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Original article

Polymorphisms within Exon 9, But Not Intron 8, of the Vitamin D Receptor Gene Are Associated with Asthma

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Abstract

Objective(s)
Deregulation of the immune system through allied factors and cytokine responses are thought to be important contributors to the pathogenesis of asthma. Vitamin D3 and its nuclear receptor appear to be factors that maybe involved in regulating i
mmune responses during the progression of asthma. The aim of this study was to investigate the association between polymorphisms in intron 8 and exon 9 of the vitamin D receptor (VDR) and this disease.
Materials and Methods
This study was performed on 100 asthmatic patients and 100 healthy controls. PCR-RFLP was performed to examine polymorphisms in intron 8 and exon 9 of VDR gene.
Results
Our results showed a statistically significant difference in the Taq-1 evaluated genotypes of exon 9 of the VDR gene when comparing healthy patients to asthmatic patients.
Conclusion
Based on our results, it can be concluded that VDR and its functional polymorphisms may play an important role in the pathogenesis of asthma.

Keywords: Asthma, Polymorphism, VDR

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Introduction

Asthma is a multifactorial respiratory disease caused by acute and chronic bronchial inflammation resulting in airway obstructions of various degrees (1). Bronchial hyper-responsiveness (BHR) and increased total serum IgE levels are the main characteristics associated with the asthma phenotype (2). The airway inflammation underlying asthma is driven by a hyperactive immune response (3); hence, factors that regulate this system can affect asthma pathogenesis (4). Recent evidence demonstrated that the interaction of 1, 25-dihydroxy vitamin D (the active form of vitamin D) and its nuclear receptor (VDR) has supportive and regulatory impacts on the immune system (5, 6). The immunoregulatory effects of 1, 25-dihydroxy vitamin D and VDR gene polymorphisms on immune responses were demonstrated by several investigators. For example, Evans *et al* showed that vitamin D had a regulatory effect on dendritic cells (7). Gorman *et al* also reported that 1,25-dihydroxy vitamin D enhanced the ability of ectopically transferred regulatory T cells to modulate Th2-driven asthmatic responses (8). Several studies also showed that VDR polymorphisms are associated with several immune-related diseases, and that the interaction between vitamin D and its receptor modulates immune cell differentiation (5, 6, 9). The VDR gene consists of 8 introns and 9 exons and functional polymorphisms within intron 8 and exon 9 influence the expression of VDR gene (10). Due to the critical role of VDR in immune responses, we aimed to examine polymorphisms of VDR in asthmatic patients.

Materials and Methods

Subjects

We studied 100 unrelated asthmatic patients, from February 2009 to June 2009 at the Rafsanjan University of Medical Sciences. The mean age of the patients was 48 years (range 15-79) and they were selected by convenient method based on the aims of this study. Assessment of socio-economic conditions were measured based on the level of education (diploma: weak, under graduate: moderate and post graduate: high) and monthly income (under $250: weak, $250-$1000: moderate and more than $1000: high). Healthy control cases were selected from the Rafsanjanese population with matched sex, age and socio-economical status. Pregnant women and patients with a history of cigarette use were excluded from the study. Asthma was diagnosed according to the American Thoracic Society (ATS) criteria. Patients were classified into 2 groups, allergic and non-allergic, according to the clinical findings and history of the patients. Hundred genetically unrelated controls with normal spirometric values and no respiratory symptoms (refer to Table 1) were matched with the patients for sex and ethnicity. The study protocol was approved by the ethics committee of the Rafsanjan University of Medical Sciences, and written informed consent was obtained from all participants prior to sample collection. Characteristics of the subjects are summarized in Table 1.

Genomic DNA extraction

To extract genomic DNA, peripheral blood was collected on EDTA and genomic DNA was prepared using a commercial kit according to the manufacture's guidelines (Bioneer, South Korea). Extracted DNA was aliquoted for each sample and stored at -20 °C for further use.

Detection of polymorphisms

VDR gene polymorphisms within intron 8 and exon 9 were analyzed as previously described (6). In brief, primers were designed to flank a known *T*aq-1 polymorphism (rs731236, SNP identifier from the National Center for Biotechnology Information database) carried within exon 9 and a known *A*pa-1 polymorphism (rs7975232) with intron 8 of the VDR gene. Amplicons were subjected to restriction digestion with the appropriate enzyme and the products separated on an agarose gel. Alleles were scored according to the fragment patterns. Alleles digested by *T*aq-1 or *A*pa-1 were scored as *T* and *A* alleles respectively whereas alleles not digested by *T*aq-1 or *A*pa-1 were scored as *t* and *a* alleles respectively (Figures 1 and 2).

Results

Polymorphisms within the VDR gene were scored according to PCR-RFLP of exon 9 and

intron 8. An example of a typical *T*aq-1 digestion of exon 9 and how it is scored is shown in Figure 1. Figure 2 shows a typical example of how the *A*pa-1 polymorphism within intron 9 is scored.

