Original Article

Effects of genetic polymorphisms in Vitamin D metabolic pathway on Vitamin D level and asthma control in South Indian patients with bronchial asthma

Manju Rajaram[1], Sandhiya Selvarajan[2], Revathy Neelamegan[3], Sadishkumar Kamalanathan[4], Vikneswaran Gunaseelan[5], Alphienes Stanley Xavier[2], Saibal Das[2], Vignesh Karthikeyan[6], Vinodkumar Saka[1], Adithan Chandrasekaran[2]

[1]*Department of Pulmonary Medicine, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India,* [2]*Department of Clinical Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India,* [3]*Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India,* [4]*Department of Endocrinology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India,* [5]*Department of Clinical Research, Narayana Hrudalya, Bengaluru, Karnataka, India,* [6]*Centre for Biotechnology, Cell Signaling Laboratory, Anna University, Chennai, Tamil Nadu, India*

ABSTRACT

Objectives: The study was designed to evaluate the single-nucleotide polymorphisms (SNPs) of genes involved in Vitamin D actions (rs2228570) and metabolic pathways (rs2248137 and rs10766197) and their associations with serum 25-hydroxy Vitamin D (25(OH)D) level and asthma control in South Indian patients with bronchial asthma. **Materials and Methods:** One hundred and two patients of South Indian origin with bronchial asthma either naive to inhaled corticosteroids (ICSs) or not receiving ICS for ≥1 month were included and were treated with ICS (beclomethasone 200 μg twice daily) for 8 weeks. One hundred and one unrelated healthy South Indians were used as controls. Pulmonary function test and fractional exhaled nitric oxide were used to assess asthma control. Serum 25(OH)D levels (chemiluminescence immunoassay) and SNPs in Vitamin D pathway (real-time polymerase chain reaction) were assessed. The associations of SNPs and serum 25(OH)D with asthma control was determined using linear regression. All analyses were performed using SPSS (version 19) and "SNPStats." $P < 0.05$ was considered as statistically significant. **Results:** Vitamin D receptor (VDR) polymorphism (rs2228570) was found to be protective against asthma ($P = 0.022$), while there were no significant associations between the other two SNPs and asthma. Similarly, poor correlation and insignificant associations between the SNPs and serum 25(OH)D levels were observed in both cases and controls. There were also insignificant associations between the SNPs and asthma control. **Conclusion:** VDR polymorphism (rs2228570) was found to be protective against asthma in South Indians, while other genes involved in the metabolic pathway of Vitamin D did not show associations with asthma.

KEY WORDS: Associations, bronchial asthma, inhaled corticosteroids, single-nucleotide polymorphisms, Vitamin D

Address for correspondence: Dr. Sandhiya Selvarajan, Department of Clinical Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry - 605 006, India. E-mail: sandhiyaselvarajan@gmail.com

Access this article online

Quick Response Code:

Website: www.lungindia.com

DOI: 10.4103/lungindia.lungindia_23_19

How to cite this article: Rajaram M, Selvarajan S, Neelamegan R, Kamalanathan S, Gunaseelan V, Xavier AS, *et al*. Effects of genetic polymorphisms in Vitamin D metabolic pathway on Vitamin D level and asthma control in South Indian patients with bronchial asthma. Lung India 2019;36:483-91.

INTRODUCTION

Bronchial asthma, a chronic inflammatory disease characterized by hyperresponsiveness of bronchial tree and reversible airway narrowing, manifests in the form of breathlessness, wheezing, cough, and chest tightness. This disease affects nearly 300 million people worldwide, with an estimated prevalence of 3%–38% and 2%–12% among the children and adults, respectively.[1] In India, the prevalence of asthma is about 18 million, affecting >2% of the population above 15 years of age.[2] Vitamin D, the sunshine hormone, is known for its vital role in the maintenance of bone health and calcium-phosphorus balance. In recent times, the beneficial effect of Vitamin D has been being explored in various health conditions including inflammatory diseases, cancer, autoimmune diseases, and cardiovascular and respiratory diseases.[3,4] Vitamin D has been found to offer defense against respiratory infections, improve lung function, and inhibit airway smooth muscle proliferation.[5-8] Further, Vitamin D has been shown to improve steroid responsiveness by enhancing their anti-inflammatory actions with resultant reduction in asthma exacerbations.[7,9-12]

Corticosteroids, both inhalational and systemic, play a major role in the management of asthma. Among them, inhaled corticosteroids (ICSs) are widely used in the control of persistent asthma. Although the majority of the patients with asthma respond to corticosteroids, nearly 5%–10% depict resistance to therapy.[13] Steroid resistance is shown to have poor prognosis owing to reduced asthma control and accelerated deterioration of lung function.[14] One of the explanations given for the varied clinical response to ICS is diminished sensitivity to anti-inflammatory effect of glucocorticoids.[15] In an experimental model of corticosteroid resistance, Vitamin D has been found to restore the immunosuppressive functions and anti-inflammatory effects of dexamethasone,[16] which was further confirmed in a clinical study showing anti-inflammatory and corticosteroid enhancing actions of Vitamin D in the monocytes of patients with steroid resistance asthma.[17] Likewise, an Indian study done in asthmatic children aged 1–15 years has shown between serum 25-hydroxy Vitamin D (25(OH)D) insufficiency and level of asthma control.[18]

Vitamin D (cholecalciferol) synthesized primarily in the skin following sunlight exposure undergoes hydroxylation at positions 25 and 1 in the liver as well as kidney, respectively, to become 1,25-dihydroxycholecalciferol, an active metabolite. The metabolism of Vitamin D depends on the gene CYP2R1 encoding 25-hydroxylase enzyme and CYP27B1 coding 1-α-hydroxylase enzyme.[19] The active Vitamin D enters the cells and exerts its actions by binding to Vitamin D receptor (VDR) encoded by VDR gene. CYP24A1 gene codes 24-hydroxylase enzyme responsible for deactivating the active form of Vitamin D.[16]

Cumulative observations have implicated the role of Vitamin D pathway in immune responses and asthma.[16,20] VDR, which has been shown to be expressed in respiratory epithelium,[21] is the primary binding receptor for 1,25-dihydroxy Vitamin D3 ($1,25(OH)_2D3$).[22] It has also been mapped to chromosome 12q, an area of the genome with multiple loci previously associated with asthma.[23] CYP24A1 encodes mitochondrial $1,25(OH)_2D3$ 24-hydroxylase. Expressed in many tissues, including bronchial smooth muscle,[24] it initiates the degradation of $1,25(OH)_2D3$ by hydroxylation.[25] CYP2R1 encodes Vitamin D 25-hydroxylase, a microsomal hydroxylase enzyme that converts Vitamin D into the active ligand for the VDR.[26] The localization and function of these genes suggest an active role in airway function and respiratory diseases, including asthma.[16]

The polymorphisms in the genes encoding various enzymes involved in Vitamin D actions and metabolic pathways [Figure 1] have been studied for their associations with asthma.[12,16] The associations of these genetic polymorphisms with asthma control have not been established among the Indian patients. Hence, this study aimed to evaluate the single-nucleotide polymorphisms (SNPs) of genes involved in Vitamin D actions (FokI) (VDR [rs2228570, C > T]) and metabolic pathways (CYP24A1 [rs2248137, C > G] and CYP2R1 [rs10766197, G > A]) and their associations with serum 25(OH)D level and asthma control in South Indian patients with bronchial asthma.

