *VDBP* genetic variants and asthma susceptibility. The impact of *VDBP* genetic polymorphism on asthma susceptibility appears to be intricately linked to vitamin D levels. These findings emphasize the importance of considering both genetic and environmental factors in unraveling the complexities of asthma etiology.

The strengths of our study lie in its cross-ethnic design and inclusion of diverse populations with distinct genetic backgrounds and environmental exposures. However, certain limitations should be acknowledged, such as the relatively small sample size in some ethnic groups and the potential influence of unexplored environmental factors. The collected samples were constrained by varying sampling conditions across regions and inconsistent age distributions, preventing us from exploring differences between childhood and adult asthma. While asthma diagnosis may not be unequivocally established in preschool children, the association between vitamin D or *VDBP* and asthma is relevant to both adult and pediatric asthma. Future research with larger and more diverse cohorts, along with a more comprehensive exploration of environmental influences, will be crucial to refining our understanding of the intricate interplay between genetic variants, vitamin D, and asthma susceptibility. Overall, our study contributes valuable insights into the complex relationship between genetic variants, vitamin D levels, and asthma across diverse populations, paving the way for more targeted investigations in this evolving field of research.

In conclusion, our results carry significant implications for understanding the complex interplay between genetic factors, vitamin D levels, and asthma susceptibility. The observed differences among ethnic groups highlight the importance of considering regional variations in vitamin D exposure and genetic predispositions.

Competing interests

There are no potential conflicts of interest, including full disclosure of any financial arrangements between any authors.

Acknowledgements and Funding

The study was funded by the Mutual Funds of Latvia-Lithuania -Taiwan Cooperation Project “Comparative study of vitamin D and its receptor gene polymorphisms in Lithuanian, Latvian, and Taiwanese children and adults with atopic dermatitis and asthma “, the Research Council of Lithuania (project reg. no. P-LLT-20-1, P-LLT-20-4), the Latvian Science Council and the National Science and Technology Council of Taiwan (109-2923-B-039 -001 -MY3). We acknowledge the Genome Database of Latvian Population, Latvian Biomedical Research and Study Center for providing data and DNA samples. The study was also supported by grants (1JA8) from the Center for Allergy, Immunology, and Microbiome (A.I.M.), China Medical University Hospital, Taichung, Taiwan; DMR-112-043, DMR-113-114, and DMR-112-205 from the China Medical University Hospital, Taichung, Taiwan.

Authors’ contributions

- JYW, LSHW, NP, BG, NS and MHH designed the study, supervised experiments and wrote and edited the manuscript
- BG, SM and JYW collected the study subjects
- LSHW, MHH, PCC, IRR, DB, LT and RU was responsible for the experiments including measurement of vitamin D and genotyping
- JYW, LSHW, MHH, PCC, SP, IS and IT conducted the statistical analysis the statistical analysis
- All authors read and approved the final version of the manuscript.

Availability of Data and Materials

The datasets generated during and analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

References

1. Gupta A, Sjoukes A, Richards D, Banya W, Hawrylowicz C, Bush A, et al. Relationship between serum vitamin D, disease severity, and airway remodeling in children with asthma. Am J Respir Crit Care Med. 2011; 184(12):1342-9.
2. Hollams EM, Hart PH, Holt BJ, Serralha M, Parsons F, de Klerk NH, et al. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. Eur Respir J. 2011;38(6):1320-7.
3. Chishimba L, Thickett DR, Stockley RA, Wood AM. The vitamin D axis in the lung: a key role for vitamin D-binding protein. Thorax. 2010; 65(5):456-62.
4. Wood AM, Bassford C, Webster D, Newby P, Rajesh P, Stockley RA, et al. Vitamin D-binding protein contributes to COPD by activation of alveolar macrophages. Thorax. 2011;66(3):205-10.
5. Metcalf JP, Thompson AB, Gossman GL, Nelson KJ, Koyama S, Rennard SI, et al. Gcglobulin functions as a cochemotaxin in the lower respiratory tract. A potential mechanism for lung neutrophil recruitment in cigarette smokers. Am Rev Respir Dis. 1991;143(4 Pt 1):844-9.

