The Vitamin D Receptor Gene Polymorphisms in Asthmatic Children: A Case-Control Study

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Background: The association between vitamin D receptor (VDR) polymorphisms and the risk of asthma remains unclear. This study aimed to investigate the effect of VDR gene polymorphisms and VDR mRNA expression levels on respiratory function, nitric oxide levels in expiratory air, and serum vitamin D levels in children with asthma.

Materials and Methods: The study included 80 healthy children (control group) and 100 asthmatic children (asthma group) between the age of 5 and 18 years. The VDR genotypes (*Apa*I, *Taq*I, and *Fok*I) and VDR mRNA levels were determined in all groups.

Results: There was no statistically significant difference in vitamin D levels between the asthma group and the control group ($P > 0.05$). A significant association was found between both genotype (CC) of the *Taq*I polymorphism [odds ratio (OR) = 0.2, 95% confidence interval (CI) (0.07–0.5), $P = 0.003$] and genotype (CA) of *Apa*I polymorphisms [OR = 0.2, 95% CI (0.07–0.8), $P = 0.02$], and asthma risk. In addition, when single-nucleotide polymorphism allelic frequencies between asthma and control groups were compared there is no significant association ($P > 0.05$). When compared to control group, VDR mRNA expression in asthma group decreased in genotypes CC and CA of *Apa*I and in genotypes TT and TC of *Taq*I ($P < 0.05$).

Conclusion: The results provide supporting evidence for an association between *Taq*I and *Apa*I polymorphisms and asthma susceptibility.

Keywords: asthma, vitamin D, exhaled nitric oxide, VDR gene polymorphism, VDR mRNA expression

Introduction

ASTHMA IS A MULTIFACTORIAL DISEASE, with many genetic and environmental factors playing a role in its etiology. In recent years, gene polymorphisms in asthma have been examined, and several sensitivity loci, including 2q, 5q, 6p, 11q, 12q, and 13q regions, have been identified.[1] Recent studies reported that vitamin D played a role in the pathogenesis of many inflammatory diseases, including asthma, and that the vitamin D receptor (VDR) appeared to be a candidate gene for asthma. The effects of vitamin D on the immune system are generally in the form of immunological tolerance, immunomodulation, and immunosuppression. Specifically, vitamin D targets genes encoding proinflammatory and anti-inflammatory cytokines, which play a role in the development of airway inflammation.[1,2]

Risk factors for asthma and vitamin D deficiency, 2 common conditions, are similar and include urban-style life, obesity, and undernutrition.[3–5] Due to the link between asthma and

vitamin D deficiency, a number of studies have investigated the relationship between genes involved in vitamin D metabolism and asthma phenotypes. Most of these studies provided support for the idea that vitamin D provided protection against asthma and other allergic diseases and a strong association between vitamin D levels and asthma development, asthma severity, and pulmonary function.[3,4,6–8] However, some studies found no relationship between asthma and vitamin D levels.[9,10]

In humans, the VDR is found on the long arm of chromosome 12 and is composed of 8 introns and 9 exons. The most important single-nucleotide polymorphisms (SNPs) in the VDR gene are *Fok*I, *Apa*I, *Eco*RV, *Bsm*I, *Taq*I, *Tru9*I, *3′ UTR*, *Cdx2*, and *poly*A. Of these, the most studied SNPs are *Fok*I, *Bsm*I, *Apa*I, and *Taq*I.[11] Several epidemiological studies reported an association between 1 or more VDR polymorphisms and asthma,[12–19] whereas other studies found no such association.[20–22]

This study aimed to investigate the effect of VDR gene polymorphisms (*Apa*I, *Taq*I, and *Fok*I) and VDR mRNA

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expression levels on respiratory function, nitric oxide levels in expiratory air, and serum vitamin D levels in children with asthma.