MATERIALS AND METHODS

This study protocol was approved by Institutional Ethics Committee (Human Studies), Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry.

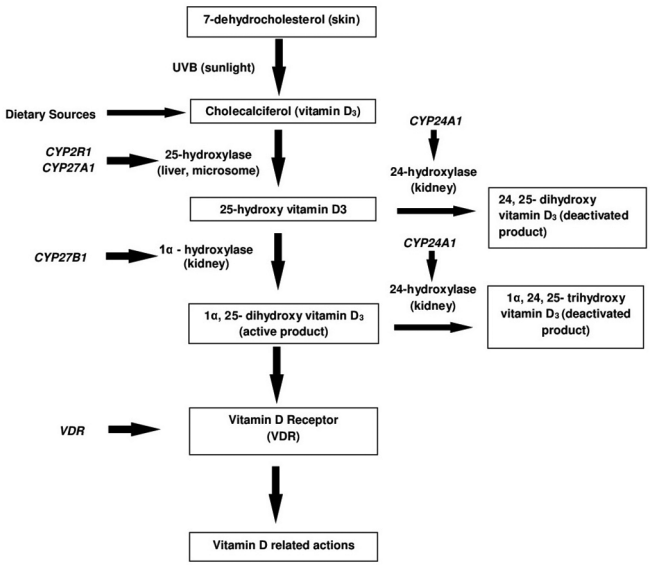

Figure 1: Genes encoding the enzymes involved in Vitamin D action and metabolic pathway

Study population

Patients attending the Outpatient Department of Pulmonary Medicine, JIPMER, Puducherry, were screened for eligibility, and written informed consent was obtained from all the study participants. Patients of South Indian origin (residing in South India for the past three generations and speaking any South Indian language as mother tongue), aged between 18 and 50 years, belonging to either gender, diagnosed with mild-to-moderate persistent asthma and either naive to ICS therapy or without ICS treatment at least for the past 1 month were included in the study. Pregnant women, lactating mothers, and patients on leukotriene antagonists, anti-immunoglobulin E (IgE) therapy, or steroid-based medications for other indications were excluded. Patients nor conforming to previously described spirometric criteria defining asthma[27] were excluded. Unrelated healthy South Indians were used as controls.

Study methods

The eligible patients were treated with ICS (beclomethasone 200 μg twice daily) for 8 weeks.

Assessment of parameters

For the eligible patients, pulmonary function test was done using spirometer (Medikro® SpiroStar USB) at the baseline and after 8 weeks of starting ICS (follow-up visit) to assess FEV_1. Similarly, fractional exhaled nitric oxide (FeNO) was measured in the exhaled breath, both at the baseline and during follow-up visit, using Nitric Oxide Breath Monitor (Bedfont®) to evaluate airway inflammation. Blood sample was collected from both responders and nonresponders for estimation of serum 25(OH)D levels and three SNPs (VDR [rs2228570, C > T], CYP24A1 [rs2248137, C > G], and CYP2R1 [rs10766197, G > A]). The same parameters were assessed in healthy controls.

Serum 25-hydroxy Vitamin D estimation

Estimation of serum 25(OH)D was done using standardized chemiluminescence immunoassay.

Genotyping

DNA was extracted from whole blood by conventional phenol–chloroform method,[28] and genotyping (VDR [rs2228570, C > T], CYP24A1 [rs2248137, C > G], and CYP2R1 [rs10766197, G > A]) was carried out with real-time polymerase chain reaction using SNP Genotyping Assay kits (TaqMan®), as per manufacturer's instructions.

Statistical analysis

Continuous data were expressed as mean ± standard deviation. Categorical data including frequency of genotypes and alleles were expressed as numbers and percentages. Comparison of continuous variables was carried out using independent Student's *t*-test or one-way analysis of variance. Bonferroni correction was done for multiple comparisons. Genotype frequencies of SNPs were assessed for Hardy–Weinberg equilibrium using Chi-square test.[29] The distribution of genotypes between the two groups was also compared using Chi-square test. The association of the different SNPs and haplotypes with asthma was evaluated using unconditional logistic regression model. The associations of the different SNPs with serum 25(OH) D level in asthma patients and healthy controls were calculated using linear regression, and Pearson's correlation was also calculated. The associations of the different SNPs and serum 25(OH)D with asthma control was determined using linear regression with sex, body mass index, duration of asthma, severity of asthma, history of allergic rhinitis and allergic dermatitis, smoking and alcohol status, and usage of ICS (naïve or not) as covariates. Pearson's correlations between serum 25(OH)D level and posttreatment changes in FEV_1 and FeNO were calculated. Pearson's correlation was also estimated between the posttreatment deterministic parameters of asthma control (percentage change in FEV_1) and FeNO score from baseline till the end of 8 weeks' treatment). The asthma patients were divided as responders and nonresponders to treatment by the change in FeV_1 from baseline.[30] The serum 25(OH)D levels across the genotype distribution between the responders and nonresponders as well as the healthy controls were compared. All analyses were performed using SPSS (version 19) (IBM, New York, USA) and "SNPStats."[31] $P < 0.05$ was considered as statistically significant.

RESULTS

The baseline demographic characteristics of asthma patients and healthy volunteers have been enumerated in Table 1. The frequencies of the genotypes were in Hardy–Weinberg equilibrium in both the cases and controls. The distribution of genotypic and allelic frequencies among asthma patients and healthy controls is shown in Table 2. The associations of the three SNPs with asthma have been depicted in Figure 2. VDR polymorphism (rs2228570, C > T) was found to be protective against asthma ($P = 0.022$). However, there were no significant associations with the other two SNPs (CYP24A1 [rs2248137, C > G] and CYP2R1 [rs10766197, G > A]) and asthma. There were no significant associations between any of the haplotypes and asthma [Figure 3].