6. Disanto G, Ramagopalan SV, Para AE, Handunnetthi L. The emerging role of vitamin D binding protein in multiple sclerosis. J Neurol. 2011; 258(3):353-8.
7. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013;369(21):1991-2000.
8. Fawzy MS, Abu AlSel BT. Assessment of Vitamin D-Binding Protein and Early Prediction of Nephropathy in Type 2 Saudi Diabetic Patients. J Diabetes Res. 2018;2018:8517929.
9. Agnello L, Scazzone C, Lo Sasso B, Bellia C, Bivona G, Realmuto S, et al. VDBP, CYP27B1, and 25-Hydroxyvitamin D Gene Polymorphism Analyses in a Group of Sicilian Multiple Sclerosis Patients. Biochem Genet. 2017;55(2):183-92.
10. Fu L, Yun F, Oczak M, Wong BY, Vieth R, Cole DE. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. Clin Biochem. 2009;42(10-11):1174-7.
11. Li F, Jiang L, Willis-Owen SA, Zhang Y, Gao J. Vitamin D binding protein variants associate with asthma susceptibility in the Chinese Han population. BMC Med Genet. 2011;12:103.
12. Zhang Y, Wang Z, Ma T. Associations of Genetic Polymorphisms Relevant to Metabolic Pathway of Vitamin D3 with Development and Prognosis of Childhood Bronchial Asthma. DNA Cell Biol. 2017;36(8): 682-92.
13. Fawzy MS, Elgazzaz MG, Ibrahim A, Hussein MH, Khashana MS, Toraih EA. Association of group-specific component exon 11 polymorphisms with bronchial asthma in children and adolescents. Scand J Immunol. 2019;89(3):e12740.
14. Nasiri-Kalmarzi R, Abdi M, Hosseini J, Tavana S, Mokarizadeh A, Rahbari R. Association of vitamin D genetic pathway with asthma susceptibility in the Kurdish population. J Clin Lab Anal. 2020;34(1): e23039.
15. Leung TF, Wang SS, Tang MF, Kong AP, Sy HY, Hon KL, et al. Childhood asthma and spirometric indices are associated with polymorphic markers of two vitamin D 25-hydroxylase genes. Pediatr Allergy Immunol. 2015; 26(4):375-82.
16. Wu LS, Sjakste T, Sakalauskas R, Sitkauskiene B, Paramonova N, Gasiuniene E, et al. The burden of allergic asthma in children: a landscape comparison based on data from Lithuanian, Latvian, and Taiwanese populations. Pediatr Neonatol. 2012;53(5):276-82.
17. Sjakste T, Paramonova N, Wu LS, Zemeckiene Z, Sitkauskiene B, Sakalauskas R, et al. PSMA6 (rs2277460, rs1048990), PSMC6 (rs2295826, rs2295827) and PSMA3 (rs2348071) genetic diversity in Latvians, Lithuanians and Taiwanese. Meta Gene. 2014;2:283-98.
18. Paramonova N, Wu LS, Rumba-Rozenfelde I, Wang JY, Sjakste N, Sjakste T. Genetic variants in the PSMA6, PSMC6 and PSMA3 genes associated with childhood asthma in Latvian and Taiwanese populations. Biopolymers and Cell. 2014;30(5):377-87.
19. Munkhbayarlakh S, Kao HF, Hou YI, Tuvshintur N, Bayar-Ulzii B, Narantsetseg L, et al. Vitamin D plasma concentration and vitamin D receptor genetic variants confer risk of asthma: A comparison study of Taiwanese and Mongolian populations. World Allergy Organ J. 2019; 12(11):100076.
20. Burney PG, Luczynska C, Chinn S, Jarvis D. The european community respiratory Health survey. Eur Respir J. 1994;7(5):954-60.
21 Pearce N, Sunyer J, Cheng S, Chinn S, Björkstén B, Burr M, et al. Comparison of asthma prevalence in the ISAAC and the ECRHS. ISAAC steering committee and the european community respiratory Health survey. International study of asthma and allergies in childhood. Eur Respir J. 2000;16(3): 420–6.
22 Bastyte D, Tamasauskiene L, Golubickaite I, Ugenskiene R, Sitkauskiene B. Vitamin D receptor and vitamin D binding protein gene polymorphisms in patients with asthma: a pilot study. BMC Pulm Med. 2023;23(1):245.
23. Laufs J, Andrason H, Sigvaldason A, Halapi E, Thorsteinsson L, Jonasson K, et al. Association of vitamin D binding protein variants with chronic mucus hypersecretion in Iceland. Am J Pharmacogenomics. 2004;4(1):63-8.
24. Filiou A, Hoyer A, Holmdahl I, Chakraborty S, van Hage M, Nordlund B, et al. Vitamin D receptor genetic variant associated with asthma in Swedish school-children. Clin Exp Allergy. 2023;53(10): 1045-9.
25. Ismail MF, Elnady HG, Fouda EM. Genetic variants in vitamin D pathway in Egyptian asthmatic children: a pilot study. Hum Immunol. 2013;74(12):1659-64.
26. Rozmus D, Ciesielska A, Plominski J, Grzybowski R, Fiedorowicz E, Kordulewska N, et al. Vitamin D Binding Protein (VDBP) and Its Gene Polymorphisms-The Risk of Malignant Tumors and Other Diseases. Int J Mol Sci. 2020;21(21):7822.
27. Ovesen L, Andersen R, Jakobsen J. Geographical differences in vitamin D status, with particular reference to European countries. Proc Nutr Soc. 2003;62(4):813-21.
28. Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet. 1993;92(2):183-8.
29. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int. 2005;77(1):15-22.
30. Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, et al. The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin D status. Cancer Epidemiol Biomarkers Prev. 2008;17(6):1339-43.