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Original Article

Association of vitamin D receptor gene polymorphisms in North Indian children with asthma: a case-control study

Nidhi Awasthi[1], Shally Awasthi[1], Shivani Pandey[2], Sarika Gupta[1]

[1]Department of Pediatrics, King George's Medical University Lucknow, India; [2]Department of Biochemistry, King George's Medical University, Lucknow, India

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Abstract: Asthma is a complex genetic disease. Vitamin D and vitamin D receptor (*VDR*) gene polymorphisms are involved in asthma pathogenesis. However, accurate inflammatory mechanisms and their role in *VDR* gene polymorphisms are unclear. The objective of this study was to investigate the association of *VDR* gene polymorphisms, *ApaI*, *FokI*, *TaqI*, and *BsmI* with asthma as compared to controls. Children (age 5-15 years) with a history of respiratory symptoms (wheeze, shortness of breath and chest tightness) were recruited as cases. Age matched children admitted with central nervous system disorders (encephalitis/seizures) without any respiratory complaints were recruited as controls after parental consent. Children with a clinical diagnosis of cystic fibrosis, congenital heart disease and whose parents did not consent for participation in the study were excluded. *VDR* gene polymorphisms were genotyped using PCR-RFLP method. One hundred and sixty asthmatics and one hundred controls were enrolled in this study. Mean age of the cases was 103.29±32.7 months and controls 94.24±30.52 months. Children with heterozygous (AC) genotype [OR=1.83, 95% CI=1.01-3.32, p=0.046] of ApaI polymorphism were found to be associated with the risk of asthma. Our findings suggest that *ApaI* polymorphism of *VDR* gene may contribute to asthma susceptibility among children.

Keywords: Asthma, polymorphism, Vitamin D receptor, inflammation, children, North India

Introduction

Asthma is a complex, chronic respiratory disease with a strong genetic predisposition. As per the latest update of World Health Organization -2016 Bulletin, 235 million people across the globe suffer from asthma. According to the same estimates, there were 417,918 deaths attributable to asthma in 2016. In India, 10-15% of children aged 5-11 years are asthmatics [1]. Gene-environment interaction increases the complexity of asthma [2].

Vitamin D has emerged as one of the contributing factors in the asthma pathogenesis as it plays a significant role in both innate and as well as adaptive immunity [3]. Additionally, low vitamin D status has been found to be associated with higher severity of asthma and impaired lung function in children and adults [4-7]. Over 900 genes are regulated by vitamin D through vitamin D receptor (VDR) [8]. VDR belongs to a nuclear transcription receptor family, so it mediates the effects of the biologically active form of vitamin D 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] [9]. After activation, VDR modulates the transcription level of target genes, which participate in T helper (Th) cell development and Th cytokine profile change [10]. Genome and transcriptome-wide association studies showed that vitamin D signaling is actively involved in inflammatory responses, including gene expression, secretion of pro-inflammatory cytokines [11]. These lead to airway hyper-responsiveness as a result of inflammation and mucus obstruction and clinically manifests as exacerbation of asthma [12]. VDR is encoded by the *VDR* gene [13]. In humans, *VDR* gene is found on the long arm of chromosome 12, a position that has been linked to asthma and allergic conditions [14, 15]. A num-

ber of single nucleotide polymorphisms (SNPs) have altered vitamin D mechanism and have been associated with pathogenesis of asthma and other respiratory diseases [16].

Multiple studies have examined the association of *VDR* gene polymorphism with asthma in various populations, but findings among different populations are not uniform [17-25]. Mainly, *VDR* gene polymorphisms, *ApaI, TaqI* and *FokI* polymorphisms were found to be associated with asthma in various populations [17-19]. However, data on the association of *VDR* gene polymorphisms with pediatric asthma is limited in Indian population, possibly data reported from north India is still lacking. Therefore in this study our primary objective was to access the association of *VDR* gene polymorphisms (*ApaI* or rs7975232, *FokI* or rs2228570, *TaqI* or rs-731236, *BsmI* or rs1544410) with asthma in children in Northern India. Our secondary objective was to assess the association of above polymorphisms with asthma control.

Methods and materials

This was a hospital based case-control study conducted at the outpatient and inpatient ward of the Department of Pediatrics, King George's Medical University (KGMU) Lucknow, a tertiary care public hospital in North India, from Oct 2016-Oct 2019 after obtaining ethical clearance from the institutional ethics committee (letter no. 575/Ethics/R-Cell-16). Written, informed consent was obtained from each parent or guardian.

In this study, 260 children (160 asthmatic children/cases and 100 controls) were recruited. The eligibility criteria of the cases was as follows: (1) Age 5-15 years, (2) History of respiratory symptoms such as wheeze, shortness of breath, chest tightness, (3) Cough that varies over time and in intensity together with the variable expiratory airflow limitation, (4) First episode of wheeze or positive family history of asthma in parents or sibling. Children with clinical diagnosis of cystic fibrosis, congenital heart disease and whose parents did not consent for participation in the study were excluded.

