Influence of vitamin D receptor gene *Fok*I and *Apa*I polymorphisms on glucocorticoid response in patients with asthma

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Background: Glucocorticoid (GC)-resistant asthma, a complex disease phenotype, has a high morbidity and mortality and takes up a disproportionate share of healthcare costs. The aim of this work was to assess serum 25-hydroxyvitamin D (25(OH)VD) levels in steroid-resistant, steroid-sensitive patients with asthma and in healthy controls, and to investigate the association between the vitamin D receptor gene (*VDR*) *Fok*I and *Apa*I polymorphisms and GC resistance in patients with asthma.

Methods: This case-control study included 70 patients with severe bronchial asthma and 30 apparently healthy controls. Atopic status was determined by skin-prick test reaction to the most common locally-encountered allergens. A GC reversibility test was performed to differentiate between GC-sensitive and GC-resistant asthma. For all subjects, analysis of the *VDR Fok*I and *Apa*I polymorphisms by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and measurement of serum 25(OH)VD levels by enzyme-linked immunosorbent assay (ELISA) were performed.

Results: The frequencies of *Fok*I polymorphism genotypes and alleles differed significantly between patients with asthma and controls. The frequencies of the TT genotype and T allele carriers were significantly higher among patients with asthma than among controls, and also among GC-resistant patients with asthma than among GC-sensitive patients with asthma. Additionally, serum 25(OH)VD levels differed significantly among the 3 *VDR Fok*I polymorphic genotypes in GC-resistant patients with asthma; the highest level was detected in the TT genotype. No significant differences in *Apa*I were found.

Conclusion: We found a possible association between the *Fok*I T allele and GC resistance in patients with asthma. Variations in *VDR Fok*I might also play a role in 25(OH)VD levels. © 2019 ARS-AAOA, LLC.

Key Words:
bronchial asthma; glucocorticoid resistance; vitamin D receptor; vitamin D; single nucleotide polymorphism; *Fok*I; *Apa*I

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Bronchial asthma has become the world's most common chronic respiratory disease, and its incidence and mortality have been on the rise in recent years.[1] Glucocorticoids (GCs) are currently the gold standard for controlling inflammation in patients with asthma.[2] However, up to 50% of patients with asthma may not respond well to inhaled corticosteroids (ICSs), and up to 25% patients with difficult to control asthma may not respond well to oral GCs.[3] This makes GC resistance in asthma a challenging healthcare problem associated with significant morbidity and life-threatening disease progression.[4] Although multiple molecular mechanisms that contribute to GC resistance have been described, the exact mechanism of insensitivity has not been fully elucidated and is an area of active research.[4]

Vitamin D has numerous effects on the immune system. It modulates T cell proliferation, resulting in a switch from a T helper 1 (Th1) phenotype to a Th2 phenotype. Furthermore, vitamin D can inhibit the synthesis, secretion, and release of Th1 cell anti-inflammatory cytokines (interleukin 4 [IL-4] and IL-10), while inducing those of Th2 cell pro-inflammatory cytokines (IL-1, tumor necrosis factor α [TNF-α], interferon γ [IFN-γ]).[5] Vitamin D deficiency has been linked to several autoimmune diseases, including

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systemic lupus erythematosus,[6] multiple sclerosis,[7] and myasthenia gravis,[8] as well as thyroid diseases[9] and inflammatory bowel disease.[10]

It has been reported that reduced vitamin D levels are associated with asthma severity[11] and increased airway hyperreactivity, leading to increased requirements for higher-dose corticosteroids.[12,13] In patients with steroid-resistant asthma, there appear to be defects in GC-mediated gene transcription of anti-inflammatory mediators such as IL-10 and mitogen-activated protein kinase phosphatase-1 (MKP-1).[14,15] These changes may be reverted by vitamin D.[16] Vitamin D also could inhibit IL-17A,[17] "regulated upon activation, normal T-cell expressed and secreted" (RANTES), and fractalkine secretion,[18] adding to the mechanism for reversal of steroid resistance. Furthermore, it has been reported that vitamin D may prevent the conversion of CD8+ T cells from IFN-γ–producing to IL-13–producing cells, a pathogenic type that has been detected in GC-resistant patients with asthma.[19]