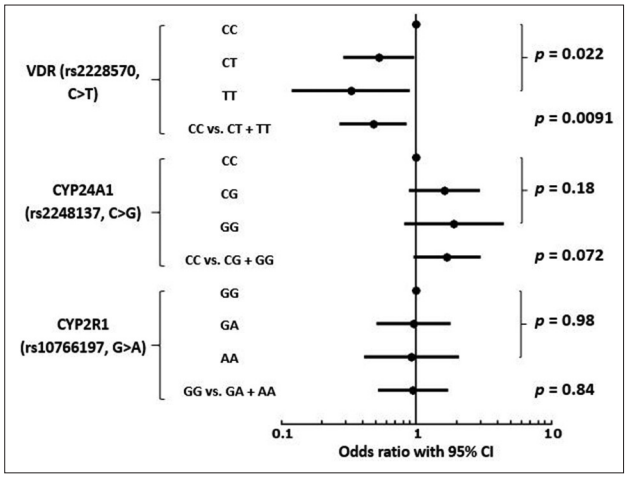

Figure 2: Association of the polymorphisms with asthma

Table 1: Baseline demographic characteristics of asthma patients and healthy controls

Characteristics	Overall	Serum 25(OH)D level at baseline (ng/ml)			*P**
		<20 (deficiency)	20-30 (insufficiency)	>30 (normal)	
Asthma patients (*n*=102)					
n (%)	102 (100)	42 (41.20)	35 (34.30)	25 (24.50)	
Age (years), mean±SD	37.07±8.96	38.79±8.63	32.49±7.71	40.60±8.82	<0.05
Males/females, *n* (%)	22 (21.57)/80 (78.43)	8 (36.36)/34 (42.5)	7 (31.82)/28 (35)	7 (31.82)/18 (22.5)	0.269/0.269
BMI (kg/m^2)	23.64±4.60	24.23±4.91	23.43±4.26	22.94±4.58	0.52
Duration of asthma (years), mean±SD	5.12±4.34	4.88±4.57	5.66±4.35	4.76±4.04	0.66
History of smoking, *n* (%)	6 (100)	3 (2.94)	1 (0.98)	2 (1.96)	0.467
History of alcohol intake, n (%)	3 (100)	1 (0.98)	2 (1.96)	0 (0.00)	0.556
ICS naïve patients	42 (100)	19 (18.63)	13 (12.75)	10 (9.80)	0.958
Serum 25(OH)D level at baseline (ng/ml), mean±SD	23.05±10.54	13.76±3.48	23.70±2.75	37.78±7.65	<0.05
FEV_1 at baseline (%) (L), mean±SD	58.5±16.93	54.83±15.43	60.31±17.81	62.12±17.55	0.17
FVC at baseline (%) (L), mean±SD	65.35±17.4	61.97±16.42	67.28±19.10	68.32±16.18	0.26
FeNO at baseline (ppb), mean±SD	98.5±36.81	95.89±39.86	102.60±31.36	97.16±39.47	0.72
Healthy volunteers (*n*=101)					
n (%)	101 (100)	82 (81.19)	18 (17.82)	1 (0.99)	
Age (years)	32.58 (8.23)	31.81 (7.84)	35.167 (8.90)	49	<0.05
Males/females, *n* (%)	60 (59.41)/41 (40.59)	45 (75)/37 (90.24)	14 (23.33)/4 (9.76)	1 (1.67)/0	0.456/0.456
BMI (kg/m^2), mean±SD	25.08±4.12	25.07±4.09	25.13±4.42	24.39	0.99
Serum 25(OH)D level at baseline (ng/ml), mean±SD	15.04±5.89	13.07±4.38	23.13±2.65	32.21	<0.05

*Among the three groups with respect to serum 25(OH)D level at baseline. BMI: Body mass index, FeNO: Fractional exhaled nitric oxide in exhaled breath, FEV_1: Forced expiratory volume in 1 s, FVC: Forced vital capacity, ICS: Inhaled corticosteroids, SD: Standard deviation, 25(OH)D: 25-hydroxy Vitamin D

Table 2: Distribution of genotypic and allelic frequencies among asthma patients and healthy controls

SNPs	Asthma patients (*n*=102)	Healthy controls (*n*=101)	*P*
VDR (rs2228570, A>G)			
Genotypes, *n* (%)			
CC	43 (42.2)	61 (60.4)	0.487
CT	44 (43.1)	33 (32.7)	
TT	15 (14.7)	7 (6.9)	
Alleles (%)			
C	36.3	23.3	
T	63.7	76.7	
CYP24A1 (rs2248137, C>G)			
Genotypes, *n* (%)			
CC	48 (47.1)	35 (34.7)	0.859
CG	41 (40.2)	48 (47.5)	
GG	13 (12.7)	18 (17.8)	
Alleles (%)			
C	67.2	58.4	
G	32.8	41.6	
CYP2R1 (rs10766197, G>A)			
Genotypes, *n* (%)			
GG	31 (30.4)	32 (31.7)	0.876
GA	51 (50.0)	50 (49.5)	
AA	20 (19.6)	19 (18.8)	
Alleles (%)			
G	55.4	56.4	
A	44.6	43.6	