Age matched children hospitalized with central nervous system (CNS) disorders (encephalitis/seizures) without any respiratory complaints were recruited as controls. The symptoms of recruited children with encephalitis were as follows: acute onset of fever, change in mental status, (such as; confusion, disorientation or inability to talk) and without any history of seizures; while the children with seizure disorders had unusual behavior, sensations, loss of awareness, blinking and staring, loss of muscle tone, stiffening of limbs. Excluded were those who had clinical diagnosis of acute respiratory illness, cystic fibrosis, congenital heart disease.

GINA (Global Initiative for Asthma) guidelines were used to determine the serious exacerbations and asthma control level (i.e. well controlled, partially controlled and uncontrolled) in asthmatic children (GINA 2019) [26]. As per this criteria, children were categorized as having "uncontrolled asthma" when they had 3-4 of these following symptoms: (1) daytime symptoms of asthma more than twice per week, (2) night waking, (3) reliever needed for asthma symptoms more than twice per week, (4) limited activity due to asthma. Children with "Partially controlled" asthma had 1-2 of the above symptoms. Children had "well controlled" asthma when they had none of the above four symptoms.

Data collection was done on predesigned and pretested questionnaire. Parents were asked for the demographic and socioeconomic status. Environmental data including residential details, no. of rooms, no. of windows, cooking environment and fuel used for cooking, were also taken. Smoking (per day consumption), history of passive smoking was noted. Immunization was recorded for cases and controls from the immunization card. In case the immunization card was unavailable/lost, parents were asked about the immunization status of the recruited child. Clinical data and complications were noted from the medical history of the recruited subjects. Anthropometric details of the subjects were also measured. Weight was measured in kilograms by electronic weighing machine. Height was measured in centimeter by stadiometer and digits were corrected to one decimal unit.

Sample collection and genotyping

Two milliliter of venous blood sample was withdrawn from peripheral vein of each participant under aseptic conditions and collected in an

ethylenediaminetetraacetic acid (EDTA) vial for genotyping. DNA extraction was done by using phenol chloroform method. Quality estimation was done on 0.8% agarose gel electrophoresis. Purity and quantity checking was done by using spectrophotometer. Four polymorphisms, rs7975232 or *ApaI,* rs2228570 or *FokI,* rs-731236 or *TaqI* and rs1544410 or *BsmI* were selected for screening with at least 10% minor allele frequency in Indian population.

All the selected polymorphisms were genotyped by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP). PCR amplification was done by using reported primers (Saadi *et. al.* 2009). PCR (Applied Biosystems, Foster City, USA) was performed by using following conditions: initial denaturation was done at 95°C for 2 min followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for *FokI* and *BsmI* and 57.5°C for *ApaI* and 54.5°C for *TaqI*, for 15 seconds, and extension at 72°C for 30 seconds. The final extension of the reaction was ended at 72°C. Amplified PCR products of each polymorphism *ApaI (*662 bp*), FokI (*270 bp*), TaqI (*690 bp*)* and *BsmI (*613 bp*)* were confirmed on 2% agarose gel electrophoresis.

Sample size

Online sample size estimator (OSSE) was used for sample size calculation. Sample size was estimated on the basis of previous research study [17]. Minor allele frequency was used 5% for cases and in controls it was taken 15%. Power of the study was taken 80% and significant level was taken 5%. Case to control ratio was 2:1.

Statistical analysis

All the data were double entered in MS Excel. Statistical analysis was done using INSTAT 3.0 and SPSS (Statistical package for the social science) software (version 16.0). Frequency distribution of the variables was checked by univariate analysis. All the categorical variables were analyzed by using Fisher exact test and Chi-square test. Odds ratios (ORs) were calculated using binary logistic regression analysis. All the continuous variables were represented as mean, SD and analyzed by Independent t-test. Hardy Weinberg Equilibrium (HWE) was checked for all cases and controls. *P* value less than 0.05 was considered significant.

Results

We recruited total 260 subjects (160 asthmatics and 100 controls) in between the time period of October 2016 to October 2019. **Table 1** shows the baseline and clinical characteristics of the cases and controls. The mean age of cases and controls were 103.29±32.7 and 94.24±30.52 months respectively. Among cases, 70% (112/160) were males. Almost half (55%, 88/160) cases and 48% (48/100) controls were completely immunized and were not found to be associated with asthma (*P*=0.272). Cases that lived in joint families and born with preterm deliveries were found to be associated with asthma (*P*<0.05).