For vitamin D to function properly, its receptor must be produced and function correctly. The vitamin D receptor (VDR) is a nuclear protein, composed of 437 amino acids, and is encoded by the vitamin D receptor gene (*VDR*) located on chromosome 12, which has been linked to asthma or related phenotypes in different populations.[20] *VDR* is composed of 11 exons and spans 75 kilobases (kb). More than 900 single-nucleotide polymorphisms (SNPs) have been identified in this gene, including coding and noncoding regions. Most of these SNPs are concentrated in exons 2 and 3, which are responsible for encoding the DNA binding domain. Alterations in these 2 exons modify the zinc finger that binds to DNA, generating a deformation at the receptor, which prevents the vitamin from binding.[21] Changes in the 5′ region of the *VDR* gene promoter can alter expression patterns and messenger RNA (mRNA) levels, whereas variations in the 3′ untranslated region (3′ UTR) may affect mRNA stability and the efficiency of protein translation.[22] In this case-control study, we tested 2 polymorphisms of the *VDR* gene that are known to have a specified extent for asthma and/or other pulmonary diseases, as described in earlier research among different populations: rs2228570 (*Fok*I)[23] and rs7975232 (*Apa*I).[24]

The *VDR Fok*I SNP is located in exon 2 and results in the substitution of thymine (T) by the cytosine (C) at the first ATG site (ATG to ACG). This substitution affects the VDR protein, resulting in the synthesis of a 3–amino acid shorter protein compared to the one synthesized in the presence of the T allele.[25] In vitro study showed a 1.7-fold higher activity of the shorter form of the VDR protein.[25] The *VDR Apa*I SNP is located in intron 8 and results in the substitution of a C allele for an A allele. This SNP may have a possible influence on mRNA stability.[26]

Our first aim was to evaluate serum 25-hydroxyvitamin D (25(OH)VD) levels in steroid-resistant, steroid-sensitive patients with asthma, and in healthy controls. The second aim was to investigate the influence of the *VDR Fok*I and *Apa*I polymorphisms on GC resistance in patients with asthma.

Patients and methods

Subjects and sample collection

Prior to initiation, written informed consent was obtained from the study participants after explaining the aim and procedures of the study and ensuring the confidentiality of the data. The study was carried out after the approval of the Ain Shams University Ethics Committee. This case-control study was conducted at Ain Shams University Hospital from April 2017 to April 2019, and included 70 genetically unrelated patients with asthma. The patients were recruited from the Allergy and Clinical Immunology outpatient clinic. Asthma diagnosis was established according to Global Initiative for Asthma (GINA) guidelines.[27] Atopy was defined when there was a positive skin-prick test (SPT) reaction to at least 1 of the common environmental allergens. Exclusion criteria included any similar clinical manifestations not following GINA guidelines, occupational asthma with ongoing antigenic exposure, gastroesophageal reflux, food allergy, aspirin or nonsteroidal anti-inflammatory drug (NSAID) sensitivity, pregnancy, cigarette smoking, and established patients with asthma suffering from immunocompromised or chronic inflammatory diseases such as cancer, diabetes, or tuberculosis (TB). Furthermore, patients using drugs that might induce resistance to GCs (including rifampicin, phenobarbital, phenytoin, and ephedrine) and subjects with signs of viral infections, either generalized or affecting the respiratory tract, were also excluded from the study. A total of 30 genetically unrelated healthy subjects with negative SPT reactions, no respiratory symptoms, and no family history for allergy admitted to the hospital during the same period were enrolled as the control group.

Two venous blood samples (5 mL each) were obtained by venipuncture from each participant. The first sample was collected into a gel Vacutainer tube (Becton Dickinson, Oxford, UK). Blood was allowed to clot and serum was separated by centrifugation (3500 rpm, 1200 g, 15 minutes, 25°C) and then stored in aliquots at −20°C until used for measurement of serum 25(OH)VD levels. The second sample was collected on a K3EDTA Vacutainer tube (Becton Dickinson) and blood was used for genotyping of *VDR Fok*I and *Apa*I polymorphisms.

