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Vitamin D plasma concentration and vitamin D receptor genetic variants confer risk of asthma: A comparison study of Taiwanese and Mongolian populations

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ABSTRACT

Background: Recent reports have suggested that lower vitamin D serum levels are associated with susceptibility to and severity of asthma in different white populations, which may be due to a lack of sunlight exposure, genetic polymorphism of vitamin D pathway genes, and dietary intake. We investigated the associations between vitamin D concentration, genetic polymorphism of the vitamin D receptor (VDR), and asthma traits in Mongolian and Taiwanese populations that inhabited two different geographical areas.

Methods: In total, 328 Han Taiwanese subjects and 381 Mongolian subjects were enrolled, and their vitamin D serum levels assayed. Genomic DNA of 178 Han Taiwanese subjects and 90 Mongolian subjects was obtained from blood samples. Single-nucleotide polymorphisms (SNPs) of VDR, *ApaI* (rs7975232), *TaqI* (rs731236), *BsmI* (rs1544410) and *FokI* (rs2228570), were selected for genotyping. Logistic regression analyses were performed to detect an association between allergic asthma status and the interaction of the VDR SNP and serum vitamin D concentration in the case–control samples.

Results: We observed a significantly lower vitamin D level in the Mongolian subjects as compared with the Taiwanese population. In particular, in the population under 14 years of age, the serum vitamin D level was significantly higher in the Taiwanese population, in both non-asthmatic and asthmatic subjects, than in the Mongolian non-asthmatic and asthmatic subjects, respectively ($P < 0.01$). Moreover, the vitamin D level in the asthmatic children was significantly lower than that in the non-asthmatic children in both the Taiwanese and Mongolian populations ($P < 0.01$, respectively). Furthermore, we found that the rs2228570 genotype (OR, 3.763) of the VDR SNP and the vitamin D concentration (lower than 40 ng/ml, OR: 38.938) both contribute to increased susceptibility to bronchial asthma.

Conclusion: Our results demonstrated an association between vitamin D concentration and the risk of asthma in two populations of differing ethnicity living in different geographical areas. This

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information implies a potential role of vitamin D in the prevention and treatment of asthma worldwide.

Keywords: Asthma, Vitamin D, Single-nucleotide polymorphism

INTRODUCTION

Bronchial asthma is one of the most common chronic respiratory diseases, of increasing prevalence and financial burden, with more than 300 million sufferers worldwide.[1] This disease affects individuals in all countries and all ethnic groups; however, the prevalence rate of asthma has been reported to vary significantly among different regions.[2] A number of epidemiologic studies have been performed to compare the burden of asthma, and these studies have reported striking differences in asthma prevalence between countries and geographical areas.[3] Without doubt, in addition to racial differences, environment and lifestyle, such as dietary preference, also play significant roles in the development of asthma.[4,5] Despite these confounding factors, few comparison studies have been conducted on the prevalence of bronchial asthma according to ethnicity, geographical area, and cultural differences across various countries and populations.[6,7]

Vitamin D is a nutrient and hormone that is key to the metabolism of calcium and phosphorus. Vitamin D is primarily acquired and produced in the skin upon sunlight exposure or absorbed from dietary intake (e.g., from oily fish and dairy products), and from supplements as secondary sources. Several studies have found that low cord blood vitamin D levels are associated with increased risks of wheezing and recurrent lung symptoms in young children, but not with asthma.[8–10] A recent systematic review found that several prospective studies that measured the 25-hydroxyvitamin D [25(OH)D] level in cord blood at birth or during pregnancy did not identify any association between the 25(OH)D level and asthma in children aged 4–8 years.[11] However, higher serum levels of 25(OH)D were found to be associated with a reduced risk of asthma exacerbation.[11] In the Childhood Asthma Management Program (CAMP) study, a higher risk of severe asthma exacerbation leading to an emergency room (ER) visit or hospitalization was associated with vitamin D insufficiency [25(OH)D $<$ 30 ng/mL].[12] As the vitamin D serum level is not only affected by diet and supplements, but also by climate and lifestyle, whether or not vitamin D can be considered a good biomarker for asthma susceptibility across different geographical regions and ethnic populations is still unproved.