Environmental factors, residential area, no. of rooms, distance of heavy traffic, type of road (busy road with vehicular traffic, near highway) and industrial factory nearby residence were studied and found to be associated with asthma (*P*<0.05). Among cases, availability of animals 38.7% (62/160), curtains 82.5% (132/160) and carpets 38.13% (61/160) in house were not found to be associated with asthma (*P*>0.05). Data related to Cooking environment was also recorded. Separate cooking space (*P*=0.008) and exclusive use of liquefied petroleum gas (LPG) for cooking (P=0.037) were found to be significantly associated with presence of asthma. Garden/farming around the house (*P*=0.001) and indoor smoking by family members (*P*=0.027) were also found to be associated (**Table 1**).

Genetic association of VDR gene polymorphisms

The genotypic and allelic proportions of the *VDR* gene polymorphisms in asthmatics and controls are discussed in **Table 2**. The genotypic frequency distribution of CC, AC and AA genotypes of *ApaI* polymorphism in the cases were, 30.62%, 45.0% and 24.38% as compared to controls 46%, 34% and 20% respectively. Children with heterozygous genotype (AC) [OR= 1.83, 95% CI=1.01-3.32, *P*=0.046] genotype of *ApaI* polymorphism were found to be associated with the risk of asthma. However, no significant association was observed among *FokI* (rs2228570), *TaqI* (rs731236), *BsmI* (rs154-4410) with asthma.

Table 3 depicts the genetic models among asthmatics and controls. In multivariate logistic

Table 1. Baseline and clinical characteristics of asthmatic cases and controls

Variables	Asthmatics (N=160)	Controls (N=100)	*p* value
Age (months) (m ± SD)	103.29±32.7	94.24±30.52	0.026
Sex			0.242
Males	112 (70.0)	63 (63.0)	
Weight (kg) (M ± SD)	24.87±10.20	18.73±5.24	<0.000
Height (cm) (M ± SD)	126.03±15.57	113.32±10.80	<0.000
Consanguinity			
Yes	27 (16.88)	14 (14.0)	0.536
Family type			
Nuclear	47 (29.37)	55 (55.0	Ref
Joint	113 (70.63)	45 (45.0)	<0.000
Preterm birth	60 (37.5)	20 (20.0)	0.003
Mode of delivery			
Normal	110 (68.75%)	77 (77.0)	Ref
Caesarean	50 (31.25%)	23 (23.0)	0.149
No. of siblings	2.40±1.07	1.9±0.88	<0.000
Duration of exclusive breast feeding in months			
<6 months	84 (52.5)	57 (57.0)	Ref
≥ months	76 (47.5)	43 (43.0)	0.478
Belongs to			
Urban	86 (53.75)	33 (33.0)	Ref
Rural	74 (46.25)	67 (67.0)	0.001
No. of rooms in house	3.83±2.49	3.12±1.31	0.008
No. of windows in house	7.10±4.96	6.8±2.63	0.574
Garden/Farming field around the house	110 (68.75)	49 (49.0)	0.001
Separate cooking space	115 (71.87)	86 (86.0)	0.008
Exclusive use of liquefied petroleum gas for cooking	123 (76.87)	65 (65.0)	0.037
Distance of residence from heavy traffic (km)	2.22±2.58	1.4±0.60	0.002
Road type near residence			
●→Road of occasional traffic	109 (68.12)	45 (45.0)	Ref
●→Busy road with vehicular traffic	23 (14.38)	20 (20.0)	0.03
●→Near highway	28 (17.5)	35 (35.0)	0.000
Industrial factory in nearby area	27 (16.88)	8 (8.0)	0.041
Indoor smoking by family members	58 (38.25)	25 (25.0)	0.027

regression analysis, dominant model of *FokI* polymorphism [CC vs CT+TT, OR=2.08, 95% CI=1.03-4.19, *P*=0.04] was found to be associated with the increased risk of asthma.

Association of VDR gene polymorphisms with asthma control

In our study, asthma control levels were classified on the basis of GINA guidelines. Among cases, 42.5%, (68/160) had well controlled, 32.5% (52/160) had partially controlled and 25% (40/160) had uncontrolled asthma. Genotypic distribution of *VDR* gene polymorphisms among asthma level control groups of asthma and controls is detailed in **Table 4**. We compared each sub-group of asthma level control with control group independently.