Materials and Methods

Patients' study design

This was a case–control study conducted between January 2012 and June 2012 in Turkish Children. The study consisted of 100 children with asthma (asthma group) and 80 healthy children (control group) between 5 and 18 years of age. All the participants in the asthma group had been diagnosed with long-term asthma at least 1 year before and were receiving regular prophylactic treatment. The diagnosis and classification of the clinical severity of asthma were based on clinical symptoms and lung function according to the Global Initiative for Asthma (GINA) guidelines. They were diagnosed according to the clinical manifestations (cough, wheezing, shortness of breath, and exercise intolerance) in agreement with GINA guidelines and confirmed by spirometry.[1] Patients receiving vitamin D therapy or antiepileptic drugs and those with chronic diseases (eg, cardiorespiratory, metabolic, or endocrine diseases) other than asthma were excluded from the study. We recruited 80 healthy children as control group from the emergency department of our hospital. The children in the control group did not have any history of chronic disease, including asthma, other respiratory disease, liver disease such as hepatitis, renal/urologic disease, bone disease, and metabolic disease. Neither the asthma nor the control group subjects had received vitamin D supplementation in the past 3 years.

Measures

Lung function was measured by maximum expiratory flow volume loops (ZAN 100; Spiromed, Oberthulba, Germany), recording the test with the highest forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), peak expiratory flow (PEF), forced expiratory flow between 25% and 75% of vital capacity (FEF 25%–75%), and FEV1/FVC ratio values as the best of 3 tests. The values were expressed as percent of the predicted for the child's height, age, and gender. These measurements were performed according to the standards of the European Respiratory Society (ERS) and the American Thoracic Society (ATS). Fractional exhaled nitric oxid (FeNO) was analyzed with NioxMinor[®] from Aerocrine (NIOX System; Aerocrine, Sweden) and was performed following the recommendations of the ERS and ATS. In accordance with the ERS/ATS recommendations, low FeNO was defined as a level ≤20 ppb, an intermediate level as 21–34 ppb, and a high level as ≥35 ppb.

Background information regarding the diagnosis, treatment, and monitoring of asthma was obtained from clinical records and parents. Nutritional status was defined according to the patient's body mass index (BMI). The children were categorized as normal weight (BMI in <85th percentile), overweight (BMI in ≥85th and <95th percentiles), or obese (BMI in ≥95th percentile). Serum 25(OH)D was measured using a commercially available kit (Dia Sorin, Saluggia, Italy), and values were reported in nanograms per milliliter. In descriptive analysis, vitamin D levels were categorized as sufficient (>30 ng/mL), insufficient (20–30 ng/mL), or deficient (<20 ng/mL) on the basis of previous recommendations.[23]

A venous blood sample was collected from all subjects for analysis of VDR gene polymorphisms and VDR gene expression, and serum was separated and stored at −80°C until used in the analysis. DNA was isolated from the blood samples using a Pure Link Genomic DNA isolation kit (Catalog No. K182002; Invitrogen, Carlsbad, CA), and DNA polymorphisms were detected using the ABI Prism StepOnePlus™ Real Time System (Applied Biosystems, Foster City, CA) and TaqMan probes. Three different specific and well-characterized VDR SNPs, namely *Fok*I, *Apa*I, and *Taq*I, were assessed. According to allele 1 and allele 2 discrimination, the genotypes were considered homozygous normal (AA), heterozygous mutant (CA), and homozygous mutant (CC) for *Apa*I; homozygote normal (TT), heterozygote mutant (TC), and homozygote mutant (CC) for *Taq*I; and homozygote normal (CC), heterozygote mutant (TC), and homozygote mutant (TT) for *Fok*I.

The amount of RNAs obtained for assessing VDR gene expression was measured using a Qubit device (Invitrogen), following separation of DNA samples using an RNA isolation kit (Invitrogen Ambion[®] RNA Mini Kit-Catalog No. 12183018A; Invitrogen). cDNA synthesis was performed using a high-capacity cDNA synthesis kit (Invitrogen). To analyze VDR gene expression, the real-time polymerase chain reaction was performed using a TaqMan Gene Expression Assay (Invitrogen). Beta-actin was used a housekeeping gene. VDR gene polymorphisms and VDR mRNA expression were studied according to manufacturer's recommendations.[23]

The clinical research ethics committee of our university approved this study, and written informed consent was obtained from all the subjects and their parents.