The gene encoding the vitamin D receptor (VDR), being a transcription factor that alters the transcription of target genes responsible for a wide spectrum of biologic responses, is the most widely-studied target along the vitamin D pathway.[13–16] VDR is primarily activated by the binding of its primary ligand, 1,25-dihydroxyvitamin D 3 ($1,25(OH)_2D_3$),[17] and it is expressed in the majority of immune cells, including B and T lymphocytes, monocytes, macrophages, and dendritic cells.[18] Among the known VDR polymorphisms, the most common single-nucleotide polymorphisms (SNPs) that influence VDR expression within the immune system include *BmsI* (rs1544410), *ApaI* (rs7975232), *TaqI* (rs731236) and *FokI* (rs10735810).[19] *BsmI*, *ApaI* and *TaqI* have been shown to be in strong linkage disequilibrium (LD) in several populations.[20]

Mongolia and Taiwan, with different ethnic backgrounds, are located at different latitudes of the Asian northern hemisphere, with markedly different degrees of sunshine, temperature variation, and dietary habits; hence, it is interesting and of academic importance to compare factors including the vitamin D serum level and genetic polymorphisms of VDR that are related to increased susceptibility to asthma in both countries. We believe the results of this study may indicate a potential role of vitamin D in the prevention, or in the future, the treatment of bronchial asthma in different ethnic populations and geographical regions.

MATERIALS AND METHODS

Study population and clinical evaluation

Our study population consisted of asthmatic children ranging from 5 to 18 years of age. The non-asthma subjects included children and adults without a history of asthma. The study protocol was approved both by the Ethical and Clinical Trial Committee of National Cheng-Kung University Hospital, Tainan, Taiwan, and the School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia. A signed informed consent form was required from all participants or their guardians after completing a modified British Medical Society respiratory questionnaire, which was identical to the European Community Respiratory Health Survey (ERCHS). This survey presented similar results to those of the ISAAC and ERCHS, pertinent to the diagnosis and assessment of asthma.[21,22] Pulmonary function was evaluated using standard methods, including spirometry before and after the administration of two puffs of inhaled salbutamol (200 μg/puff). To diagnose the presence of asthma, the following criteria had to be met: (1) a history of wheezing and experiencing shortness of breath during or without concurrent respiratory infections; (2) chronic coughing for more than one month, as well as the presence of wheezing, as observed by a physician; and (3) a bronchodilator test confirming a 15% increase in the FEV1. Non-asthma controls were defined as neither having a history of asthma, as per criteria (1) above, nor being diagnosed with asthma, as per criteria (2). Other evaluations included skin prick tests to examine responsiveness to 6 common aeroallergens, a differential blood count (including total eosinophil count), the level of total serum IgE, and the levels of IgE specific to house dust and mixed pollens, using the Unicap system (Pharmacia, Diagnostic, Sweden). A positive skin test was defined as the presence of $\geq$1 reaction with a wheal diameter $\geq$5 mm. Total serum IgE was measured by solid-phase immunoassay (Pharmacia IgE EIA; Pharmacia Diagnostics). Non-allergy subjects were defined as having a total serum IgE $<$200 and negative skin tests. All Taiwanese subjects were Han-Taiwanese and lived in Taiwan. The Mongolian study subjects, enrolled from an outpatient department, included asthmatic children and adults, and their clinical status was assessed in the same way as for the Taiwanese study group.

Measurement of total IgE and allergen-specific IgE

Serum samples were collected from the study subjects to obtain the total IgE and allergen-specific IgE sensitization profile using multiplex *in vitro* allergen sensitization diagnostic kits, BioIC ®.[23] Serum total IgE levels was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocols (IgE ELISA kits; cat. no. BMS2097; eBioscience).

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

The serum level of 25(OH)D was measured by sandwich EIA using a commercial kit (cat.no. AC-57SF1; Immunodiagnostic Systems, Fountain Hills, AZ, USA).