Evaluation of the polymorphism within exon 9 of the VDR gene polymorphisms by *T*aq-1 restriction enzyme digestion showed that the prevalence of *T/T* genotype was 6 (6%) in patients and 18 (18%) in controls. Our results also showed that *T/t* genotype of the VDR gene was 48 (48%) and 35 (35%) in patients and controls, respectively and that the *t/t* genotype of was found to be 46 (46%) in patients and 47 (47%) in controls. Statistical analysis showed that the difference regarding these genotypes was significant (*P*= 0.032) (Table 2). The frequency of *T* alleles was 60 (30%) and 71 (35. 5%) in patients and controls, respectively. One hundred and forty (70%) patients contained *t* alleles whereas the frequency of this allele was 129 (64. 5%) in controls.

Statistical analysis showed that the difference in these genotypes was not significant (*P*= 0.291) (Table 2).

The findings of intron 8 of VDR by *A*pa-1 restriction enzyme indicated that 7 (7%) of patients and 17 (17%) of the controls had the *A/A* genotype. Fifty eight of the patients (58%) and 56 of the controls (56%) had the *A/a* genotype. The frequency of the *a/a* allele in patients and controls was 35 (35%) and 27 (27%), respectively. Statistical analysis revealed no significant difference between the groups regarding these genotypes (*P*= 0.026) (Table 2). Our results also showed that the frequency of the *A* allele was 72 (36%) and 90 (45%) in patients and controls, respectively. 128 (64%) *a* alleles were seen in patients and the frequency of this allele was 110 (55%) in controls. Statistical analysis showed that the difference in these genotypes was not significant (*P*= 0.291) (Table 2).

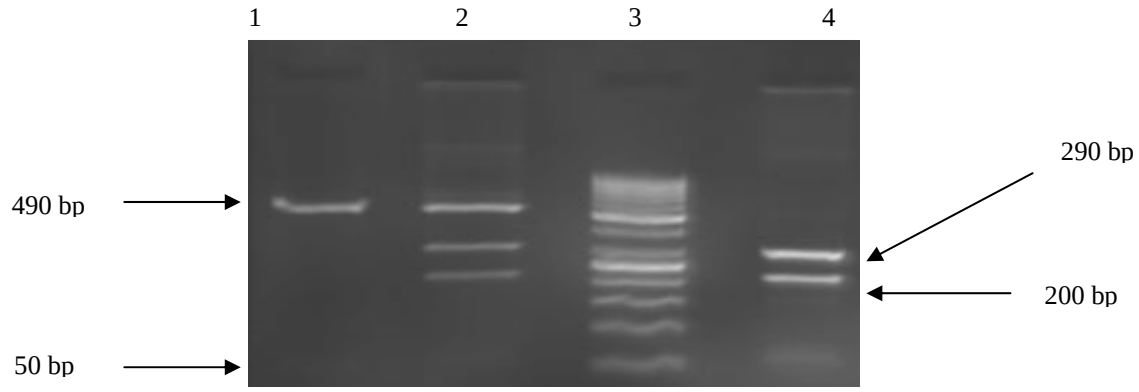

Figure 1. An ethidium bromide stained agarose gel showing PCR-RFLP analysis of exon 9 of the VDR gene. The gel illustrates *T*aq-1 digestion of the VDR exon 9 amplicon which contains a known *T*aq-1 RFLP. Lane 1: homozygotic PCR product, which is not susceptible to *T*aq-1 digestion (*t/t*). Lane 2: heterozygotic digestion (*T/t*). Lane 3: 50 bp ladder marker and lane 4: homozygotic digestion (*T/T*).

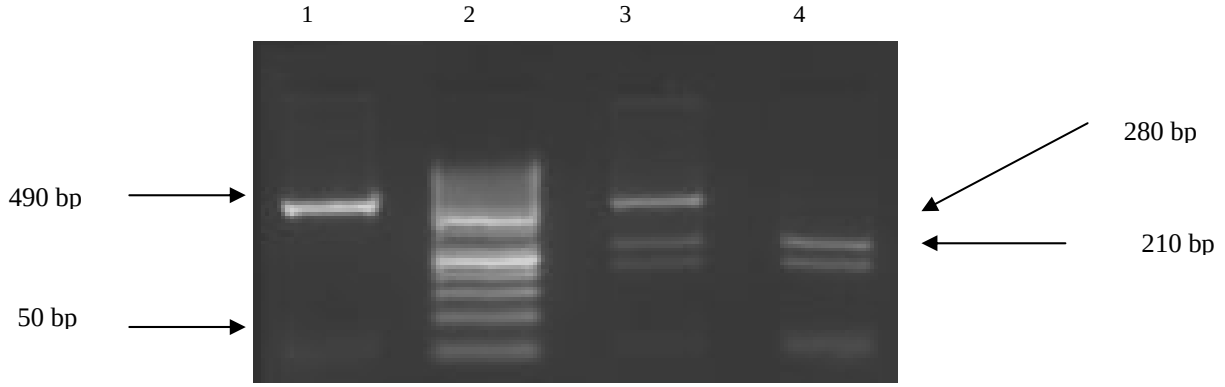

Figure 2. An ethidium bromide stained agarose gel showing PCR-RFLP analysis of intron 8 of the VDR gene. The gel illustrates *A*pa-1 digestion of the VDR intron 8 amplicon which contains a known *A*pa-1 RFLP. Lane 1: homozygotic PCR product, which is not susceptible to *A*pa-1 digestion (*a/a*). Lane 2: 50 bp ladder marker and lane Lane 3: heterozygotic digestion (*A/a*). 4: homozygotic digestion (*A/A*).