Statistical analysis

All statistical analyses were performed using the SPSS statistical package, version 21.0 (IBM, SPSS, Inc., Chicago, IL). The characteristics of the 2 patient groups and control group were compared using a chi-square test and *t*-test for categorical and continuous variables, respectively. A two-tailed *P* value of ≤0.05 was considered statistically significant. Genetic risks were assessed by Binary Logistic regression analysis calculating odds ratios (ORs), with their 95% confidence intervals (95% CIs). A chi-square test was performed for Hardy–Weinberg equilibrium (HWE) determination. All genotypes were tested for HWE. The obtained genotype and allele frequencies were compared against the values predicted by the HWE using a chi-square test. Linkage disequilibrium was calculated using Haploview 4.2 software package (Daly Lab, Cambridge, MA). In our study, sample size and formal power calculation were performed as follows: when a power analysis was performed at 80% power and at 0.05 significance level for 25 (OH) vitamin D levels measured in the study, it was calculated that at least 80 optimum 100 cases should be included in the study. An 80% power had been obtained with *post hoc* power analysis according to the effect size. The effect size for three VDR polymorphisms of 25 (OH) Vitamin D levels was 0.319 according to the *F*-test.

Results

Among the asthma group, there were 52 (52%) males and 48 (48%) females. The mean age was 9.5 ± 2.8 (5–16) years. In the control group, there were 42 (51%) males and 38

(49%) females, and the mean age was 9.5 ± 2.5 (5–14) years. There was no statistically significant difference in the age, sex, parental relationship, family history, birth pattern, premature birth history, breastfeeding, use of vitamin D, BMI, or serum vitamin D levels between the asthma group and control group ($P > 0.05$). There was also no statistically significant difference in vitamin D deficiency, insufficiency, or sufficiency between the both groups ($P > 0.05$) (Table 1).

Comparison of the genotype frequencies of the 3 polymorphisms (*Apa*I, *Taq*I, and *Fok*I) in the asthma and control groups revealed no statistically significant difference in the genotype frequencies of *Fok*I polymorphisms ($P > 0.05$). However, a significant association was found between both genotype (CC) of the *Taq*I polymorphism [OR = 0.2, 95% CI (0.07–0.5), $P = 0.003$] and genotype (CA) of *Apa*I polymorphisms [OR = 0.2, 95% CI (0.07–0.8), $P = 0.02$], and asthma risk (Table 2). In addition, there was no statistically significant difference in the allele frequencies of *Apa*I, *Taq*I, and *Fok*I polymorphisms in either the asthma group or those in the control group (Table 3) ($P = 0.05$). There was no statistically significant relationship between vitamin D levels and VDR gene polymorphisms in any of the groups ($P > 0.05$). *Apa*I, *Taq*I, and *Fok*I polymorphisms were not significantly associated with the number of asthma attacks, age at asthma onset, asthma severity, asthma control level, asthma treatment steps, FeNO levels, absolute eosinophil count, serum vitamin D level, positive skin prick test, serum total IgE level, FEV1, FEV1/FVC, and PEF values in the asthma group ($P > 0.05$).

None of the 3 categories of vitamin D levels showed a statistically significant association with age at onset of asthma, asthma severity, asthma treatment steps, number of asthma attacks, skin prick test positivity, serum IgE level, absolute eosinophil count, and FeNO parameters in asthma groups ($P > 0.05$). However, the FEV1 and PEF parameter levels were statistically higher in the sufficiency category of Vitamin D level compared to the other 2 groups ($P = 0.04$ and $P = 0.02$, respectively) (Table 4).

The subjects with mRNA expression of $\geq 2 \mu g/mL$ in the VDR gene were evaluated for gene expression. Among the

TABLE 2. FREQUENCY AND DISTRIBUTION OF GENOTYPES FOR THE VITAMIN D RECEPTOR POLYMORPHISMS IN ASTHMATIC AND CONTROL SUBJECTS

	Asthmatics n (%)	Controls n (%)	P	OR (95% CI)
*Apa*I				
AA	18 (18)	26 (32.5)	0.2	0.6 (0.2–1.4)
CA	60 (60)	42 (52.5)	0.02	0.2 (0.07–0.8)
CC	22 (22)	12 (15)	0.06	
*Taq*I				
CC	8 (8)	20 (25)	0.003	0.2 (0.07–0.5)
TC	61 (61)	32 (40)	0.3	0.6 (0.1–1.9)
TT	31 (31)	28 (35)	0.002	
*Fok*I				
TT	9 (9)	4 (5)	0.8	0.9 (0.4–1.8)
TC	33 (33)	28 (35)	0.2	0.4 (0.1–1.7)
CC	58 (58)	48 (60)	0.5	

The reference genotype for the *Apa*I polymorphism is CC. The reference genotype for the *Taq*I polymorphism is TT.