DNA preparation

Genomic DNA was extracted from blood samples of 178 Han Taiwanese subjects and 90 Mongolian subjects using a QIAamp DNA blood kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The extracted genomic DNA was analyzed by agarose gel electrophoresis, quantified by spectrophotometry, and stored at −80 °C until use.

SNP genotyping

SNPs of the VDR gene, *ApaI* (rs7975232), *TaqI* (rs731236), *BsmI* (rs1544410) and *FokI* (rs2228570), were selected for genotyping. All selected SNPs were genotyped using the high-throughput, 384-microtiter plate MassARRAY™ System, SEQUENOM®, according to the manufacturer's protocol. In brief, DNA containing the SNP site of interest was amplified, followed by performance of the homogenous MassEXTEND™ (hME) assay, in which label-free primer extension chemistry was used to generate allele-specific diagnostic products. Each allele-specific diagnostic product had a unique molecular weight, and this could be distinguished through the application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry. SNPs with a call

rate lower than 90% were excluded from the statistical analysis.

Statistical analysis

The quality of the genotype data was evaluated by testing for Hardy-Weinberg equilibrium (HWE) proportions and Mendelian inheritance consistency. The χ^2 test was used to detect associations between allergic asthma and each SNP. Logistic regression analyses were performed to detect an association between allergic asthma status and interaction of the VDR SNP and the serum vitamin D concentration in the case–control samples. In the logistic regression models, the SNP genotype was coded 0 and 1, indicating whether the risk genotype was carried or not carried by individual subjects.

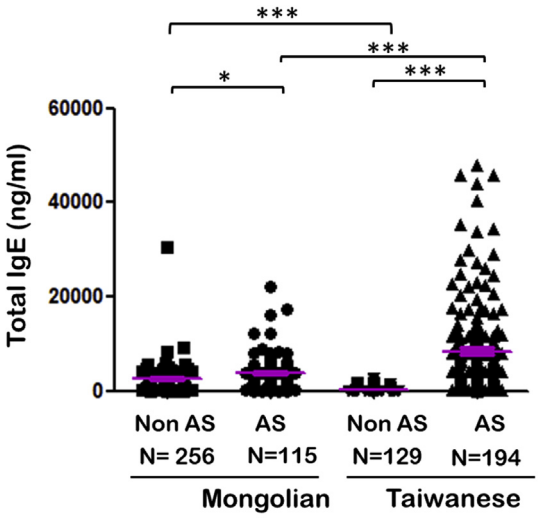
RESULTS

Total IgE and vitamin D levels in the Mongolian and Taiwanese study subjects

In total, 323 Han Taiwanese subjects (194 asthma patients and 129 non-asthma controls) and 371 Mongolian subjects (115 asthma patients and 256 non-asthma controls) were enrolled, and blood samples were collected from all subjects for conduction of a serum Total IgE and vitamin D assay. Of the enrolled subjects, genomic DNA was extracted from blood samples from 178 Han Taiwanese and 90 Mongolians. The demographic data and clinical information of the study subjects are presented in Table 1. There were more male asthma patients in the Taiwanese subjects than in the Mongolian subjects. The average age of the non-asthma subjects was higher than that of the asthma subjects in both the Taiwanese and

	Taiwanese (323)		Mongolian (371)		
	Non-asthma	Asthma	Non-asthma	Asthma	*P* value (between Asthma groups)
Sex (M/F)	21/108	129/64	140/116	79/36	0.730
Age (years; mean ± SD)	28.11 ± 9.67	7.77 ± 4.09	27.75 ± 13.16	11.94 ± 10.28	<0.0001
FEV_1 (pred%)	N/A	92.2 ± 9.3	N/A	89.3 ± 11.5	<0.0001
ICS used (%)	N/A	68.1	N/A	23.5	<0.0001
Total IgE(ng/ml)	297.9 ± 36.9	8376 ± 712.9	2540 ± 385.7	3813 ± 329.8	<0.0001
HDM (SPT%)	N/A	69.1	N/A	13.0	<0.0001
Mugwort (SPT%)	N/A	0.01	N/A	17.4	<0.0001
Sensitization to:					
1. Any mite or cockroach	N/A	77.3	N/A	24.3	<0.0001
2. Any pollen	N/A	19.1	N/A	51.3	<0.0001
3. Any mold	N/A	31.9	N/A	13.0	<0.0001
4. Any animal	N/A	18.1	N/A	26.9	<0.0001
Vitamin D blood concentration (mean ± SD)	68.88 ± 2.57	31.13 ± 1.77	14.34 ± 1.59	12.56 ± 0.75	<0.0001