Heterozygous (AC) genotype of *ApaI* polymorphism was found to be associated with well controlled [OR=2.30, 95% CI=1.13-4.67, *P*=0.020] and partially controlled [OR=2.41, 95% CI=1.09-5.32, *P*=0.026] subgroup of asthma level control. Mutant allele (A) of *ApaI* polymorphism was found to be associated with partially

Table 2. Genotypic and allelic distribution of *VDR* gene polymorphisms among asthmatics and controls

Gene/SNP	Controls (N=100) (n%)	Asthmatics (N=160) (n%)	Adjusted OR, 95% CI, *p* value
ApaI (rs7975232)			
CC	46 (46.0)	49 (30.62)	Ref
AC	34 (34.0)	72 (45.0)	1.83 (1.01-3.32) 0.046
AA	20 (20.0)	39 (24.38)	1.74 (0.85-3.53) 0.12
C	126 (63.0)	170 (53.1)	Ref
A	74 (37.0)	150 (46.9)	0.88 (0.26-2.98) 0.849
FokI (rs2228570)			
CC	47 (47.0)	55 (34.38)	Ref
CT	37 (37.0)	66 (41.25)	1.28 (0.71-2.30) 0.396
TT	16 (16.0)	39 (24.37)	1.83 (0.89-3.76) 0.099
C	131 (65.5)	176 (55.0)	Ref
T	69 (34.5)	144 (45.0)	1.48 (0.42-5.16) 0.535
TaqI (rs731236)			
TT	80 (80.0)	108 (67.5)	Ref
TC	12 (12.0)	33 (20.6)	1.81 (0.86-3.80) 0.116
CC	8 (8.0)	19 (11.9)	1.77 (0.71-4.37) 0.215
T	172 (86.0)	249 (77.8)	Ref
C	28 (14.0)	71 (22.2)	6.17 (0.77-49.57) 0.087
BsmI (rs1544410)			
GG	78 (78.0)	114 (71.25)	Ref
GA	17 (17.0)	32 (20.0)	1.02 (0.51-2.03) 0.950
AA	5 (5.0)	14 (8.75)	1.80 (0.60-5.40) 0.292
G	173 (86.5)	260 (81.2)	Ref
A	27 (13.5)	60 (18.8)	0.20 (0.02-1.64) 0.135

controlled [OR=1.63, 95% CI=1.01-2.64, *P*=0.043]. Mutant homozygous genotype (TT) [OR=3.81, 95% CI=1.40-10.39, *P*=0.006] of *FokI* polymorphism was found to associated with the uncontrolled subgroup of asthma level control. Mutant allele (T) of *FokI* polymorphism was found to be associated with the well controlled [OR=1.59, 95% CI=1.01-2.48, *P*=0.040] and uncontrolled [OR=2.20, 95% CI=1.30-3.73, *P*=0.003] subgroup of asthma. Heterozygous (TC) [OR=3.05, 95% CI=1.19-7.79, *P*=0.016] genotype of *TaqI* polymorphism was found to be associated with uncontrolled subgroup of asthma level control. Mutant allele (C) of *TaqI* polymorphism was found to associated with partially controlled [OR=1.84, 95% CI=1.00-3.38, *P*=0.046] and uncontrolled subgroup [OR=2.18, 95% CI=1.15-4.14, *P*=0.014] of asthma. However mutant allele (A) of *BsmI* polymorphism, was found to be associated [OR=2.13, 95% CI=1.17-3.89, *P*=0.012] with partially controlled subgroup of asthma.

Table 5 shows the genetic models among asthma level control groups and controls. In *ApaI* polymorphism, dominant model [CC Vs AC+AA] was found to be associated with well controlled [OR=2.04, 95% CI=1.06-3.93, *P*=0.030] and partially controlled [OR=2.31, 95% CI=1.11-4.79, *P*=0.022] however over-dominant model [CC+AA vs AC] was found to be associated with only well controlled [OR=1.94, 95% CI=1.03-3.64, *P*=0.038] subgroup of asthma.

Dominant [CC vs CT+TT, OR=2.66, 95% CI=1.17-6.02, *P*=0.016] and recessive [CC+CT vs TT, OR=2.52, 95% CI=1.07-5.91, *P*=0.029] models of *FokI* polymorphism were found to be associated with uncontrolled subgroup of asthma.

In *TaqI* polymorphism, dominant model [TT vs TC+CC] was found to be associated with partially controlled [OR=2.11, 95% CI=1.00-4.49, *P*=0.048] and uncontrolled [OR=2.66, 95% CI=1.19-5.93, *P*=0.014] subgroup of asthma, however over-dominant model [TT+CC vs TC] was found to be associated with uncontrolled [OR=2.78, 95% CI=1.10-6.97, *P*=0.025] subgroup of asthma. Dominant model [GG vs GA+AA] of *BsmI* polymorphism was found to associated with the partially controlled [OR=2.21, 95% CI=1.06-4.60, *P*=0.031] sub-group of asthma.

Haplotype analysis

Haplotype analysis was conducted between cases and controls using SHesis software (available online: http://analysis.bio-x.cn/myAnalysis.php) (**Table 6**). Among eleven haplotytpes: ACCG, ACTA, ACTG, ATCG, ATTA, ATTG, CCCG, CCTA, CCTG, CTCG, CTTG, increased risk of asthma was found with presence of CCTG haplotype [OR=1.80, 95% CI=1.20-2.69, *P*=0.003].