Table 1. Clinical and socio-economic parameters of asthma patients and healthy controls. N/A: Not assessed, FEV_1: Forced expiratory volume in 1 second, FVC: Forced vital capacity, PEF: Peak expiratory flow, $FEF_{25-75\%}$: Forced Expiratory Flow or average flow of air coming out of the lung during the middle portion (25-75%) of the expiration, L/% predicted: data are measured by liter and are shown as percentage of predicted volume.

Characteristics		Controls	Asthmatics
Sex / M/F ratio		27/73	31/69
Age / yrs		43±14	48±12
Assessment of at least 2 symptoms (cough, wheeze, and shortness of breath)		N/A	+
% of group with Allergy		N/A	70.3%
Positive family history of asthma		N/A	43.2%
Smoking history		Nil	Nil
FEV_1 (% predicted)		87.3±14	79.2±21
FEV_1/FVC		88.1±11	82.3±9
PEF (L/% predicted)		91.6±21	74.6±19
$FEF_{25-75\%}$		84.7±17	62.6±34
FVC (L/% predicted)		93.4±8	79.9±19
socio-economic	weak	24 (24%)	21 (21%)
	moderate	44 (44%)	49 (49%)
	high	32 (32%)	30 (30%)

Table 2. Frequency of genotypes and alleles within intron 8 and exon 9 of the VDR gene in asthmatic patients and controls.

Condition	Patients	Control	*P* value
Genotype			
A/A n (%)	7 (7%)	17 (17%)	
A/a n (%)	58 (58%)	56 (56%)	*P*= 0.032
a/a n (%)	35 (35%)	27 (27%)	
T/T n (%)	6 (6 %)	18 (18%)	
T/t n (%)	48 (48%)	35 (35.8%)	*P*= 0.101
t/t n (%)	46 (46%)	47 (47%)	
Alleles			
T n (%)	60 (30%)	71 (35. 5%)	*P*= 0.291
T n (%)	140 (70%)	129 (64. 5%)	
A n (%)	72 (36%)	90 (45%)	*P*= 0.087
A n (%)	128 (64%)	110 (55%)	

Discussion

Several studies have reported the significant divergent modulatory roles of vitamin D on the immune system, specially its immune suppressive effects during pregnancy (7). When vitamin D binds to its intracellular receptor (VDR) it causes changes in the expression of VDR which in turn lead to alterations in the regulatory functions of vitamin D on immune system (11). In our current study, we demonstrated that there is an association between *T*aq-1 evaluated polymorphisms in exon 9 of the VDR gene with asthma. Previous studies demonstrated that intron 8 and exon 9 polymorphisms of VDR have an impact on its expression (12) and it has been shown that by using a recombinant VDR luciferase reporter gene, that these polymorphisms are associated with VDR mRNA stability (13), suggesting that these polymorphisms may influence the immune system by regulating VDR expression levels. Our results showed that the *T*aq-1 evaluated genotypes are associated with asthma and it is possible that the reported changes in VDR mRNA stability caused by these polymorphisms could be responsible for the deregulation of the immune system typical seen in asthma patients. Raby *et a*l showed that polymorphisms in the VDR gene were associated with asthma in a complex manner (9). Another study also demonstrated that the *T* allele of *T*aq-1 and the *a* allele of *A*pa-1 are associated with asthma in the American population (14). Saadi *et al* reported that only *A*pa-1 alleles are associated with asthma in

Chinese population (15). Therefore, it may be concluded that *T*aq-1 and possibly *A*pa-1 polymorphisms are important in asthma through their effects on VDR expression. On the other hand, a study by Vollmert and colleagues, using *F*okI (polymorphism in exon 2, rs2228570), demonstrated that there is no link between polymorphisms in the VDR gene and asthma (16). However, Fang *et al* (17) in their study also failed to show any association of this SNP and asthma. The discrepancy between our findings and the results from the studies carried out by Voomert *et al*, and Fang *et al* (16, 17) may be related to the different SNP polymorphism evaluated in our study or may reflect the differences in the studied populations in terms of genetic background or their exposure to environmental factors.

Conclusion

In summary, our results suggest that due to the polymorphisms in the VDR gene in asthmatic patients, the production and stability of mRNA of the VDR gene may be reduced; hence, vitamin D regulation of the immune system may be compromised. It is plausible that this leads to expanded helper T-lymphocyte cytokine 2 (Th2) immune responses in asthmatic patients leading to onset of the disease.

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The authors declare that they have no conflict of interests.

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