The reference genotype for the *Fok*I polymorphism is CC. The binary logistic regression test for statistical analysis has been used.

A, adenine; C, cytosine; CI, confidence interval; OR, odds ratio; T, thymine.

asthmatics, mRNA expression was $\geq 2 \mu g/mL$ in 71 patients (30/39 allergic asthma and 41/61 nonallergic asthma), and it was $\geq 2 \mu g/mL$ in 37 cases in the control group. mRNA expression was 4.82 ± 0.97 (2.45–6.87) $\mu g/mL$ in the asthmatics and 5.86 ± 1.63 (2.33–7.87) $\mu g/mL$ in the controls. Compared with the control group, we observed that mRNA expression of VDR decreased in the asthma group ($P = 0.0001$). In addition, we analyzed the relationship between SNPs and VDR mRNA expression in the asthmatics and controls. The mRNA expression levels in the CC and CA genotypes of *Apa*I decreased significantly statistically low in the asthmatic group compared to the control group ($P = 0.01$ and $P = 0.02$, respectively). Similarly, mRNA

TABLE 1. DEMOGRAPHIC AND LABORATORY CHARACTERISTICS OF THE SUBJECTS

Characteristic	Asthma group (n = 100)	Control group (n = 80)	P^a
Age (year) (mean ± SD)	9.5 ± 2.8	9.5 ± 2.5	0.7
Gender, n (%)			
Males	52 (52)	42 (52.5)	0.8
Females	48 (48)	38 (47.5)	0.7
BMI (kg/m^2) (mean ± SD)	17.8 ± 3.4	17.4 ± 3.1	0.4
Breastfeeding, n (%)	92 (92)	70 (87.5)	0.3
Use of supplemental vitamin D^b, n (%)	59 (59)	43 (54.4)	0.6
25-OH vitamin D (ng/mL) (mean ± SD)	22.2 ± 12.3	21.7 ± 6.9	0.8
Vitamin D status, n (%)			
Vitamin D deficiency	45 (58.4)	32 (41.6)	0.2
Vitamin D insufficiency	35 (47.9)	38 (52.1)	0.8
Vitamin D sufficiency	20 (66.7)	10 (33.3)	0.07
Method of birth, n (%)			
Caesarean	32 (32)	24 (30)	0.8
Vaginal delivery	68 (68)	56 (70)	0.8
Consanguinity, n (%)	42 (42)	31 (38.8)	0.2

[a]The distribution of the data was normal according to variance analysis test. The independent sample *t*-test for continuous variables and the Pearson's chi-square test for categorical variables have been used.
[b]Use of supplemental vitamin D in childhood period (400 IU/day).

BMI, body mass index; SD, standard deviation.

TABLE 3. FREQUENCY AND DISTRIBUTION OF ALLELES
FOR THE VITAMIN D RECEPTOR POLYMORPHISMS
IN ASTHMATIC AND CONTROL SUBJECTS

	Asthmatics n (%)	Controls n (%)	P	OR (95% CI)
ApaI			0.05	
A	96 (49)	94 (59)		1.5 (0.9–2.3)
C	104 (51)	66 (41)		
TaqI			0.2	
T	123 (61.5)	88 (55)		1.3 (0.8–1.9)
C	77 (38.5)	72 (45)		
FokI			0.5	
C	149 (74.5)	124 (77.5)		1.1 (0.7–1.9)
T	51 (25.5)	36 (22.5)		

The binary logistic regression test for statistical analysis has been used.

expression levels in the TT and TC genotypes of *TaqI* were significantly lower in the asthma group compared with that in the controls ($P = 0.0001$ and $P = 0.0001$, respectively). However, there was no statistically significant relationship for VDR mRNA expression levels in the *FokI* genotype between asthma and control groups ($P > 0.05$) (Table 5).