Table 3. Association of *VDR* gene polymorphisms among Asthmatics and controls analyzed by different genetic models

Gene/SNP	Controls (N=100) (n%)	Asthmatics (N=160) (n%)	Adjusted OR, 95% CI, *p* value
ApaI (rs7975232)			
CC	46 (46.0)	49 (30.62)	Ref
AC+AA (Dominant)	54 (54.0)	111 (69.38)	1.83 (0.93-3.58) 0.07
CC+AA vs	66 (66.0)	88 (55.0)	Ref
AC (Over-dominant)	34 (34.0)	72 (45.0)	1.20 (0.52-2.76) 0.65
CC+AC	80 (80.0)	121 (75.62)	Ref
AA (Recessive)	20 (20.0)	39 (24.38)	0.82 (0.36-1.88) 0.65
FokI (rs2228570)			
CC	47 (47.0)	55 (34.37)	Ref
CT+TT (Dominant)	53 (53.0)	105 (65.62)	2.08 (1.03-4.19) 0.04
CC+TT	63 (63.0)	94 (58.75)	Ref
CT (Over-dominant)	37 (37.0)	66 (41.25)	0.52 (0.23-1.19) 0.124
CC+CT	84 (84.0)	121 (75.62)	Ref
TT (Recessive)	16 (16.0)	39 (24.37)	1.89 (0.83-4.28) 0.124
TaqI (rs731236)			
TT	80 (80.0)	108 (67.5)	Ref
TC+CC (Dominant)	20 (20.0)	52 (32.5)	1.75 (0.73-4.22) 0.206
TT+CC	88 (88.0)	127 (79.4)	Ref
TC (Over-dominant)	12 (12.0)	33 (20.6)	1.47 (0.36-5.88) 0.583
TT+TC	92 (92.0)	141 (88.12)	Ref
CC (Recessive)	8 (8.0)	19 (11.9)	0.67 (0.17-2.71) 0.58
BsmI (rs1544410)			
GG	78 (78.0)	114 (71.2)	Ref
GA+AA (Dominant)	22 (22.0)	46 (28.8)	1.91 (0.66-5.53) 0.23
GG+AA	83 (83.0)	128 (80.0)	Ref
GA (Over-dominant)	17 (17.0)	32 (20.0)	0.53 (0.15-1.88) 0.33
GG+GA	95 (95.0)	146 (91.2)	Ref
AA (Recessive)	5 (5.0)	14 (8.8)	1.86 (0.53-6.55) 0.33

Discussion

The present case-control study was conducted to investigate the association of *VDR* gene polymorphisms *(ApaI, FokI, TaqI, BsmI)* with asthma in North Indian children. In this study, heterozygous (AC) genotype of *ApaI* polymorphism was found to be associated with increased risk of asthma. Among genetic models (dominant, over-dominant, recessive models), dominant model of *FokI* polymorphism was found to be associated with increased risk of asthma.

VDR is a ligand dependent transcription factor it binds with vitamin D then heterodimerizes with the retinoid X receptor (RXR) and forms an active complex which moves to the nucleus to bind with vitamin D response elements (VDRE) on the genome [27]. VDRs show dynamic activities in pulmonary tissues [28]. *VDR* gene is located on the long arm of the 12th chromosome at the position 12q13.14 and contain six promoter regions. *ApaI* and *BsmI* both polymorphisms are located on intron 8 and influence protein expression by changing stability of mRNA. *TaqI* polymorphism is found on 9th exon and its silent codon alteration is characterized by the substitution of C with T, resulting in ATC to ATT codon alteration. In second exonic position of *VDR* gene, the alteration in the single *FokI* restriction site can lead to the alteration in ATG start codon [20].

We found *ApaI* polymorphism to be associated with increased risk of asthma. Similar findings were reported in pediatric population from Turkey, Ireland, China [18, 20, 29]. In contrast to our findings, *ApaI* polymorphism was not found to be associated with asthma in children in Tunisian, Greece, Chile American populations [19, 25, 30]. Various studies have reported diverse findings on role of *ApaI* polymorphism in asthma in adults as well as in adolescents [17, 22, 31].