Measurement of serum 25(OH)VD levels

Because 25(OH)VD is the main circulating form of vitamin D, it is widely used to assess vitamin D status. Vitamin D deficiency was defined as a 25(OH)VD serum level of <20 ng/mL, whereas vitamin D insufficiency was defined as a 25(OH)VD serum level of 20 to 30 ng/mL, and finally, vitamin D sufficiency was defined as a 25(OH)VD serum level of >30 ng/mL.[28] Serum 25(OH)VD levels were estimated using an enzyme-linked immunosorbent assay

(ELISA) kit supplied by Calbiotech, Inc. (El Cajon, CA; catalog# VD220B).

GC reversibility test

First, spirometry was done to diagnose patients with asthma. A GC reversibility test was then carried out to stratify GC-resistant patients with asthma (n = 34) from GC-sensitive patients with asthma (n = 36). A failure to show improvement in forced expiratory volume in the first second (FEV1) of 15% after an adequate course of GCs (oral prednisolone 40 mg/day for 2 weeks), despite reversibility (>15%) with β2-agonists, defines GC-resistant asthma. On the contrary, improvement of 30% in FEV1 following identical doses of GCs defines GC-sensitive asthma. Spirometry and GC reversibility test were carried out at the Pulmonary Functions Laboratory, Ain Shams University Hospital. Assessment followed the standards of the American Thoracic Society (ATS) and the European Respiratory Society (ERS).[29]

Detection of *VDR Fok*I and *Apa*I polymorphisms by restriction fragment length polymorphism analysis

DNA extraction

DNA extraction was done using the QIAamp DNA Mini Kit supplied by Qiagen (Hilden, Germany). DNA in the sample was liberated using proteinase K solution and lysis buffer. Released DNA was bound exclusively and specifically to the QIAamp membrane in the presence of binding buffer under appropriate salt and pH conditions. Denatured protein and other contaminants were removed with several washing procedures. The DNA was then eluted from the membrane with elution buffer.

Polymerase chain reaction

For detection of *VDR Fok*I (T>C) and *Apa*I (C>A) polymorphisms, whose amplified products are 265-basepair (bp) and 740-bp fragments, respectively, amplification was performed using a thermal cycler. Both polymerase chain reaction (PCR) products were carried out in a volume of 50 µL containing 5 µL genomic DNA, 25 µL of the ready to use master mix supplied by Qiagen, 2.5 µL (25 pmol) forward primer, 2.5 µL (25 pmol) reverse primer, and 15 µL deionized water. The test primers were chosen according to Despotovic et al.[30] and prepared by Promega (Madison, WI).

For SNP *Fok*I:

*Fok*I forward primer = 5′-AGCTGGCCCTGGCAC TGACTCTGCGTCT-3′
*Fok*I reverse primer = 5′-ATGGAAACACCTTGCT TCTTCTCCCTC-3′

For SNP *Apa*I:

*Apa*I forward primer = 5′-CAGAGCATGGACAGG GAGCAA-3′
*Apa*I reverse primer = 5′-GAGACCTCAGCCATGA GGAGTTGC-3′

PCR conditions were an initial denaturation step at 94°C for 5 minutes; 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds; then final extension at 72°C for 5 minutes.

Restriction fragment length polymorphism

Restriction digestion for VDR FokI SNP. The digestion mixture for *Fok*I was performed using a 0.5-µL (5000 units/mL) *Fok*I restriction enzyme supplied by New England BioLabs (Beverly, MA); restriction site: (5′GGATG $(N)_9 \downarrow$ 3′) (3′CCTAC$(N)_{13}\uparrow$ 5′). The enzyme was added to 5 µL PCR-amplified product, 18.5 µL deionized water, and 1 µL buffer (10× of 60mM Tris-HCL), followed by a gentle mix. Then the tubes were placed on a heat block for 4 hours at 37°C. The enzyme was inactivated at 65°C for 20 minutes. As experimental control, a no-enzyme "mock" digest was used.