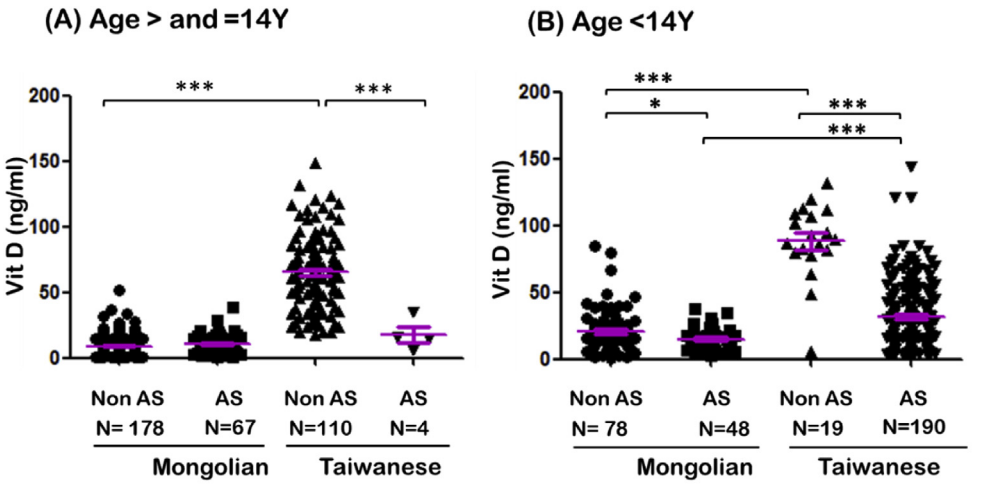
Table 1. Demographic data and clinical information of the study subjects **One outlier with a vitamin D blood concentration of 155 ng/ml in the Mongolian sample was excluded*



Total IgE (ng/ml)	Mongolian	Taiwanese
Non-asthma	2540 ± 385.7	297.9 ± 36.9
asthma	3813 ± 329.8.	8376 ± 712.9

Fig. 1 Comparison of the total IgE levels in the Taiwanese and Mongolian study populations. The level of total IgE was significantly higher in the asthmatic subjects than non-asthmatics in the Taiwanese ($p < 0.001$) as well as in Mongolian populations ($p < 0.05$). Moreover, the total IgE level was significantly higher in the non-asthmatics in Mongolian populations as compared with the non-asthmatic subjects in Taiwan ($p < 0.01$)

Mongolian groups. Non-asthma subjects of a greater age (than the asthma subjects) were enrolled in order to decrease the false negative rate of asthma status. Fig. 1 shows that the level of total IgE was significantly higher in the asthmatic subjects than non-asthmatics in the Taiwanese ($p < 0.001$) as well as in Mongolian populations ($p < 0.05$). Moreover, the total IgE level was significantly higher in the non-asthmatics in Mongolian populations as compared with the non-asthmatic subjects in Taiwan ($p < 0.01$). The serum vitamin D concentration of the non-asthma subjects was significantly higher than that of the asthma subjects in the Taiwanese group. Almost all Mongolian subjects, including asthma patients and non-asthma controls, had a serum vitamin D concentration lower than 20 ng/ml. As shown in Fig. 2, in the study population over 14 years of age, the vitamin D level was significantly higher in the non-asthmatic Taiwanese subjects than in the same Mongolian population ($P < 0.01$); it was also higher than in the asthmatic Taiwanese subjects ($p < 0.01$). In the study population under 14 years of age, the serum vitamin D level was significantly higher in the Taiwanese population, both non-



Vit.D (ng/ml)	Mongolian	Taiwanese
Non-asthma	8.90 ± 0.55	65.48 ±2.68
asthma	10.50 ± 0.83	17.60 ±6.25