In current study, dominant model of *FokI* polymorphism was found to be associated with the increased risk of asthma. Similar to our findings, *FokI* polymorphism was found to be associated with increased risk of asthma in Tunisian pediatric population [19]. In contrast to our findings, *FokI* polymorphism was found to be associated with the lower risk of asthma in south Indian adults and Egyptian Children [21, 24]. Similarly, case-control study conducted in

Table 4. Genotypic distribution of *VDR* gene polymorphisms among asthma control groups of asthma and controls

Polymorphisms	Controls (N=100)	Well controlled (N=68)	Partially controlled (N=52)	Uncontrolled (N=40)	Controls Vs Well controlled		Controls Vs Partially controlled		Controls Vs Uncontrolled	
					p value	OR, 95% CI	*p* value	OR, 95% CI	*p* value	OR, 95% CI
rs7975232 (*ApaI*)										
CC	46 (46.0)	20 (29.41)	14 (26.92)	15 (37.5)	-	Ref	-	Ref	-	Ref
AC	34 (34.0)	34 (50.0)	25 (48.07)	13 (32.5)	0.020	2.30 (1.13-4.67)	0.026	2.41 (1.09-5.32)	0.718	1.17 (0.49-2.78)
AA	20 (20.0)	14 (20.59)	13 (25.0)	12 (30.0)	0.276	1.61 (0.68-3.81)	0.102	2.13 (0.85-5.35)	0.192	1.84 (0.73-4.63)
C	126 (63.0)	74 (54.4)	53 (50.9)	43 (53.7)	-	Ref	-	Ref	-	Ref
A	74 (37.0)	62 (45.6)	51 (49.1)	37 (46.3)	0.115	1.42 (0.91-2.22)	0.043	1.63 (1.01-2.64)	0.152	1.46 (0.86-2.47)
FokI (rs2228570)										
CC	47 (47.0)	25 (36.76)	20 (38.46)	10 (25.0)	-	Ref	-	Ref	-	Ref
CT	37 (37.0)	24 (35.29)	25 (48.07)	17 (42.5)	0.581	1.21 (0.60-2.47)	0.212	1.58 (0.76-3.29)	0.087	2.15 (0.88-5.27)
TT	16 (16.0)	19 (27.94)	7 (13.46)	13 (32.5)	0.053	2.23 (0.97-5.08)	0.957	1.02 (0.36-2.88)	0.006	3.81 (1.40-10.39)
C	131 (65.5)	74 (54.4)	65 (62.5)	37 (46.3)	-	Ref	-	Ref	-	Ref
T	69 (34.5)	62 (45.6)	39 (37.5)	43 (53.7)	0.040	1.59 (1.01-2.48)	0.604	1.13 (0.69-1.86)	0.003	2.20 (1.30-3.73)
TaqI (rs731236)										
TT	80 (80.0)	50 (73.53)	34 (65.38)	24 (60)	-	Ref	-	Ref	-	Ref
TC	12 (12.0)	10 (14.70)	12 (23.07)	11 (27.5)	0.534	1.33 (0.53-3.31)	0.056	2.35 (0.96-5.76)	0.016	3.05 (1.19-7.79)
CC	8 (8.0)	8 (11.76)	6 (11.54)	5 (12.5)	0.373	1.60 (0.56-4.53)	0.320	1.76 (0.56-5.47)	0.225	2.08 (0.62-6.96)
T	172 (86.0)	110 (80.9)	80 (76.9)	59 (73.7)	-	Ref	-	Ref	-	Ref
C	28 (14.0)	26 (19.1)	24 (23.1)	21 (26.3)	0.209	1.45 (0.80-2.60)	0.046	1.84 (1.00-3.38)	0.014	2.18 (1.15-4.14)
BsmI (rs1544410)										
GG	78 (78.0)	54 (79.41)	32 (61.54)	28 (70)	-	Ref	-	Ref	-	Ref
GA	17 (17.0)	10 (14.70)	14 (26.92)	8 (20)	0.708	0.84 (0.36-1.99)	0.091	2.00 (0.88-4.55)	0.573	1.31 (0.50-3.37)
AA	5 (5.0)	4 (5.88)	6 (11.54)	4 (10)	0.999	1.15 (0.29-4.50)	0.082	2.92 (0.83-10.27)	0.261	2.22 (0.55-8.89)
G	173 (86.5)	118 (86.8)	78 (75.0)	64 (80.0)	-	Ref	-	Ref	-	Ref
A	27 (13.5)	18 (13.2)	26 (25.0)	16 (20.0)	0.944	0.97 (0.51-1.85)	0.012	2.13 (1.17-3.89)	0.172	1.60 (0.81-3.16)

Table 5. Association of *VDR* gene polymorphisms among asthma control groups of asthma and controls analyzed by different genetic models