Restriction digestion for VDR ApaI SNP. The digestion mixture for *Apa*I was performed using a 0.5-µL (50,000 units/mL) *Apa*I restriction enzyme supplied by New England BioLabs; restriction site: (5′GGGCC ↓C 3′) (3′ C↑CCGGG 5′). The enzyme was added to 5 µL PCR-amplified product, 18.5 µL deionized water, and 1 µL buffer (10× of 60mM Tris-HCL), followed by a gentle mix. Then the tubes were placed on a heat block for 4 hours at 25°C. The enzyme was inactivated at 65°C for 20 minutes. As experimental control, a no-enzyme "mock" digest was used.

DNA analysis by gel electrophoresis. Amplified product of DNA samples and restriction fragments were run on 2% agarose gel (Promega) for 30 minutes at 100 V, stained with ethidium bromide (Amresco, Germany). A 100-bp DNA ladder (Promega) was also run to identify the site of bands. The gel was examined under an ultraviolet transilluminator (Biometra GmbH, Göttingen, Germany).

Genotypes of *VDR Fok*I (T>C) and VDR *Apa*I (C>A) polymorphisms were defined as follows, according to Despotovic et al.[30]

For *VDR Fok*I (T>C) polymorphism (Fig. 1)

Homozygous CC genotype if the band was not divided: 265 bp.
Homozygous TT genotype if the band is divided into 2 parts: 196 bp and 69 bp.
Heterozygous CT genotype if it yields 3 bands: 265 bp, 196 bp, and 69 bp.

For *VDR Apa*I (C>A) polymorphism (Fig. 2)

Homozygous AA genotype if the band was not divided: 740 bp

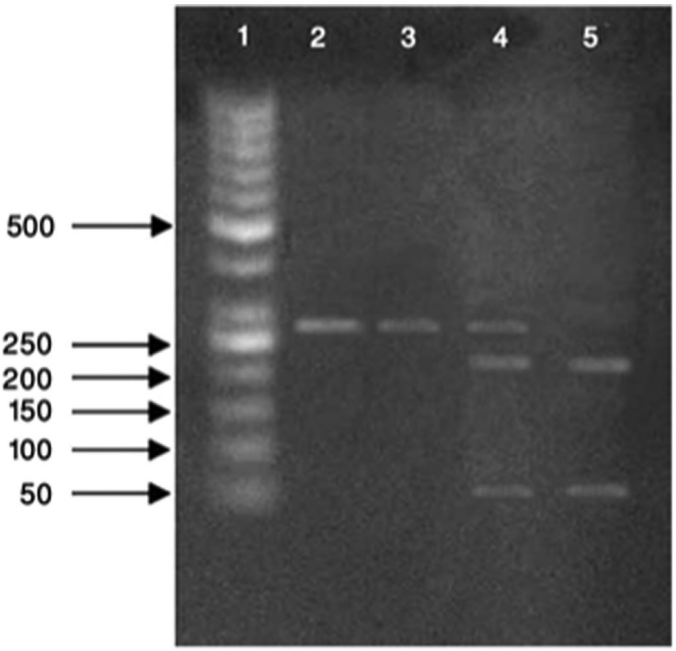

FIGURE 1. Agarose gel electrophoresis of *VDR Fok*I (T>C) fragments stained with ethidium bromide. Lane 1: 100-bp ladder. Lane 2 represents unrestricted fragment; 1 band = 265 bp (experimental control: no enzyme "mock"). Lane 3 shows homozygous (CC) genotype; 1 band = 265 bp. Lane 4 shows heterozygous (CT) genotype; 3 bands = 265 bp, 196 bp, and 69 bp. Lane 5 shows homozygous (TT) genotype; 2 bands = 196 bp and 69 bp.