Vit.D (ng/ml)	Mongolian	Taiwanese
Non-asthma	20.89 ±1.79	88.56 ±6.47
asthma	15.04 ±1.14	31.41 ±1.80

Fig. 2 Comparison of the serum vitamin D levels in the asthmatic Taiwanese and Mongolian subjects over 14 years of age (A) and under 14 years of age (B). In the study population aged over 14, the level of vitamin D was significantly higher in the non-asthmatic subjects in the Taiwanese population than in the Mongolian population ($P < 0.01$), and was also higher than that in the asthmatic Taiwanese subjects ($P < 0.01$). In the study population under 14, the serum vitamin D level was significantly higher in the Taiwanese population, both non-asthmatic and asthmatic subjects, than in the Mongolian non-asthmatic and asthmatic subjects, respectively ($P < 0.01$). Moreover, the vitamin D level was significantly lower in the asthmatic children as compared with the non-asthmatic children in both the Taiwanese and Mongolian populations ($P < 0.01$, respectively). Mean vitamin D levels in each groups were shown in the tables under the respective figures

SNP ID	Genotype	Taiwanese		*P* value	Mongolian		*P* value
		Asthma	Non-Asthma		Asthma	Non-Asthma	
rs7975232[a] (*ApaI*)	CC	31 (61%)	63 (51%)	0.426	35 (54%)	13 (52%)	0.981
	AC	15 (29%)	49 (40%)		23 (35%)	9 (36%)	
	AA	5 (10%)	11 (9%)		7 (11%)	3 (12%)	
rs731236[a] (*TaqI*)	TT	44 (86%)	109 (89%)	0.666	54 (83%)	21 (84%)	0.916
	CT	7 (14%)	14 (11%)		11 (17%)	4 (16%)	
rs1544410 (*BsmI*)	CC	44 (86%)	105 (82%)	0.620	54 (83%)	21 (84%)	0.916
	CT	7 (14%)	20 (16%)		11 (17%)	4 (16%)	
		0	2 (2%)				
rs2228570 (*FokI*)	GG	20 (39%)	28 (23%)	0.065	28 (43%)	9 (36%)	0.748
	AG	20 (39%)	63 (50%)		28 (43%)	13 (52%)	
	AA	11 (22%)	36 (27%)		9 (14%)	3 (12%)	

Table 2. Association of VDR polymorphism with allergic asthma *a. Missing data due to failed genotyping, 4 for rs7975322 and 4 for rs731236*

asthmatic and asthmatic subjects, than in the Mongolian non-asthmatic and asthmatic subjects, respectively ($P < 0.01$). Moreover, the vitamin D level in the asthmatic children was significantly lower than that in the non-asthmatic children in both the Taiwanese and Mongolian populations ($P < 0.01$, respectively).

VDR genotypic associations with asthma traits

The genotype distributions of the selected SNPs are presented in Table 2. Only rs2228570 (VDR missense polymorphism) showed marginal significance in the Taiwanese group, and the rs2228570 GG genotype frequency was slightly higher in the Mongolian group (41.1%) than in the Taiwanese group (27.0%) (Table 2). Table 3 presents the VDR polymorphism rs2228570 and vitamin D concentrations in the Taiwanese subjects. The recessive model of the rs2228570 GG genotype (*vs.* AG + AA) is associated with a risk of asthma ($P < 0.02$). Moreover, a lower vitamin D blood concentration, below the cut-off point of 40 ng/ml, was also found to be related to a high risk of asthma ($p < 0.0001$) (Table 3). According to multifactorial analysis by logistic regression, the rs2228570 genotype (GG genotype, OR: 3.763, 95%CI: 13.8–109.9) and the

	Non-asthma	Asthma	*P* value
rs2228570			
GG	28	20	0.02
AG + AA	99	31	
Vitamin D conc.			
<40 ng/ml	20	45	<0.0001
≥40 ng/ml	103	6	
Multifactorial analysis			
Risk factors	*P* value	Odds ratio	95% confidence interval
Vit. D < 40 ng/ml	<0.0001	38.94	13.80, 109.89
rs2228570 GG	0.012	3.767	1.34, 10.58