Polymorphisms	Controls (N=100)	Well controlled (N=68)	Partially controlled (N=52)	Uncontrolled (N=40)	Controls Vs Well controlled		Controls Vs Partially controlled		Controls Vs Uncontrolled	
					p value	OR, 95% CI	*p* value	OR, 95% CI	*p* value	OR, 95% CI
rs7975232 (*ApaI*)										
CC	46 (46.0)	20 (29.41)	14 (26.9)	15 (37.5)	-	Ref	-	Ref	-	Ref
AC+AA (Dominant)	54 (54.0)	48 (70.59)	38 (73.1)	25 (62.5)	0.030	2.04 (1.06-3.93)	0.022	2.31 (1.11-4.79)	0.359	1.42 (0.66-3.01)
CC+AA vs	66 (66.0)	34 (50)	27 (51.92)	27 (67.5)	-	Ref	-	Ref	-	Ref
AC (Over-dominant)	34 (34.0)	34 (50)	25 (48.07)	13 (32.5)	0.038	1.94 (1.03-3.64)	0.091	1.79 (0.90-3.56)	0.865	0.93 (0.42-2.05)
CC+AC	80 (80.0)	54 (79.41)	39 (75)	28 (70)	-	Ref	-	Ref	-	Ref
AA (Recessive)	20 (20.0)	14 (20.59)	13 (25)	12 (30)	0.925	1.03 (0.48-2.23)	0.478	1.33 (0.60-2.95)	0.203	1.71 (0.74-3.95)
FokI (rs2228570)										
CC	47 (47.0)	25 (36.8)	20 (38.46)	10 (25)	-	Ref	-	Ref	-	Ref
CT+TT (Dominant)	53 (53.0)	43 (63.26)	32 (61.54)	30 (75)	0.188	1.52 (0.81-2.86)	0.314	1.41 (0.71-2.80)	0.016	2.66 (1.17-6.02)
CC+TT	63 (63.0)	44 (64.70)	27 (51.92)	23 (57.5)	-	Ref	-	Ref	-	Ref
CT (over-dominant)	37 (37.0)	24 (35.3)	25 (48.07)	17 (42.5)	0.821	0.92 (0.48-1.76)	0.187	1.57 (0.79-3.10)	0.545	1.25 (0.59-2.65)
CC+CT	84 (84.0)	49 (72.06)	45 (86.54)	27 (67.5)	-	Ref	-	Ref	-	Ref
TT (Recessive)	16 (16.0)	19 (27.94)	7 (13.46)	13 (32.5)	0.061	2.03 (0.95-4.32)	0.678	0.81 (0.31-2.13)	0.029	2.52 (1.07-5.91)
TaqI (rs731236)										
TT	80 (80.0)	50 (73.53)	34 (65.4)	24 (60)	-	Ref	-	Ref	-	Ref
TC+CC (Dominant)	20 (20.0)	18 (26.47)	18 (34.6)	16 (40)	0.325	1.44 (0.69-2.98)	0.048	2.11 (1.00-4.49)	0.014	2.66 (1.19-5.93)
TT+CC	88 (88.0)	58 (85.29)	40 (76.92)	29 (72.5)	-	Ref	-	Ref	-	Ref
TC (Over-dominant)	12 (12.0)	10 (14.70)	12 (23.07)	11 (27.5)	0.609	1.26 (0.51-3.11)	0.075	2.20 (0.90-5.32)	0.025	2.78 (1.10-6.97)
TT+TC	92 (92.0)	60 (88.2)	46 (88.46)	35 (87.5)	-	Ref	-	Ref	-	Ref
CC (Recessive)	8 (8.0)	8 (11.8)	6 (11.54)	5 (12.5)	0.414	1.53 (0.54-4.30)	0.474	1.50 (0.49-4.58)	0.407	1.64 (0.50-5.36)
BsmI (rs1544410)										
GG	78 (78.0)	54 (79.4)	32 (61.54)	28 (70)	-	Ref	-	Ref	-	Ref
GA+AA(Dominant)	22 (22.0)	14 (20.6)	20 (38.46)	12 (30)	0.826	0.91 (0.43-1.95)	0.031	2.21 (1.06-4.60)	0.318	1.51 (0.66-3.46)
GG+AA	83 (83.0)	58 (85.3)	38 (73.08)	32 (80)	-	Ref	-	Ref	-	Ref
GA (Over-dominant)	17 (17.0)	10 (14.7)	14 (26.92)	8 (20)	0.691	0.84 (0.35-1.97)	0.149	1.79 (0.80-4.02)	0.675	1.22 (0.47-3.10)
GG+GA	95 (95.0)	64 (94.11)	46 (88.5)	36 (90)	-	Ref	-	Ref	-	Ref
AA (Recessive)	5 (5.0)	4 (5.9)	6 (11.54)	4 (10)	0.999	1.18 (0.30-4.59)	0.139	2.47 (0.71-8.54)	0.275	2.11 (0.53-8.30)

Table 6. Distribution of genotyped *VDR* gene polymorphism's haplotypes and its association with asthma