SNPs: Single-nucleotide polymorphisms, VDR: Vitamin D receptor

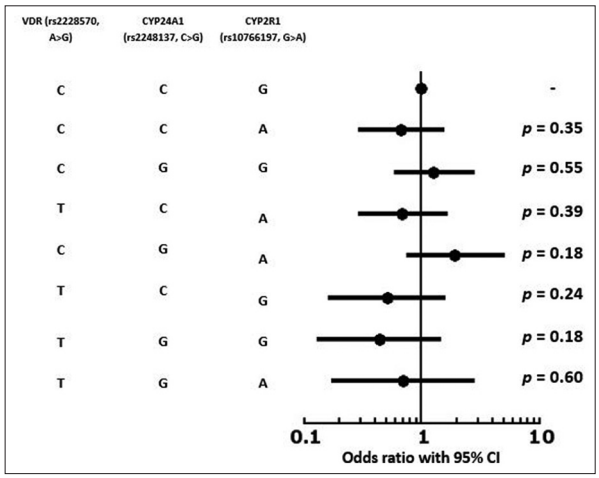

Figure 3: Haplotype associations with asthma

There were poor correlations and insignificant associations between the SNPs and serum 25(OH)D levels in both the groups taken together [Table 3]. Table 4 shows the associations of the three SNPs with asthma control, as diagnosed by posttreatment changes in FEV_1 and FeNO score, which were all statistically insignificant. Pearson's correlation between serum 25(OH)D level and posttreatment changes in FEV_1 was –0.19 and between serum 25(OH)D level and changes in FeNO score was −0.18. Posttreatment, Pearson's correlation between the percentage change in FEV_1 and FeNO score was −0.026. Serum 25(OH)D level in the responders (61/102) was significantly lower as compared to that of the nonresponders (41/102) (20.57 ± 11.57 vs. 25.53 ± 9.51, $P < 0.05$). Although the distribution of CYP24A1 (rs2248137, C > G) and CYP2R1 (rs10766197, G > A) SNPs did not vary between the responders and nonresponders, the distribution of VDR (rs2228570, A > G) was significantly different ($P < 0.05$) between these two groups. The serum 25(OH)D levels in the responders and nonresponders were significantly different from that in the healthy controls across different genotypes [Table 5].

DISCUSSION

In this study, we have tried to evaluate the associations between Vitamin D levels and polymorphisms of genes involved in Vitamin D actions (rs2228570) and metabolic pathways (rs2248137 and rs10766197) on asthma control

Table 3: Association of the single-nucleotide polymorphisms with serum 25-hydroxy Vitamin D level in asthma patients and healthy controls

SNPs	Genotypes	Serum 25(OH)D level (ng/ml)						Pearson's r^2	*P*
		Asthma patients (*n*=102)			Healthy controls (*n*=101)				
		<20	20-30	>30	<20	20-30	>30		
VDR (rs2228570, A>G)	CC	13	16	14	50	10	1	0.009	0.180
	CT	22	16	6	26	7	0		
	TT	7	3	5	6	1	0		
CYP24A1 (rs2248137, C>G)	CC	22	20	6	25	10	0	0.001	0.660
	CG	14	18	9	42	6	0		
	GG	6	3	4	15	2	1		
CYP2R1 (rs10766197, G>A)	GG	15	8	8	29	3	0	0.003	0.428
	GA	17	21	13	37	12	1		
	AA	10	6	4	16	3	0		

SNPs: Single-nucleotide polymorphisms, 25(OH)D: 25-hydroxy Vitamin D, VDR: Vitamin D receptor

Table 4: Associations of the single-nucleotide polymorphisms with asthma control (*n*=102)

Parameters	Genotypes	Serum 25(OH)D level (ng/ml), mean±SD	Posttreatment response (*P*)	
			Change in FEV_1	Change in FeNO
SNPs				
VDR (rs2228570, A>G)	CC	26.0±11.53	0.767	0.504
	CT	20.34±9.18		
	TT	22.47±9.55		
CYP24A1 (rs2248137, C>G)	CC	22.71±10.14	0.793	0.786
	CG	23.37±10.62		
	GG	23.23±12.37		
CYP2R1 (rs10766197, G>A)	GG	20.55±9.13	0.744	0.486
	GA	23.27±9.70		
	AA	24.21±12.40		
Overall serum 25(OH)D level		23.05±10.54	0.769	0.683

FeNO: Fractional exhaled nitric oxide in exhaled breath, FEV_1: Forced expiratory volume in 1 s, SNPs: Single-nucleotide polymorphisms, 25(OH)D: 25-hydroxy Vitamin D, VDR: Vitamin D receptor, SD: Standard deviation

Table 5: Genotype distribution between the responders and nonresponders

SNPs	Genotypes	Asthma patients (*n*=102)				Healthy controls (*n*=101)	*P*
		Responders (*n*=61), *n* (%)	Serum 25(OH)D level (ng/ml) in responders, mean±SD	Nonresponders (*n*=41), *n* (%)	Serum 25(OH)D level (ng/ml) in nonresponders, mean±SD	Serum 25(OH) D level (ng/ml), mean±SD	
VDR	CC	21 (34.42)	24.0±11.79	22 (53.66)	28.0±11.27	15.15±6.21[a,b]	<0.05*
(rs2228570, A>G)	CT	33 (54.09)	19.74±8.28	11 (26.83)	20.94±10.08	14.98±5.93[a,b]	
	TT	10 (16.39)	21.97±7.52	5 (12.19)	22.97±11.58	15.05±5.89[a,b]	
CYP24A1	CC	31 (50.82)	21.08±12.01	17 (41.46)	24.34±8.27	13.76±4.27[a,b]	0.89*
(rs2248137, C>G)	CG	25 (40.98)	20.79±11.0	16 (14.63)	25.95±10.24	16.85±7.12[a,b]	
	GG	5 (8.19)	22.56±12.08	8 (1.95)	23.9±12.66	15.91±8.96[a,b]	
CYP2R1	GG	13 (21.31)	21.01±9.98	18 (43.90)	20.09±8.28	16.22±7.82[a,b]	0.80*
(rs10766197, G>A)	GA	30 (49.18)	20.98±10.0	21 (51.22)	25.56±9.40	15.56±6.31[a,b]	
	AA	8 (13.11)	22.04±12.04	12 (29.27)	26.38±12.76	11.88±3.76[a,b]	
Overall serum 25(OH)D level (ng/ml), mean±SD			20.57±11.57	23.05±10.54	25.53±9.51	15.04±5.89[a,b]	<0.05#

**P* value for genotype distribution between responders and nonresponders, *#P* value for serum 25(OH)D level between responders and nonresponders, aSignificantly different from the responders, bSignificantly different from the nonresponders. SNPs: Single-nucleotide polymorphisms, 25(OH)D: 25-hydroxy Vitamin D, VDR: Vitamin D receptor, SD: Standard deviation

as well as response to ICS in South Indian patients. The genotype and allele frequencies in the three SNPs studied were not significantly different among asthma patients and healthy controls. However, the frequency was different from Indians of other states and global populations, as shown in Tables 6 and 7.[32-39]

Vitamin D has been shown to be associated with respiratory viral infections and asthma.[12] Recent studies have shown that low Vitamin D levels are linked to increased asthma severity in older children[40] and increased prenatal Vitamin D intake may reduce childhood asthma incidence.[41] In our study, among the three SNPs studied, VDR polymorphism (rs2228570) was found to be protective against asthma. Variants within VDR, CYP2R1, and CYP24A1 genes have been shown to be associated with asthma in the Caucasians[23,42] and Egyptians.[43] Some previous studies have raised the susceptibility of VDR gene to be a candidate gene for childhood asthma,[44,45] and it has been shown that