Table 3. VDR polymorphism rs2228570 and vitamin D concentration in the Taiwanese population

vitamin D concentration (lower than 40 ng/ml, OR: 38.938, 95%CI: 1.34–10.58) both contributed to increased susceptibility to allergic asthma in the Taiwanese group. Table 4 shows the joint effects of VDR polymorphism rs2228570 and the serum vitamin D concertation in the Taiwanese group. Subjects with the GG genotype of rs2228570 combined with a serum vitamin D concentration <40 ng/ml were at greatest risk of bronchial asthma (OR: 147.3, 95%CI: 27.4–793.8).

DISCUSSION

In this study, we investigated the serum vitamin D concentration and genetic variations of VDR, and their associations with bronchial asthma, in Taiwanese and Mongolian populations. We found that a low serum vitamin D concentration (<40 ng/ml) and VDR polymorphism of the GG genotype of rs2228570, conferred a high risk of bronchial asthma in the Taiwanese population. The susceptibility to bronchial asthma conferred by the serum vitamin D concentration was found to be stronger than the effect conferred by VDR genetic polymorphism. Almost all the Mongolian subjects had a very low serum vitamin D concentration (<20 ng/ml). Genetic variants of VDR were not found to be associated with asthma in the Mongolian population.

Vitamin D deficiency (<20 ng/ml) and insufficiency (21–29 ng/ml) are currently on the rise globally, and are associated with increased asthma morbidity.[24–26] Epidemiological data suggested that low levels of vitamin D during pregnancy and early life contribute to the development of childhood wheezing and asthma.[9] Studies have shown that vitamin D plays a role in fetal lung development and maturation, as well as maintaining lung structure and function.[27] In a US nationwide study that examined vitamin D insufficiency, asthma and lung function among US children and adults, it was found that vitamin D insufficiency was associated with current asthma and wheezing, a lower FEV1, and the forced vital capacity (FVC) in both adults and children.[28] Interestingly, this National Health and Nutrition Examination Survey also showed that over years in which vitamin D insufficiency decreased, the prevalence of asthma also decreased.[28]

In addition to genetic factors, the main factors that determine the 25(OH)D serum level are skin pigmentation, sun exposure, age, gender, latitude of residence (higher latitudes result in decreased opportunity for vitamin D skin synthesis), diet and vitamin D fortification.[24,26] As compared with the Taiwanese group, our results showed very low vitamin D serum levels in the Mongolian group, who were mainly enrolled from Ulaanbaatar; this result is consistent with previous reports on populations from the same area.[29] In fact, it has been reported that vitamin D supplementation improved winter-related atopic dermatitis among Mongolian children.[30] Effective vitamin D intervention has been strongly recommended for the Mongolian adult population, particularly among women and residents of Ulaanbaatar.[29] Based on our study and aforementioned studies,[29,30] the Mongolian population is likely to suffer generalized vitamin D deficiency, regardless of individual genetic variants of VDR, and therfore, it will be at greater risk of susceptibility to allergic diseases and bronchial asthma.

Interaction term	Odds ratio (95% confidence interval)	*P* value
rs2228570* vitamin D concentration		
GG *Vit. D < 40 ng/ml	147.33 (27.35, 793.77)	<0.0001
AG + AA * Vit. D < 40 ng/ml	34.67 (9.60, 125.24)	<0.0001
GG * Vit. D ≥ 40 ng/ml	3.12 (0.59, 16.45)	0.180
AG + AA * Vit. D ≥ 40 ng/ml	1	

Table 4. Joint effects of VDR polymorphism rs2228570 and vitamin D concentration in the Taiwanese population