Discussion

In this study, we found no significant difference between the asthma and control groups in terms of *FokI* polymor-

TABLE 5. THE EVALUATION OF VITAMIN D RECEPTOR
mRNA EXPRESSION LEVELS (μG/mL) ACCORDING
TO SINGLE-NUCLEOTIDE POLYMORPHISM GENOTYPES
IN ASTHMA AND CONTROL GROUPS

SNP	Asthma (n = 71) (mean ± SD)	Control (n = 37) (mean ± SD)	P
ApaI (n)			
AA	(15) 4.63 ± 0.75	(10) 4.97 ± 0.90	0.7
CA	(42) 4.74 ± 1.02	(21) 6.43 ± 1.65	0.02
CC	(14) 5.26 ± 0.94	(6) 5.44 ± 1.91	0.01
TaqI (n)			
TT	(20) 5.22 ± 0.90	(12) 6.76 ± 1.14	0.0001
TC	(46) 4.72 ± 0.98	(17) 5.96 ± 1.47	0.0001
CC	(5) 4.14 ± 0.61	(8) 4.37 ± 1.66	0.7
FokI (n)			
CC	(7) 4.87 ± 0.78	—	—
TC	(21) 4.66 ± 0.97	(8) 4.84 ± 2.10	0.5
TT	(43) 4.69 ± 1.00	(29) 4.77 ± 1.39	0.6

The independent sample *t*-test for data analysis has been used.
SNP, single-nucleotide polymorphism.

phism genotypes. However, a significant association was found between both genotype (CC) of the *TaqI* polymorphism and genotype (CA) of *ApaI* polymorphisms, and asthma risk. A number of previous studies also reported a statistically significant association between asthma and *TaqI* and *ApaI* polymorphisms.[12–19,24,25] In agreement with the results of this study, Arababadi et al.[26] showed that *TaqI*

TABLE 4. RELATIONSHIP BETWEEN DEMOGRAPHIC, CLINICAL, AND LABORATORY CHARACTERISTICS
AND 25(OH)D LEVEL CATEGORIES

Parameter	Category of 25(OH)D			P
	Sufficiency	Insufficiency	Deficiency	
Age of asthma onset (year) (mean ± SD)	6.6 ± 2.7	4.9 ± 3.4	6.6 ± 3.8	0.09
Asthma severity, n (%)				
Mild	15 (75)	17 (48.6)	28 (62.2)	0.3
Moderate	3 (15)	13 (37.1)	10 (22.2)	
Severe	2 (10)	5 (14.3)	7 (15.6)	
Asthma control, n (%)				
Controlled	17 (85)	21 (60)	27 (60)	0.3
Partially controlled	1 (5)	7 (20)	10 (22.2)	
Uncontrolled	2 (10)	7 (20)	8 (17.8)	
Treatment step, n (%)				
Treatment step 2	11 (55)	15 (42.9)	20 (44.5)	0.7
Treatment step 3	7 (35)	11 (31.4)	15 (33.3)	
Treatment step 4	2 (10)	9 (25.7)	10 (22.2)	
Number of asthma attacks (mean ± SD)	7.8 ± 7.9	12.2 ± 8.7	11.2 ± 10.7	0.8
FEV1 (mean ± SD) (%)	94.5 ± 19.8	81.3 ± 21.7	83.7 ± 16.2	0.04
PEF (mean ± SD) (%)	87.0 ± 25.9	79.8 ± 24.2	71.8 ± 16.1	0.02
Positive skin prick test, n (%)	5 (12.8)	15 (38.5)	19 (48.7)	0.3
Total IgE level (mean ± SD)	446.2 ± 563.6	286.4 ± 370.5	341.1 ± 560.9	0.5
Peripheral eosinophilia (mean ± SD)	354.5 ± 157.5	364.6 ± 299.2	265.8 ± 214.5	0.1
FeNO (ppb) (mean ± SD)	20.0 ± 8.9	27.7 ± 17.3	27.8 ± 22	0.2
FeNO n (%)				
≤20 ppb	12 (60)	16 (45.7)	26 (57.8)	0.6
21–34 ppb	5 (25)	9 (25.7)	8 (17.8)	
≥35 ppb	3 (15)	10 (28.6)	11 (24.4)	