Table 6: Comparison of allele and genotype frequencies in other populations (values are expressed as number [percentage])

SNPs	Genotype/allele	AFR (*n*=661)	AMR (*n*=347)	EAS (*n*=504)	EUR (*n*=503)	SAS (*n*=489)	Present study	
							Asthma patients (*n*=102)	Healthy controls (*n*=101)
VDR (rs2228570, A>G)	CC	435 (65.8)	95 (27.4)	175 (34.7)	203 (40.4)	263 (53.8)	43 (42.2)	61 (60.4)
	CT	202 (30.6)	169 (48.7)	237 (47.0)	220 (43.7)	193 (39.5)	44 (43.1)	33 (32.7)
	TT	24 (3.6)	83 (23.9)	92 (18.3)	80 (15.9)	33 (6.7)	15 (14.7)	7 (6.9)
	Chi-square test	NS	*P*<0.05	*P*<0.05	*P*<0.05	NS	-	-
	C	1072 (81.1)	359 (51.7)	587 (58.2)	626 (62.2)	719 (73.5)	37 (36.3)	24 (23.3)
	T	250 (18.9)	335 (48.3)	421 (41.8)	380 (37.8)	259 (26.5)	65 (63.7)	77 (76.7)
CYP24A1 (rs2248137, C>G)	CC	48 (7.3)	161 (46.4)	183 (36.3)	172 (34.2)	189 (38.7)	48 (47.1)	35 (34.7)
	CG	267 (40.4)	151 (43.5)	232 (46.0)	255 (50.7)	231 (47.2)	41 (40.2)	48 (47.5)
	GG	346 (52.3)	35 (10.1)	89 (17.7)	76 (15.1)	69 (14.1)	13 (12.7)	18 (17.8)
	Chi-square test	*P*<0.05	*P*<0.05	NS	NS	NS	-	-
	C	363 (27.5)	473 (68.2)	598 (59.3)	599 (59.5)	609 (62.3)	69 (67.2)	59 (58.4)
	G	959 (72.5)	221 (31.8)	410 (40.7)	407 (40.5)	369 (37.7)	33 (32.8)	42 (41.6)
CYP2R1 (rs10766197, G>A)	GG	531 (80.3)	150 (43.2)	207 (41.1)	140 (27.8)	146 (29.9)	31 (30.4)	32 (31.7)
	GA	123 (18.6)	50 (14.4)	234 (46.4)	249 (49.5)	242 (49.5)	51 (50.0)	50 (49.5)
	AA	7 (1.1)	147 (42.4)	63 (12.5)	114 (22.7)	101 (20.7)	20 (19.6)	19 (18.8)
	Chi-square test	*P*<0.05	*P*<0.05	NS	NS	NS	-	-
	G	1185 (89.6)	447 (64.4)	648 (64.3)	529 (52.6)	534 (54.6)	57 (55.4)	57 (56.4)
	A	137 (10.4)	247 (35.6)	360 (35.7)	477 (47.4)	444 (45.4)	45 (44.6)	44 (43.6)

AFR: African, AMR: American, EAS: East Asian, EUR: European, NS: Nonsignificant SAS: South Asian, SNPs: Single-nucleotide polymorphisms, VDR: Vitamin D receptor

Table 7: Comparison of allele and genotype frequencies of Vitamin D receptor polymorphism (rs2228570, A>G) with other Indian studies (values are expressed as number [percentage])

Study	Population studied	Genotype			Allele		*P* (Chi-square test)
		CC	CT	TT	C	T	
Bid *et al*., 2009 (*n*=160)	North Indian	80 (50.0)	79 (49.4)	1 (0.6)	239 (74.7)	81 (25.3)	0.0012
Bid *et al*., 2005 (*n*=346)	North Indian	152 (43.9)	170 (49.1)	24 (7.0)	474 (68.5)	218 (31.5)	0.0105
Swapna *et al*., 2011 (*n*=200)	South Indian	68 (34.0)	102 (51.0)	30 (15.0)	238 (59.5)	162 (40.5)	0.000059
Neela *et al*., 2015 (*n*=182)	South Indian	100 (55.0)	74 (40.6)	8 (4.4)	274 (75.3)	90 (24.7)	0.3291
Dasgupta *et al*., 2015 (*n*=250)	South Indian	152 (60.8)	84 (33.6)	14 (5.6)	388 (77.6)	112 (22.4)	0.8898
Selvaraj *et al*., 2004 (*n*=80)	South Indian	43 (53.8)	29 (36.2)	8 (10.0)	115 (71.9)	45 (28.1)	0.6012
Sur *et al*., 2015 (*n*=82)	East Indian	19 (23.2)	35 (42.7)	28 (34.1)	73 (44.5)	91 (55.5)	<0.00001
Present study							
Asthma patients (*n*=102)	South Indian	43 (42.2)	44 (43.1)	15 (14.7)	37 (36.3)	65 (63.7)	-
Healthy controls (*n*=101)	South Indian	61 (60.4)	33 (32.7)	7 (6.9)	24 (23.3)	77 (76.7)	-

VDR influences asthma and allergy susceptibility in a complex manner.[23] A recent study of the VDR gene in Caucasian French Canadian families demonstrated associations between six alleles (rs3782905C, rs1540339A, rs2239185C, rs2239185G, BsmIG, and TaqIT) and asthma and four alleles (rs2239185C, BsmIG, ApaIC, and TaqIT) and atopy ($P < 0.05$), respectively.[23,46] In the same population, three alleles (rs2239185C, ApaIC, and TaqIT) were significantly associated with higher IgE levels. In the Childhood Asthma Management Program cohort, the ApaI SNP was significantly associated with asthma in the overall population.[23] In another study, VDR gene polymorphisms (rs7975232, rs2239185, rs2107301, rs1540339, rs3782905, and rs2228570) were significantly associated with increased asthma severity. A recent meta-analysis has demonstrated that rs2228570, rs7975232, rs731236, and rs3782905 gene polymorphisms in VDR are associated with increased susceptibility to asthma, indicating that VDR polymorphisms could be developed as biomarkers for asthma susceptibility.[47] Another recent meta-analysis concluded that VDR gene polymorphism (rs2228570, C > T) may be connected with pediatric asthma in the Caucasian population.[48]