In contrast, in the Taiwanese group, the vitamin D serum level and genetic variants of VDR played significant roles in terms of conferring a risk of asthma. In our study, an association between the VDR *FokI* (rs2228570) genetic variant and allergic asthma was identified in the Taiwanese population, but not in the Mongolian population. A meta-analysis was conducted by Zhao et al. to examine the relationships between childhood asthma and VDR gene polymorphisms *ApaI* (rs7975232), *BsmI* (rs1544410), *FokI* (rs2228570) and *TaqI* (rs731236).[31] Of the four SNPs identified, the *ApaI* polymorphism was found to play a role in childhood asthma in Asians, the *FokI* polymorphism may be related to pediatric asthma in Caucasians, and *BsmI* polymorphism was identified as marginally contributing to susceptibility to childhood asthma. Recently, a report showed TaqI and ApaI polymorphisms were associated with asthma in Irish children.[31] Though our genetic data from Mongolian controls may contain less information due to the small sample size, the genotype frequency of the *FokI* polymorphism in the control group was similar to that reported in a previous study examining brick-tea type fluorosis.[32] The *FokI* polymorphism genotype frequencies of GG *vs.* AG + AA in the controls in our study were 36% *vs.* 64% in the Mongolian control subjects (Table 2), and were 34.5% vs. 65.5% in the Mongolian control subjects in the previous brick-tea type fluorosis study.[32]

In this study, we found that the effect of serum vitamin D concentration on susceptibility to bronchial asthma was stronger than that of VDR polymorphism in the Taiwanese group (Table 4). In the Mongolian population, who suffered vitamin deficiency, VDR polymorphism played a minor role as compared with the vitamin D serum concentration, which confers a risk of bronchial asthma.

In conclusion, our results indicated that the vitamin D serum concentration and variants of the VDR gene are major risk factors for the development of bronchial asthma in the subtropical region of Taiwan, while the vitamin D level is the major determinant of the risk of asthma in the high-altitude, temperate zone of Mongolia. Effective vitamin D intervention for Taiwanese and Mongolian populations is warranted, and more extensive studies will have to be carried out in relation to vitamin D supplementation in the Mongolian population.

Ethics approval and consent to participate
The study protocol was approved both by the Ethical and Clinical Trial Committee of National Cheng-Kung University Hospital, Tainan, Taiwan, and the School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia.

Consent for publication
All authors have seen and approved the last version.

Authors' contributions
SM, HFK, JYW and LSHW designed the study, supervised experiments and wrote and edited the manuscript; SM, NT, BB-U and JYW collected the study subjects; YIH, LN and LSHW was responsible for the experiments including measurement of vitamin D and genotyping; JYW, HFK and LSHW conducted the statistical analysis; SM and JYW was responsible for the ethical approval and the handling of the human samples for *in vitro* testing; All authors read and approved the final version of the manuscript.

Declaration of competing interest
The authors declare that they have no competing interests.

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REFERENCES

1. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004;59:469–478.