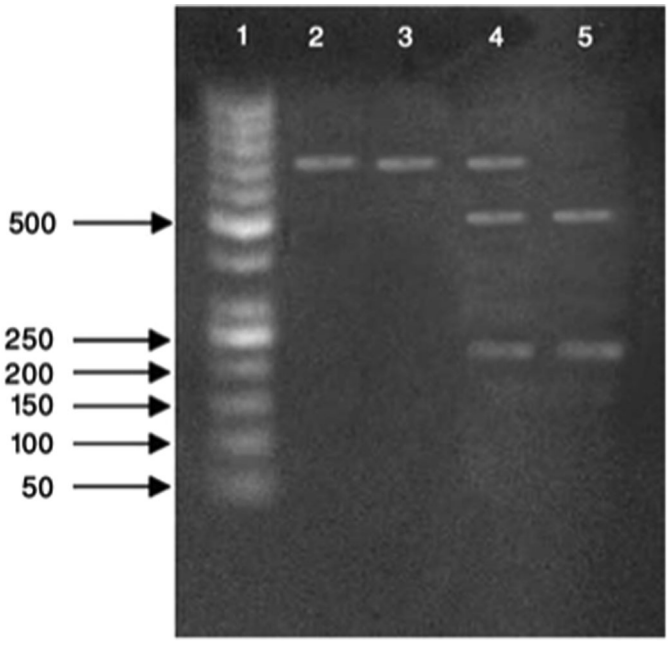

FIGURE 2. Agarose gel electrophoresis of *VDR ApaI* (C>A) fragments stained with ethidium bromide. Lane 1: 100-bp ladder. Lane 2 represents unrestricted fragment; 1 band = 740 bp (experimental control: no enzyme "mock"). Lane 3 shows homozygous (AA) genotype; 1 band = 740 bp. Lane 4 shows heterozygous (AC) genotype; 3 bands = 740 bp, 515 bp, and 225 bp. Lane 5 shows homozygous (CC) genotype; 2 bands = 515 bp and 225 bp.

Homozygous CC genotype if the band is divided into 2 parts: 515 bp and 225 bp.
Heterozygous AC genotype if it yields 3 bands: 740 bp, 515 bp, and 225 bp.

Statistical analysis

Analysis of data was performed using the SPSS program, version 20 (IBM Corp., Armonk, NY). Data are expressed as mean ± standard deviation (SD) for quantitative parametric measures, and number and percentage for categorized data. For parametric data, Student *t* test was used to compare the means of 2 groups, whereas the 1-way analysis of variance (ANOVA) test was used to compare the means of 3 groups, followed by Tukey test as a post hoc test to identify significant differences between means. Chi-square (χ^2) test was used to compare categorical data. Obtained values of genotype and allele frequencies were compared to the values predicted by Hardy-Weinberg equilibrium (HWE) using the χ^2 test. The odds ratios (ORs) as a measure of relative risk, and their 95% confidence intervals (CIs) were also calculated. The strength of the relationship between serum 25(OH)VD levels and FEV1 values was assessed using Pearson's method. All p values are 2-sided. Values of $p < 0.05$ were considered significant.

Results

Demographic, clinical, and biochemical data of the study population

This study included 70 adult patients with severe bronchial asthma (27 females, 43 males) and 30 controls. Patients with severe asthma were subdivided into 2 subgroups: GC-sensitive (n = 36) and GC-resistant (n = 34). Age, sex, body mass index (BMI), and atopic status were comparable among both subgroups. Significantly higher FEV1 values were observed among GC-sensitive patients with asthma after prednisolone therapy, as compared to GC-resistant patients with asthma (Table 1).

Frequencies of *VDR Fok*I polymorphism genotypes and alleles in the 3 groups

There was a highly significant difference in frequencies of *VDR Fok*I polymorphism genotypes and alleles between patients with asthma and controls. The TT genotype was more frequent among patients with asthma than among controls, the CC genotype was more frequent among controls than among patients with asthma, and the CT genotype did not differ in distribution between patients with asthma and controls. Moreover, almost all controls were C allele carriers in comparison to patients with asthma, whereas almost all patients with asthma were T allele carriers. Additionally, a significant difference was observed in frequencies of *VDR Fok*I polymorphism genotypes and alleles between GC-sensitive and GC-resistant patients with asthma, with a greater frequency of the TT genotype distribution as well as T allele carriers among GC-resistant

TABLE 1. Demographic, clinical, and biochemical data of the study population

Variable	GC-sensitive patients (n = 36)	GC-resistant patients (n = 34)	Control (n = 30)	*p*
Age (years), mean ± SD[a]	40.8 ± 8.5	41.9 ± 8.4	40.7 ± 7.8	0.80
Sex, n (%)[b]				
Female	14 (38.9)	13 (38.2)	13 (43.3)	0.90
Male	22 (61.1)	21 (61.8)	17 (56.7)	
BMI (kg/m^2), mean ± SD[a]	24.7 ± 3.5	24.6 ± 3.5	24.9 ± 3.4	0.94
FEV1 (%), mean ± SD[a]				
Before prednisolone	55.7 ± 2.5	54.7 ± 2.4	–	0.09
After prednisolone	69.9 ± 4.1	54.9 ± 2.5	–	<0.001
Atopic status, n (%)[b]				
Atopic	17 (47.2)	21 (61.8)	–	0.22
Non-atopic	19 (52.8)	13 (38.2)	–	