The ANOVA and chi-square tests for comparison of more than 2 groups have been used.
ANOVA, analysis of variance; FeNO, fractional nitric oxide in exhaled breath; FEV1, forced expiratory volume in 1 s; PEF, peak expiratory flow.

polymorphism was more frequent in asthmatic patients compared with that in healthy controls. In a case–control study in Cypriot adolescents, the *Taq*I homozygous genotype was associated with wheezing and asthma.[18] Maalmi et al.[14] evaluated VDR SNPs in Tunisian children with asthma and reported that *Fok*I, *Bsm*I, and *Taq*I polymorphisms were associated with asthma. Hutchinson et al.[25] found a relationship between asthma and *Taq*I and *Apa*I polymorphisms in Irish children.

Wjst[22] investigated 13 SNPs, including *Fok*I, *Taq*I, and *Apa*I polymorphisms, and failed to find a significant association between asthma and any polymorphism. Pillai et al.[20] also found no association between VDR genetic polymorphisms and asthma development in a study of African Americans. The Childhood Asthma Management Program study examined 7 VDR SNPs in 3 different ethnic groups (Caucasians, African Americans, and Hispanics) and found a significant association between *Apa*I and asthma in a genetic analysis stratified according to the ethnic group.[13] The family-based Nurses' Health Study identified a significant relationship between the *Taq*I polymorphism and asthma.[15] The same study reported that the *Taq*I polymorphism was significantly associated with serum IgE levels. Han et al.[17] suggested that *Fok*I, *Apa*I, *Taq*I, and rs3782905 polymorphisms in the VDR may contribute to asthma development. Saadi et al.[16] studied *Fok*I, *Dde*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms in a Han population in China and reported that the *Apa*I polymorphism was associated with asthma. A case–control study in Canada of 12 VDR SNPs in individuals 3–80 years of age found a statistically significant relationship among 6 of the SNPs (rs3782905, rs1540339, rs2239185, rs2239182, *Bsm*I, and *Taq*I) with asthma. In this study, researchers also reported that the T-allele of the *Taq*I polymorphism showed a statistically significant relationship with asthma and atopy.[12] In the majority of the aforementioned studies, VDR polymorphisms were considered independent risk factors for asthma susceptibility.

In a meta-analysis study evaluating the relationship between asthma and VDR SNP, including *Apa*I, *Bsm*I, *Fok*I, and *Taq*I, they found a statistically significant relationship between the homozygous genotype of *Apa*I polymorphism and childhood asthma. Stratification by ethnicity revealed a statistical association in Asians. However, the authors reported that there may be no relationship between *Taq*I polymorphism and the risk of childhood asthma. In addition, they have reported that *Fok*I polymorphism may be connected with pediatric asthma in Caucasian population and *Bsm*I polymorphism marginally contributes to childhood asthma susceptibility.[27] In another meta-analysis evaluating 8 case–control studies, *Fok*I, *Taq*I, and *Bsm*I VDR polymorphisms were significantly associated with an increased risk of asthma in some populations. In this meta-analysis, the authors suggested that serum 25(OH)D levels and various environmental factors could modify the effect of these polymorphisms on asthma development.[15]

In our study, VDR SNPs were not significantly associated with FeNO, serum 25(OH)D, IgE level, eosinophil counts, skin prick test positivity, respiratory function test parameters, or acute asthma attacks. However, some studies established a relationship between asthma and the *Taq*I polymorphism, reporting a statistically significant association between this polymorphism and FeNO, IgE levels, eosinophil counts, skin test positivity, respiratory function

test parameters, and acute asthma attacks.[12–14] Poon et al.[12] reported a relationship between 4 SNP alleles (rs2239185C, *Bsm*IG, *Apa*IC, and *Taq*IT) and atopy. They also found a significant association between high IgE levels and rs2239185C, *Apa*IC, and *Taq*IT alleles. Maalmi et al.[14] reported that VDR polymorphisms showed a statistically significant association with serum vitamin D levels and asthma severity.