However, there are few studies showing no associations between VDR gene polymorphisms and asthma.[42,49] In a study conducted in Han Chinese population, of the five studied SNPs in VDR gene (rs2228570, rs3782905, rs1544410, rs7975232, and rs731236), only rs7975232 was significantly associated with asthma.[50] In another study in Han Chinese population, polymorphisms in VDR and CYP2R1 genes were not associated with asthma.[51] A study conducted in the German population has demonstrated that VDR gene is not associated with asthma or the expression of related allergic phenotypes such as eosinophilia and changes in IgE level.[52] In a recent systematic review and meta-analysis including 483 unique studies, it was demonstrated that Vitamin D supplementation, as compared to placebo, reduced the rate of asthma exacerbations requiring treatment with systemic corticosteroids in people with a baseline serum 25(OH) D of <10 ng/ml, but Vitamin D supplementation did not

result in a statistically significant reduction in exacerbation rate in participants with a baseline serum 25(OH)D of ≥10 ng/ml. The authors could not find definitive evidence that the effects of this intervention differed across subgroups of patients.[53] Some previous authors have also suggested that FF genotype of FokI (rs2228570) in VDR gene is protective for asthma.[23,44] Interestingly, a recent study in Serbian patients has demonstrated that FF genotype and F allele of FokI (rs2228570) in VDR gene have a protective effect on asthma.[54] Likewise, our study has demonstrated that (rs2228570, C > T) is protective for asthma.

SNPs in CYP24A1 has been shown to be associated with asthma and atopy.[16] In our study, although CYP24A1 polymorphism (rs2248137, C > G) showed associations with asthma, it was not of statistical significance. In a German adult cohort, a 5-point frequent CYP24A1 haplotype (rs2296241, rs17219315, rs276942, rs2248137, and rs2248359) was significantly associated with asthma and IgE level.[42] In another study, CYP24A1 polymorphisms were significantly associated with decreased asthma control (rs2296241), higher baseline lung function (rs2248137), decreased response to bronchodilators (rs17219315, rs2248137, and rs2248359), and decrease in 25(OH)D level (rs2248137).[12]

SNPs in CYP2R1 genes are also found to be associated with asthma and atopy.[16] However, in our study, CYP2R1 (rs10766197, G > A) showed no associations with asthma. A German study reported a significant association between CYP2R1 SNP rs10766197 and IgE level.[42] In another study, CYP2R1 polymorphism (rs10766197) homozygous genotype was associated with asthma.[12] Significant associations were also found between rs10766197 of CYP2R1, rs7041 and rs4588 of CG, rs4646536 of CYP27B1, rs2228570, rs7975232, and rs1544410 of VDR, and susceptibility to and prognosis of childhood asthma.[55] In a genome-wide association study on the role of Vitamin D in asthmatic individuals, four SNPs (rs11002969, rs163221, rs1678849, and rs4864976) were found to have consistent genetic associations with asthma in selected populations.[56]

In our study, nonsignificant associations were observed between all the haplotypes and asthma. There were poor and nonsignificant correlations between the three SNPs and serum 25(OH)D levels in both asthma patients and healthy controls. Interestingly, there were nonsignificant associations between all the three SNPs and serum 25(OH) D levels with asthma control, as determined by the posttreatment changes in FEV_1 and FeNO.

It is pertinent here to mention that the endogenous serum metabolite of Vitamin D (calcitriol, $1,25(OH)_2D3$) is considered a true steroid hormone (D hormone). Regarding a possible synergism between Vitamin D and glucocorticoids, several studies have shown that $1,25(OH)_2D_3$ has significant additive effects on dexamethasone-mediated inhibition of human lymphocyte and monocyte proliferation.[57] Steroid hormones derived from Vitamin D act through classical nuclear receptors, as well as specific binding sites on the plasma membrane of target cells that are coupled to signal transduction systems.[58] One study has suggested that Vitamin D interacts with clinically relevant glucocorticoid signaling pathways.[59] Likewise, another study found an association between lower Vitamin D levels and increased ICS requirements in children, with a reduced need for anti-inflammatory controller therapy, as serum 25(OH)D levels increased.[60] An experimental model of corticosteroid resistance suggested that Vitamin D may restore the immunosuppressive function of dexamethasone.[61] Thus, Vitamin D supplementation may accentuate the anti-inflammatory function of corticosteroids in asthma.[61] It has been demonstrated clinically that Vitamin D sufficiency in patients treated with ICS is associated with improved lung function in patients with asthma.[62,63] The correlation between higher serum 25(OH)D levels and improved lung function was stronger in ICS-naïve patients than who were previously treated with ICS.[63] Likewise, in our study, there were good correlations between serum 25(OH)D level and response to ICS, as determined by posttreatment changes in FEV_1 and FeNO score [Table 4].

Our study has few limitations. First of all, the selected SNPs of genes involved in Vitamin D actions and metabolic pathways might be having a relatively minor role in the complex disease process.[64] The cumulative effects of other genetic polymorphisms in the same pathway (CYP27A1, CYP27B1, GC, etc.) or genes known to be transcriptionally regulated by Vitamin D (IL10, IL1RL1, CD28, CD86, IL8, SKIIP, etc.) as well as various environmental factors need to be studied. Second, the compliance to ICS was not supervised in our study. Third, there was a significant difference in the distribution of age according to baseline serum 25(OH)D levels in both asthma patients and healthy volunteers, keeping in mind that the effect of VDR system in immune responses might depend on age.[16] Moreover, in the present study, the asthmatic patients had a significantly higher serum 25(OH)D level than the healthy controls. This could be attributed to the inclusion of healthy controls, mostly working indoors, unlike the patients. Similarly, nonresponders had higher serum 25(OH)D level than the responders. However, based on our findings, we would like to hypothesize this to genetic polymorphisms resulting in lower Vitamin D action irrespective of higher Vitamin D level among nonresponders and responders compared to healthy controls. However, this needs to be proven in a larger study by including sufficient number of patients in all the three Vitamin D-related genetic polymorphisms mentioned. Although these were conflicting findings in converse to our hypothesis, we are not very sure about the explanations given, since all cases and controls had Vitamin D level in insufficiency range irrespective of the significant difference. There is also a possibility that this could be a common finding since Vitamin D deficiency is known to prevail all over the country even among healthy people irrespective of their exposure to sunlight.[65]

Notwithstanding these limitations, this study is a stepping stone in examining the potential associations of genetic polymorphisms involved in Vitamin D mechanism of actions as well as metabolic pathways with asthma control to ICS in a South Indian population. The findings indicate the potential value of additional pharmacogenetic testing and gene–gene interaction studies that can be in epistasis with genes involved in Vitamin D metabolism in asthma patients.