[a]Compared using ANOVA test.
[b]Compared using χ^2 test.
ANOVA = analysis of variance; FEV1 = forced expiratory volume in 1 second; GC = glucocorticoid; SD = standard deviation.

patients with asthma than among GC-sensitive patients with asthma (Table 2).

Frequencies of *VDR Apa*I polymorphism genotypes and alleles in the 3 groups

No statistically significant differences were observed in the distribution of *VDR Apa*I genotypes between patients with asthma and healthy subjects. Additionally, a nonsignificant difference was found in frequencies of *VDR Apa*I genotypes and alleles between GC-sensitive and GC-resistant patients with asthma (Table 3).

Notably, for the 2 polymorphisms tested in this study within the *VDR* gene sequence, genotype distributions were in HWE in both patients and controls ($p > 0.05$) (Tables 2 and 3).

Serum 25(OH)VD and *VDR* polymorphisms

In the complete sample (n = 100), 28 (28.0%) had sufficient levels of 25(OH)VD, 40 (40.0%) had insufficient levels, and 32 (32.0%) had deficient levels. 25(OH)VD status was similar between patients with asthma and controls ($p = 0.50$). However, serum 25(OH)VD levels were significantly lower in patients with asthma compared to controls. Moreover, 25(OH)VD levels were significantly higher in GC-resistant compared to GC-sensitive patients with asthma (Table 4).

Serum 25(OH)VD levels differed significantly among the 3 *VDR Fok*I polymorphic genotypes in GC-resistant patients with asthma ($p < 0.001$) and the highest level was detected in the TT genotype (Supporting Table 1).On the other hand, a nonsignificant association was observed between serum 25(OH)VD levels and *VDR Apa*I polymorphic genotypes and alleles (Supporting Table 1).

Noteworthy, there was no correlation between serum 25(OH)VD levels and FEV1 values before and after prednisolone therapy in both GC-resistant and GC-sensitive patients with asthma ($p > 0.05$). Additionally, a nonsignificant association was observed between the 2 polymorphisms investigated in this study and FEV1 values before and after prednisolone therapy in both GC-resistant and GC-sensitive patients with asthma ($p > 0.05$).

Discussion

Several *VDR* gene SNPs alter the function of vitamin D, thus preventing or hindering its activity, even in individuals with normal levels of the vitamin.[31] Numerous studies classified the *VDR* gene as 1 of the candidate genes of asthma[24,32,33]; however, few studies have addressed the relationship between *VDR* gene polymorphisms and response to therapy among patients with asthma.[31,34] Among the most studied *VDR* gene SNPs are those located in exon 2 (*Fok*I, rs2228570), intron 8 (*Bsm*I, rs1544410; *Apa*I, rs7975232), and exon 9 (*Taq*I, rs731236), named by the restriction enzymes used for their detection.[30] To the best of our knowledge, no previous studies have been conducted on the association between *VDR Fok*I and *Apa*I polymorphisms and GC resistance among patients with asthma. It is worth mentioning that identification of candidate genes, influencing response to treatment of multifactorial disease, helps in the development of interventional strategies which are safe, effective, necessary, and individualized.[35]

The *VDR Fok*I SNP investigated in the present study is known to have a specified extent for asthma and/or other pulmonary diseases,[23,33] not in the linkage disequilibrium (LD) with other *VDR* SNPs and considered as an

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TABLE 2. *Fok*I SNP distribution among the study groups