2. Rabe KF, Adachi M, Lai CK, et al. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. *J Allergy Clin Immunol*. 2004;114: 40–47.
3. Pearce N, Aït-Khaled N, Beasley R, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the international study of asthma and allergies in childhood (ISAAC). *Thorax*. 2007;62:758–766.
4. Han YY, Forno E, Holguin F, Celedón JC. Diet and asthma: an update. *Curr Opin Allergy Clin Immunol*. 2015;15:369–374.
5. Earl CS, An SQ, Ryan RP. The changing face of asthma and its relation with microbes. *Trends Microbiol*. 2015;23:408–418.
6. Forno E, Celedon JC. Asthma and ethnic minorities: socioeconomic status and beyond. *Curr Opin Allergy Clin Immunol*. 2009;9:154–160.
7. Wu LS, Sjakste T, Sakalauskas R, et al. The burden of allergic asthma in children: a landscape comparison based on data from Lithuanian, Latvian, and Taiwanese populations. *Pediatr Neonatol*. 2012;53:276–282.
8. Chawes BL, Bønnelykke K, Jensen PF, Schoos AM, Heickendorff L, Bisgaard H. Cord blood 25(OH)-vitamin D deficiency and childhood asthma, allergy and eczema: the COPSAC2000 birth cohort study. *PLoS One*. 2014;9. e99856.
9. Baïz N, Dargent-Molina P, Wark JD, Souberbielle JC, Annesi-Maesano I, EDEN Mother-Child Cohort Study Group. Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. *J Allergy Clin Immunol*. 2014;133:147–153.
10. Camargo Jr CA, Ingham T, Wickens K, et al. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics*. 2011;127:e180–e187.
11. Cassim R, Russell MA, Lodge CJ, Lowe AJ, Koplin JJ, Dharmage SC. The role of circulating 25 hydroxyvitamin D in asthma: a systematic review. *Allergy*. 2015;70:339–354.
12. Brehm JM, Schuemann B, Fuhlbrigge AL, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol*. 2010;126:52–58.
13. Poon AH, Laprise C, Lemire M, 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.
14. Raby B, Lazarus R, Silverman EK, et al. Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. *Am J Respir Crit Care Med*. 2004;170:1057–1065.
15. Saadi A, Gao G, Li H, Wei C, Gong Y, Liu Q. Association study between vitamin D receptor gene polymorphisms and asthma in the Chinese Han population: a case-control study. *BMC Med Genet*. 2009;10:71.
16. Maalmi H, Sassi FH, Berraies A, Ammar J, Hamzaoui K, Hamzaoui A. Association of vitamin D receptor gene polymorphisms with susceptibility to asthma in Tunisian children: a case control study. *Hum Immunol*. 2013;74:234–240.
17. Lin R, White JH. The pleiotropic actions of vitamin D. *Bioessays*. 2004;26:21–28.
18. Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone, and immunomodulator. *Nutrients*. 2018;10. E1656.
19. Kamel MM, Fouad SA, Salaheldin O, El-Razek Ael-R, El-Fatah AI. Impact of vitamin D receptor gene polymorphisms in pathogenesis of Type-1 diabetes mellitus. *Int J Clin Exp Med*. 2014;7:5505–5510.
20. Uitterlinden AG, Fang Y, van Meurs JB, Pols HA, van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene*. 2004;338:143–156.
21. Burney PG, Luczynska C, Chinn S, Jarvis D. The european community respiratory Health survey. *Eur Respir J*. 1994;5:954–960.
22. Pearce N, Sunyer J, Cheng S, 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;16:420–426.
23. Shyur SD, Jan RL, Webster JR, Chang P, Lu YJ, Wang JY. Determination of multiple allergen-specific IgE by microfluidic immunoassay cartridge in clinical settings. *Pediatr Allergy Immunol*. 2010;21(4 Pt 1):623–633.
24. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and respiratory infections: the vitamin D hypothesis. *Allergy*. 2012;67:10–17.
25. Thacher TD, Clarke BL. Vitamin D insufficiency. *Mayo Clin Proc*. 2011;86:50–60.
26. Gupta A, Bush A, Hawrylowicz C, Saglani S. Vitamin D and asthma in children. *Paediatr Respir Rev*. 2012;13:236–243.
27. Comberiati P, Tsabouri S, Piacentini GL, Moser S, Minniti F, Peroni DG. Is vitamin D deficiency correlated with childhood wheezing and asthma? *Front Biosci*. 2014;6:31–39.
28. Han YY, Forno E, Celedón JC. Vitamin D insufficiency and asthma in a US nationwide study. *J Allergy Clin Immunol Pract*. 2017;5:790–796.
29. Bromage S, Rich-Edwards JW, Tselmen D, et al. Seasonal epidemiology of serum 25-Hydroxyvitamin D concentrations among healthy adults living in rural and urban areas in Mongolia. *Nutrients*. 2016;8. E592.
30. Camargo Jr CA, Ganmaa D, Sidbury R, Erdenedelger Kh, Radnaakhand N, Khandsuren B. Randomized trial of vitamin D supplementation for winter-related atopic dermatitis in children. *J Allergy Clin Immunol*. 2014;134:831–835.
31. Hutchinson K, Kerley C, Faul J, et al. Vitamin D receptor variants and uncontrolled asthma. *Eur Ann Allergy Clin Immunol*. 2018;50:108–116.
32. Yang D, Liu Y, Chu Y, et al. Association between vitamin D receptor gene FokI polymorphism and skeletal fluorosis of the brick-tea type fluorosis: a cross sectional, case control study. *BMJ Open*. 2016;6. e011980.