CONCLUSION

This study conducted in South Indian population has shown that a SNP in the VDR gene (rs2228570, C > T), that is involved in the genomic actions of Vitamin D, is protective for bronchial asthma while the SNPs (CYP24A1 [rs2248137, C > G] and CYP2R1 [rs10766197, G > A]) in the genes involved in the Vitamin D metabolism pathway have no associations with the disease. Further studies are required in a larger Indian population on all the polymorphisms in the genes involved in Vitamin D pathway.

Financial support and sponsorship

Intramural research grant, JIPMER, Puducherry, was obtained for this work.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. The Global Asthma Report; 2014. Available from: http://www.globalasthmareport.org/. [Last accessed on 2018 Dec 31].
2. Jindal SK, Aggarwal AN, Gupta D, Agarwal R, Kumar R, Kaur T, *et al*. Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis in adults (INSEARCH). Int J Tuberc Lung Dis 2012;16:1270-7.
3. Zhang R, Naughton DP. Vitamin D in health and disease: Current perspectives. Nutr J 2010;9:65.
4. LoPiccolo MC, Lim HW. Vitamin D in health and disease. Photodermatol Photoimmunol Photomed 2010;26:224-9.
5. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and respiratory infections: The Vitamin D hypothesis. Allergy 2012;67:10-7.
6. Clifford RL, Knox AJ. Vitamin D – A new treatment for airway remodelling in asthma? Br J Pharmacol 2009;158:1426-8.
7. Jolliffe DA, Walton RT, Griffiths CJ, Martineau AR. Single nucleotide polymorphisms in the Vitamin D pathway associating with circulating concentrations of Vitamin D metabolites and non-skeletal health outcomes: Review of genetic association studies. J Steroid Biochem Mol Biol 2016;164:18-29.
8. Hall SC, Fischer KD, Agrawal DK. The impact of Vitamin D on asthmatic human airway smooth muscle. Expert Rev Respir Med 2016;10:127-35.
9. Jolliffe DA, Hanifa Y, Witt KD, Venton TR, Rowe M, Timms PM, *et al*. Environmental and genetic determinants of Vitamin D status among older adults in London, UK. J Steroid Biochem Mol Biol 2016;164:30-5.
10. Nissen J, Rasmussen LB, Ravn-Haren G, Andersen EW, Hansen B, Andersen R, *et al*. Common variants in CYP2R1 and GC genes predict Vitamin D concentrations in healthy Danish children and adults. PLoS One 2014;9:e89907.
11. Hibler EA, Jurutka PW, Egan JB, Hu C, LeRoy EC, Martinez ME, *et al*. Association between polymorphic variation in VDR and RXRA and circulating levels of Vitamin D metabolites. J Steroid Biochem Mol Biol 2010;121:438-41.
12. Pillai DK, Iqbal SF, Benton AS, Lerner J, Wiles A, Foerster M, *et al*. Associations between genetic variants in Vitamin D metabolism and asthma characteristics in young African Americans: A pilot study. J Investig Med 2011;59:938-46.
13. Adcock IM, Ito K. Steroid resistance in asthma: A major problem requiring novel solutions or a non-issue? Curr Opin Pharmacol 2004;4:257-62.
14. Jang AS. Steroid response in refractory asthmatics. Korean J Intern Med 2012;27:143-8.
15. Nimmagadda SR, Spahn JD, Leung DY, Szefler SJ. Steroid-resistant asthma: Evaluation and management. Ann Allergy Asthma Immunol 1996;77:345-55.
16. Bossé Y, Lemire M, Poon AH, Daley D, He JQ, Sandford A, *et al*. Asthma and genes encoding components of the Vitamin D pathway. Respir Res 2009;10:98.
17. Zhang Y, Leung DY, Goleva E. Anti-inflammatory and corticosteroid-enhancing actions of Vitamin D in monocytes of patients with steroid-resistant and those with steroid-sensitive asthma. J Allergy Clin Immunol 2014;133:1744-52.
18. Awasthi S, Vikram K. Serum 25 hydroxy Vitamin D insufficiency associated with bronchial asthma in Lucknow, India. Indian J Pediatr 2014;81:644-9.
19. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 2014;21:319-29.
20. Lange NE, Litonjua A, Hawrylowicz CM, Weiss S. Vitamin D, the immune system and asthma. Expert Rev Clin Immunol 2009;5:693-702.
21. Druilhe A, Zahm JM, Benayoun L, El Mehdi D, Grandsaigne M, Dombret MC, *et al*. Epithelium expression and function of retinoid receptors in asthma. Am J Respir Cell Mol Biol 2008;38:276-82.
22. Szpirer J, Szpirer C, Riviere M, Levan G, Marynen P, Cassiman JJ, *et al*. The Sp1 transcription factor gene (SP1) and the 1,25-dihydroxyvitamin D3 receptor gene (VDR) are colocalized on human chromosome arm 12q and rat chromosome 7. Genomics 1991;11:168-73.
23. Raby BA, Lazarus R, Silverman EK, Lake S, Lange C, Wjst M, *et al*. Association of Vitamin D receptor gene polymorphisms with childhood and adult asthma. Am J Respir Crit Care Med 2004;170:1057-65.
24. Bossé Y, Maghni K, Hudson TJ. 1alpha, 25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. Physiol Genomics 2007;29:161-8.
25. Sakaki T, Kagawa N, Yamamoto K, Inouye K. Metabolism of Vitamin D3 by cytochromes P450. Front Biosci 2005;10:119-34.
26. Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. De-orphanization of cytochrome P450 2R1: A microsomal Vitamin D 25-hydroxilase. J Biol Chem 2003;278:38084-93.
27. Richter DC, Joubert JR, Nell H, Schuurmans MM, Irusen EM. Diagnostic value of post-bronchodilator pulmonary function testing to distinguish between stable, moderate to severe COPD and asthma. Int J Chron Obstruct Pulmon Dis 2008;3:693-9.
28. Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech 2013;24:224-31.
29. Rodriguez S, Gaunt TR, Day IN. Hardy-weinberg equilibrium testing of biological ascertainment for mendelian randomization studies. Am J Epidemiol 2009;169:505-14.
30. Pocket Guide for Asthma Management and Prevention. Available from: http://wms-GINA-main-pocket-guide_2018-v1.0.pdf. [Last accessed on 2019 May 08].
31. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: A web tool for the analysis of association studies. Bioinformatics 2006;22:1928-9.
32. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, *et al*. A global reference for human genetic variation. Nature 2015;526:68-74.
33. Bid HK, Konwar R, Aggarwal CG, Gautam S, Saxena M, Nayak VL, *et al*. Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and type 2 diabetes mellitus: A North Indian study. Indian J Med Sci 2009;63:187-94.
34. Bid HK, Mishra DK, Mittal RD. Vitamin-D receptor (VDR) gene (Fok-I, Taq-I and Apa-I) polymorphisms in healthy individuals from North Indian population. Asian Pac J Cancer Prev 2005;6:147-52.
35. Swapna N, Vamsi UM, Usha G, Padma T. Risk conferred by FokI polymorphism of Vitamin D receptor (VDR) gene for essential hypertension. Indian J Hum Genet 2011;17:201-6.
36. Neela VS, Suryadevara NC, Shinde VG, Pydi SS, Jain S, Jonnalagada S, *et al*. Association of Taq I, Fok I and Apa I polymorphisms in Vitamin D receptor (VDR) gene with leprosy. Hum Immunol 2015;76:402-5.
37. Dasgupta S, Dutta J, Annamaneni S, Kudugunti N, Battini MR. Association