*Fok*I (T>C) polymorphism	Patients (n = 70) n (%)	Controls (n = 30) n (%)	OR (95% CI)	*p*
Genotype				0.004
CC	20 (28.6)	13 (43.3)	0.52 (0.21–1.27)	
TT	36 (51.4)	5 (16.7)	5.29 (1.81–15.41)	
CT	14 (20.0)	12 (40.0)	0.37 (0.14–0.95)	
HWE				0.15
C allele carriers[a]	34 (40.5)	25 (59.5)	0.46 (0.22–0.98)	0.04
T allele carriers[b]	50 (59.5)	17 (40.5)	2.16 (1.01–4.59)	
	GC-sensitive patients (n = 36) n (%)	GC-resistant patients (n = 34) n (%)	OR (95% CI)	*p*
Genotype				0.004
CC	16 (44.5)	4 (11.8)	0.16 (0.05–0.57)	
TT	12 (33.3)	23 (67.6)	4.18 (1.54–11.34)	
CT	8 (22.2)	7 (20.6)	0.90 (0.29–2.84)	
C allele carriers[a]	24 (54.5)	11 (26.8)	0.31 (0.12–0.76)	0.01
T allele carriers[b]	20 (45.5)	30 (73.2)	3.27 (1.31–8.13)	

[a]Subjects with CC or CT genotypes.
[b]Subjects with TT or CT genotypes.
HWE = Hardy-Weinberg equilibrium.

independent marker of the *VDR* gene, as well as it is functional and have impact on VDR protein synthesis.[30] The second selected *VDR* gene variant, *Apa*I, was studied because of its possible influence on mRNA stability.[30]

Regarding the *VDR Fok*I SNP, a highly significant difference in frequencies of *Fok*I polymorphism genotypes and alleles was observed between patients with asthma and controls; the TT genotype was more frequent in patients with asthma compared with control subjects, whereas the CC genotype was less frequent in patients with asthma compared with control subjects. Our findings were in line with several studies that evaluated the association of *VDR Fok*I SNP with asthma susceptibility. Although many studies demonstrated a protective effect of C allele on asthma development,[30,36] some studies could not confirm this finding.[24,31,35] A significantly higher prevalence of the C allele in healthy subjects could be the result of evolutional adaptation in human population because the shorter VDR protein (424 amino acids), synthesized in the presence of the CC genotype, has a higher affinity for 1,25(OH)2D3 binding and higher transcriptional activity compared to the longer protein synthesized in the presence of the TT genotype (427 amino acids).[25]

Regarding the association between the *VDR Fok*I polymorphism and resistance to steroid therapy, a significant difference was observed in frequencies of *VDR Fok*I polymorphism genotypes and alleles between GC-sensitive and GC-resistant patients with asthma, with a greater frequency of the TT genotype distribution as well as T allele carriers among GC-resistant patients with asthma than among GC-sensitive patients with asthma. Noteworthy, it was demonstrated that the presence of the T allele results in a threonine-methionine change and addition of 3 amino acids, which makes the protein less functionally active than its wild-type counterpart.[25] The association between *Fok*I and asthma control has been reported in a recent study that demonstrated an association between the *VDR Fok*I SNP C allele and the requirement of therapy to reach asthma control.[31]

In the current study, it was found that serum 25(OH)VD levels were significantly lower in patients with asthma compared to controls. However, serum 25(OH)VD levels were significantly higher in GC-resistant compared to GC-sensitive patients with asthma. This finding was partly in accord with Einisman et al.,[31] who observed a higher proportion of 25(OH)VD sufficiency in patients with asthma with uncontrolled status at step 4 of the GINA treatment compared to those with controlled status. Nevertheless, 2 studies[11,13] found an inverse relationship between 25(OH)VD levels and ICS doses, which contradicts our finding.

Furthermore, we observed that serum 25(OH)VD levels differed significantly among the 3 *VDR* gene *Fok*I polymorphic genotypes in GC-resistant patients with asthma