A number of factors may explain the variable results of VDR SNP studies in different populations. Although the effects of VDR polymorphisms on bone metabolism are similar, gene–gene and gene–environment interactions vary in different populations,[28,29] and these variations largely determine the relationship between VDR polymorphisms and asthma.[30] Due to stratification in the population, it is often difficult to detect the actual effect of genes in case–control samples, with stratification reversing, altering, or masking the true genetic effect of gene polymorphisms in research. In addition, other variables, such as ethnicity, climate, geographical features of the place, and lifestyle, may explain the inconsistent findings of different VDR SNP studies.[15,20]

According to the vitamin D workshop consensus on vitamin D nutritional guidelines published by Henry et al.,[31] ~50% of North America and Western Europe and two-thirds of the world's remaining population have vitamin D deficiency, and the prevalence of vitamin D deficiency is increasing worldwide. According to the CAMP study by Brehm et al.,[32] of 1,024 North American children with moderate to severe asthma, vitamin D levels were insufficient in 35% of these children. In a study conducted in Qatar, vitamin D levels were lower than 20 ng/mL in 68.1% of asthmatic children and in 36.1% of children in a control group, with a statistically significant difference between the 2 groups.[4] In this study, 76% of asthmatic patients and 87% of healthy controls had vitamin D insufficiency (<30 ng/mL), but the between-group difference was not statistically significant. Some studies reported similar results to our data.[5,10,33] Vitamin D deficiency in asthmatic patients may be due to many factors, such as obesity, drug use, chronic illnesses, poor nutrition, climate features, and low sun exposure. The latter may be the result of geographical location, season, lifestyle (eg, an excessive time spent remaining indoors), and type of clothing worn due to religious beliefs.[34]

Previous research reported that the *Fok*I polymorphism in the VDR gene resulted in reduced protein expression of 3 amino acids, but 1.7 times higher activity of transcription. Although the *Taq*I and *Apa*I SNPs are not functional, they are thought to be associated with other polymorphisms that are functional and to participate in a complex gene network, which enhances or inhibits the expression of VDR target genes.[35] Previous research revealed a relationship between VDR SNPs and VDR mRNA expression levels, but found no relationship between VDR mRNA expression levels and vitamin D levels.[36] Based on our results, we speculate that a significant reduction of mRNA gene expression in *Taq*I and *Apa*I polymorphisms may contribute to asthma physiopathology by inhibiting vitamin D immunomodulation and immunosuppression functions. Within the cell, active 1-25(OH)2D3 exerts its effects by 2 pathways: genomic and nongenomic. Most of the biological effects of vitamin D involve the genomic pathway. The extent of these biological effects depends on the cellular VDR content, VDR genetic

polymorphisms, and nuclear transcriptional mRNA. As the function of a gene can be assessed by measuring the level of mRNA expression, mRNA (RNA transcripts) can serve as an important biomarker for the diagnosis and course of diseases.[35,37]

This study has some limitations. First, we did not evaluate the effect of various factors, such as nutritional habits, number of hours of sunshine exposure per day, and type of clothing worn, that can influence vitamin D levels. Second, among the asthmatic patients, there were only a few cases of uncontrolled asthma and severe persistent asthma. Third, despite the presence of many functional SNPs in the VDR gene, we studied only 3 polymorphisms. We did not examine the cumulative effect of all SNPs thought to play a role in the development of asthma, other genes involved in vitamin D metabolism, or environmental risk factors that influence asthma development. These topics can be considered subjects for future study.

In conclusion, we found no statistically significant difference in vitamin D levels between the asthmatic patients and the control group. However, a significant association was found between both genotype (CC) of the *Taq*I polymorphism and genotype (CA) of *Apa*I polymorphisms, and asthma risk. In addition, mRNA gene expression was significantly reduced in *Apa*I and *Taq*I polymorphisms in asthmatic group. The results of this study provide supporting evidence for the association of *Taq*I and *Apa*I polymorphisms with asthma susceptibility. Improved knowledge of the association of *Apa*I and *Taq*I polymorphisms with asthma may contribute to a better understanding of asthma genetics.

Author Disclosure Statement

No competing financial interests exist.

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