of Vitamin D receptor gene polymorphisms with polycystic ovary syndrome among Indian women. Indian J Med Res 2015;142:276-85.
38. Selvaraj P, Kurian SM, Chandra G, Reetha AM, Charles N, Narayanan PR. Vitamin D receptor gene variants of BsmI, ApaI, TaqI, and FokI polymorphisms in spinal tuberculosis. Clin Genet 2004;65:73-6.
39. Sur D, Chakravorty R. Genetic polymorphism in the Vitamin D receptor gene and 25-hydroxyvitamin D serum levels in East Indian Women with polycystic ovary syndrome. J Mol Biomark Diagn 2015;6:247.
40. Ginde AA, Mansbach JM, Camargo CA Jr. Vitamin D, respiratory infections, and asthma. Curr Allergy Asthma Rep 2009;9:81-7.
41. Camargo CA Jr., Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, *et al*. Maternal intake of Vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. Am J Clin Nutr 2007;85:788-95.
42. Wjst M, Altmüller J, Faus-Kessler T, Braig C, Bahnweg M, André E. Asthma families show transmission disequilibrium of gene variants in the Vitamin D metabolism and Signalling pathway. Respir Res 2006;7:60.
43. Ismail MF, Elnady HG, Fouda EM. Genetic variants in Vitamin D pathway in Egyptian asthmatic children: A pilot study. Hum Immunol 2013;74:1659-64.
44. Nabih ES, Kamel TB. Association between Vitamin D receptor gene FokI polymorphism and atopic childhood bronchial asthma. Egypt J Chest Dis Tuberc 2014;63:547-52.
45. 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;74:234-40.
46. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, *et al*. Association of Vitamin D receptor genetic variants with susceptibility to asthma and atopy. Am J Respir Crit Care Med 2004;170:967-73.
47. Han JC, Du J, Zhang YJ, Qi GB, Li HB, Zhang YJ, *et al*. Vitamin D receptor polymorphisms may contribute to asthma risk. J Asthma 2016;53:790-800.
48. Zhao DD, Yu DD, Ren QQ, Dong B, Zhao F, Sun YH. Association of Vitamin D receptor gene polymorphisms with susceptibility to childhood asthma: A meta-analysis. Pediatr Pulmonol 2017;52:423-9.
49. Wjst M. Variants in the Vitamin D receptor gene and asthma. BMC Genet 2005;6:2.
50. 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.
51. 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.
52. Vollmert C, Illig T, Altmüller J, Klugbauer S, Loesgen S, Dumitrescu L, *et al*. Single nucleotide polymorphism screening and association analysis – Exclusion of integrin beta 7 and Vitamin D receptor (chromosome 12q) as candidate genes for asthma. Clin Exp Allergy 2004;34:1841-50.
53. Jolliffe DA, Greenberg L, Hooper RL, Griffiths CJ, Camargo CA Jr., Kerley CP, *et al*. Vitamin D supplementation to prevent asthma exacerbations: A systematic review and meta-analysis of individual participant data. Lancet Respir Med 2017;5:881-90.
54. Despotovic M, Jevtovic Stoimenov T, Stankovic I, Basic J, Pavlovic D. Vitamin D receptor gene polymorphisms in Serbian patients with bronchial asthma: A case-control study. J Cell Biochem 2017;118:3986-92.
55. 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;36:682-92.
56. Lasky-Su J, Lange N, Brehm JM, Damask A, Soto-Quiros M, Avila L, *et al*. Genome-wide association analysis of circulating Vitamin D levels in children with asthma. Hum Genet 2012;131:1495-505.
57. Cutolo M, Paolino S, Sulli A, Smith V, Pizzorni C, Seriolo B. Vitamin D, steroid hormones, and autoimmunity. Ann N Y Acad Sci 2014;1317:39-46.
58. Farach-Carson MC, Nemere I. Membrane receptors for Vitamin D steroid hormones: Potential new drug targets. Curr Drug Targets 2003;4:67-76.
59. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, *et al*. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. J Clin Invest 2006;116:146-55.
60. Brehm JM, Celedón JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, *et al*. Serum Vitamin D levels and markers of severity of childhood asthma in Costa Rica. Am J Respir Crit Care Med 2009;179:765-71.
61. Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DY. Decreased serum Vitamin D levels in children with asthma are associated with increased corticosteroid use. J Allergy Clin Immunol 2010;125:995-1000.
62. Wu AC, Tantisira K, Li L, Fuhlbrigge AL, Weiss ST, Litonjua A. Effect of Vitamin D and inhaled corticosteroid treatment on lung function in children. Am J Respir Crit Care Med 2012;186:508-13.
63. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. Am J Respir Crit Care Med 2010;181:699-704.
64. Brown SD, Calvert HH, Fitzpatrick AM. Vitamin D and asthma. Dermatoendocrinol 2012;4:137-45.
65. Selvarajan S, Gunaseelan V, Anandabaskar N, Xavier AS, Srinivasamurthy S, Kamalanathan SK, *et al*. Systematic review on Vitamin D level in apparently healthy Indian population and analysis of its associated factors. Indian J Endocrinol Metab 2017;21:765-75.