TABLE 3. *Apa*I SNP distribution among the study groups

*Apa*I (C>A) polymorphism	Patients (n = 70) n (%)	Controls (n = 30) n (%)	OR (95% CI)	*p*
Genotypes				0.62
AA	15 (21.5)	6 (20.0)	1.09 (0.37–3.15)	
CC	36 (51.4)	13 (43.3)	1.38 (0.58–3.27)	
AC	19 (27.1)	11 (36.7)	0.64 (0.25–1.59)	
HWE				0.16
A allele carriers[a]	34 (38.2)	17 (41.5)	0.87 (0.41–1.85)	0.72
C allele carriers[b]	55 (61.8)	24 (58.5)	1.14 (0.53–2.43)	
	GC-sensitive patients (n = 36) n (%)	GC-resistant patients (n = 34) n (%)	OR (95% CI)	*p*
Genotypes				0.41
AA	10 (27.8)	5 (14.7)	0.44 (0.13–1.48)	
CC	17 (47.2)	19 (55.9)	1.41 (0.55–3.62)	
AC	9 (25.0)	10 (29.4)	1.25 (0.43–3.59)	
A allele carriers[a]	19 (42.2)	15 (34.0)	0.70 (0.29–1.67)	0.42
C allele carriers[b]	26 (57.8)	29 (66.0)	1.41 (0.59–3.33)	

[a]Subjects with AA or AC genotypes.
[b]Subjects with CC or AC genotypes.
HWE = Hardy-Weinberg equilibrium.

TABLE 4. 25(OH)VD levels among the study groups

Group	25(OH)VD levels (ng/mL) (mean ± SD)	Category of 25(OH)VD		
		Sufficient n (%)	Insufficient n (%)	Deficient n (%)
Patients (n = 70)	24.3 ± 8.5	20 (28.6)	30 (42.8%)	20 (28.6%)
Controls (n = 30)	29.5 ± 16.1	8 (26.7)	10 (33.3%)	12 (40.0%)
p	0.03	0.50		
GC-sensitive patients (n = 36)	19.1 ± 5.4	1 (2.8)	21 (58.3%)	14 (38.9%)
GC-resistant patients (n = 34)	29.8 ± 7.6	19 (55.9)	9 (26.5%)	6 (17.6%)
p	<0.001	<0.001		

25(OH)VD = 25-hydroxyvitamin D; GC = glucocorticoid; SD = standard deviation.

and the highest level was detected in the TT genotype. This could be explained by that VDR may exert a type of feedback to try to overcome the deficient activation associated with the T allele.[31]

Our results concerning the association between steroid resistance in patients with asthma and the *Fok*I T allele as well as high serum 25(OH)VD levels are reinforced by the findings of an in vitro study which suggested that VDR may have synergistic effects on the corticosteroid receptor when activated by its ligand, vitamin D.[37]

Concerning the *VDR Apa*I SNP, nonsignificant differences were observed in the distribution of *Apa*I genotypes between patients with asthma and healthy subjects, which is in agreement with some studies[30,38]; other studies were not in agreement.[31,32] Additionally, our study failed to find an association between the *VDR Apa*I SNP and GC

resistance. Nevertheless, a recent study showed an association of *Apa*I and better asthma control.[39]

Existing studies concerning *VDR* SNPs in asthma showed contradictory results that partially may be the consequence of ethnic differences among the subjects involved in these studies. Moreover, differences between the studies could also be due to gene–environment interactions, especially in complex diseases such as bronchial asthma.

In the present study, we found no significant association between FEV1 values and *VDR* gene polymorphisms or serum 25(OH)VD levels. These findings regarding FEV1 values are in disagreement with previously published findings.[24,40] One possible explanation for these discrepancies is that all patients with asthma included in the present study had severe bronchial asthma.

One limitation of our study was that the measurement of 25(OH)D was not aligned with the standard procedures and materials according to the National Institute for Standard and Technology (NIST) target value as recommended by the Vitamin D Standardization Program (VDSP).[41] It is noteworthy that there is not univocal consensus on the reference values of vitamin D status.[42] Moreover, a substantial variability in 25(OH)D measurement continues to exist between assay methodologies.[43] Therefore, reference intervals and standardized measurement of vitamin D and other analytes such as galectin-3[44] and glycated albumin[45] still debated.

Conclusion

We observed a possible association between the *VDR Fok*I T allele and steroid-refractory asthma. Furthermore, T allele carriers had significantly higher levels of serum 25(OH)VD levels, as a compensatory mechanism to surmount the lower VDR activity conferred by the longer VDR isoform. Accordingly, exogenous administration of vitamin D might help to attain an improved response to steroids in certain genetically predisposed patients with asthma.

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