Haplotypes	Cases	Controls	Crude OR, 95% CI, *P* value
ACCG	3.04 (0.015)	12.76 (0.040)	0.35 [0.10-1.26] 0.095
ACTA	4.09 (0.020)	11.01 (0.034)	0.56 [0.17-1.77] 0.319
ACTG	34.15 (0.171)	49.52 (0.155)	1.07 [0.66-1.73] 0.771
ATCG	4.81 (0.024)	15.02 (0.047)	0.48 [0.16-1.36] 0.159
ATTA	8.38 (0.042)	20.01 (0.063)	0.62 [0.27-1.43] 0.266
ATTG	17.97 (0.090)	36.23 (0.113)	0.73 [0.40-1.34] 0.317
CCCG	9.83 (0.049)	19.25 (0.060)	0.77 [0.35-1.70] 0.524
CCTA	9.08 (0.045)	8.18 (0.026)	1.74 [0.66-4.56] 0.252
CCTG	67.44 (0.337)	68.01 (0.213)	1.80 [1.20-2.69] 0.003
CTCG	6.92 (0.035)	10.38 (0.032)	1.02 [0.38-2.73] 0.958
CTTG	28.83 (0.144)	48.84 (0.153)	0.89 [0.54-1.47] 0.653

Serbian adult population found allelic distribution of *FokI* polymorphism to be associated with decreased risk of asthma [22]. However *BsmI, ApaI, TaqI* polymorphisms were not found to be associated with asthma in Serbian population [22]. Likewise, we did not find any association of *TaqI, BsmI* polymorphisms with asthma in our study. Similar findings were reported pediatric population from Greece, China, and Chile American [25, 29, 30]. However, contrary results were reported in Turkish, Tunisian and Irish, pediatric populations [18-20].

Among cases of asthma we also accessed association of *VDR* gene polymorphisms with level of asthma control. In *ApaI* polymorphism, heterozygous (AC) genotype, dominant model were found to be associated with increased risk of asthma in well controlled and partially controlled subgroup of asthma, however, over dominant model was found to be associated with well controlled subgroup of asthma. Similarly, Iordanidou *et al*. found, mutant homozygous (aa) of *ApaI* polymorphism to be associated with well controlled asthma in children [25].

In *FokI* polymorphism, mutant homozygous (TT) genotype, dominant and recessive models were found to be associated with increased risk of uncontrolled subgroup of asthma. In *TaqI* polymorphism, heterozygous (TC) genotype was found to be associated with increased risk of uncontrolled asthma. Dominant model of *TaqI* polymorphism was found to be associated with increased risk of partially controlled and uncontrolled subgroup of asthma. However over-dominant model was found to be associated with increased risk of uncontrolled subgroup of asthma. In *BsmI* polymorphism, dominant model was found to be associated with increased risk of partially controlled subgroup of asthma. Iordanidou *et al*. could not find any association of *FokI, TaqI* and *BsmI* polymorphisms with asthma control groups [25]. To the best of our knowledge, no other study has investigated the association of *VDR* gene polymorphisms with asthma control groups in children.

Asthma is a multi-genetic complex disease, coupled with many polymorphisms in pathogenesis of asthma. Identification of genetic polymorphisms helps in prognosis of the complex disease and development of interventional strategies [32]. Thus, current study adds knowledge to the list of population studies where *VDR* gene SNPs seem to play role in the susceptibility of asthma.

These population studies highlight that no single SNP is associated with susceptibility of asthma, due to compound effects of multiple alleles and environmental factors. Available population studies of *VDR* gene polymorphisms showed inconsistent findings that may be due to variation of different ethnic groups, gene-environmental interactions. Furthermore, these differences could be due to inadequate sample size, statistical power and clinical heterogeneity. Hence, studies are needed with larger sample size in different ethnic population, which will help in examine the role of *VDR* gene with other genes involved in vitamin D metabolism.

Best of our knowledge, current study is the first case-control study accessing the association of *VDR* gene polymorphism (*ApaI, FokI, TaqI, BsmI)* in asthmatic North Indian children. Standardized classification of WHO was used for inclusion of the study subjects.

Healthy controls were not enrolled. Children with CNS disorders without respiratory complaints were included as controls. The minor allele frequency (a) of *ApaI* polymorphism in healthy adults of the same ethnicity from Lucknow [33], was reported 42% which was almost similar to our study findings, minor allele

frequency of *ApaI* polymorphism was 37% in the controls of our study. Vitamin D levels were not assessed in this study; this was one of our limitations. We conclude with our findings that *ApaI* polymorphism of *VDR* gene may contribute in asthma risk in north Indian children. Further studies will be required to explore the complicated involvement between *VDR* gene polymorphisms and environmental factors among asthmatics in diverse populations.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shally Awasthi, Head, Department of Pediatrics, King George's Medical University, Lucknow 226003, India. Tel: +91-9839221244; E-mail: shally07@gmail.com

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