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Association Analysis of Vitamin D Receptor Gene Polymorphisms in Chinese Population with Asthma

Wen-Liang Fang[1], Lin-Bo Gao[2], Wei-Bo Liang[2], Hui Xue[1], Peng Bai[2], Mei-Li Lv[1], Yan-Yun Wang[1], Bin Zhou[3], and Lin Zhang [1,2,3]

[1]Department of Immunology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2]Department of Forensic Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[3]Laboratory of Molecular Translational Medicine, West China Second University Hospital, Sichuan University, Chengdu 610041, Sichuan, China

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ABSTRACT

Several asthma susceptibility loci, including a region containing the vitamin D receptor (VDR) gene located at chromosome 12q, have been identified using genome-wide screens. Our aim is to investigate the association between single nucleotide polymorphisms (SNPs) in VDR gene and asthma.

One hundred one asthma patients and 206 healthy controls were enrolled in this study. Genotypes were determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) strategy and DNA sequencing.

The results showed that there was no significant differences in the genotype and allele frequencies of *Fok I* and *Bsm I* polymorphisms between asthma patients and the controls in the Chinese Hans (For *Fok I*: OR = 1.15, 95% CI: 0.82–1.60; for *Bsm I*: OR = 1.44, 95% CI: 0.87–2.38).

It is suggested that *Fok I* and *Bsm I* polymorphisms of VDR gene may not significantly contribute to the development of asthma in the Chinese Hans.

Key words: Asthma; Chinese; DNA Sequencing; Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP); Single nucleotide Polymorphism (SNP); Vitamin D receptor (VDR)

INTRODUCTION

Asthma is a chronic inflammatory disease of unknown etiology, mainly resulting in intermittent narrowing of the small airways in the lung, with a series of clinical manifestation of wheeze, cough and breathlessness.[1] The increased prevalence of asthma has been predominant since the latter half of the 20th century, and 155 million individuals worldwide are suffering from this disease now.[2] Like other atopic diseases, asthma is a complex disorder caused by multiple genetic and environmental factors. Over the last decades, several studies about gene polymorphisms have been emerging in asthma, and several susceptibility loci, including regions 2q, 5q, 6p, 11q, 12q, and 13q have been identified through genome-wide screens.[3]

The vitamin D receptor (VDR) is a member of the

Corresponding Author: Lin Zhang, MD, PhD;
Department of Immunology, West China School of Preclinical and Forensic Medicine, Sichuan University, NO.17 Renmin South Road (Section III),Chengdu 610041, Sichuan, China. Tel: (+86 28) 8546 9033; Fax: (+86 28) 8540 5541, E-mail: zhanglin@scu.edu.cn

steroid thyroid superfamily of nuclear receptors.[4] As a transcriptional regulatory factor, it mediates the effects of the biologically active form of vitamin D 1,25-dihydroxyvitamin D3 [1,25$(OH)_2D_3$]. Upon activation, VDR ligand/receptor complex regulates the transcription level of target genes which involve in T helper (Th) cell development and Th cytokine profile change.[5-7] Previous studies demonstrated that early exposure to a dietary with supplementary vitamin D has the potential risk to increase the morbidity of allergy and asthma later in life.[8-13] Importantly, VDR polymorphisms have been implicated in several immune and inflammatory disorders. VDR maps to the chromosome 12q, a region that has been committed to linkage with asthma and allergy-related phenotypes in genome-wide linkage analyses.[14] In addition its genetic variants have been proved to be associated with asthma in two recent studies from the U.S.[15] and Canada [16]. However, Vollmert et al[17] found no significant linkage/association between VDR gene polymorphisms and asthma or related phenotypes in a German ethnic group. In comparison to others, the single nucleotide polymorphisms (SNPs) are more valuable in the association analysis between VDR gene polymorphisms and asthma, especially in different ethnic origin.

Until now, no study has shown the relationship between VDR gene variants and asthma in Chinese. In this study, we aimed to explore association between *Fok I* and *Bsm I* polymorphisms of VDR gene and the risk of asthma in the Chinese Hans.

PATIENTS AND METHODS

Study Population

A total of 101 unrelated Hans asthma patients were recruited from West China hospital, Sichuan University from July 2005 to March 2008. The patients (42 males and 59 females) with average age of 35.9 years old showed typical clinical symptom of asthma and met the Hans subjects for health examination in this hospital only selection criterion on physical examination.[18] A total of 206 controls (96 males and 110 females) with average age of 34.7 years old were from local unrelated controls were screened for a history of asthma or other pulmonary diseases, who were matched with asthma patients for sex and age. Written informed consent was obtained from all the subjects, and the study was performed with the approval of the ethics committee of the Chinese Human Genome.

Genotyping

Genomic DNA was extracted from peripheral blood with an extraction kit (Bioteke Corporation; Perking, China) according to the manufacturer's instructions. VDR polymorphisms were identified by using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. Experimental conditions including primer sequences, reaction conditions, restriction enzymes used and length of resulting PCR products are shown (Table 1).[19] To confirm the accuracy of genotyping, partial samples were examined by DNA sequencing.

Statistical Analysis

The $\chi 2$ test and the Student's t-test were used to analyze demographic and clinical data between groups. Hardy-Weinberg equilibrium was tested with a goodness of fit $\chi 2$-test. We compared genotype and allele frequencies of *Fok I* and *Bsm I* in VDR gene between two groups using the $\chi 2$ test and Fisher's exact test when appropriate, and calculated odds ratio (OR) and 95% confidence intervals (CI) to assess the relative risk. The Statistical Package for Social Sciences (SPSS 11.5 Chicago, IL, U.S.A.) was used for all of the statistical analyses.

RESULTS

The genotype and allele frequencies of *Fok I* and *Bsm I* polymorphisms are shown (Table 2).

Table 1. Primer sequences and reaction conditions for genotyping VDR polymorphisms

Polymorphism	Primer sequence	Annealing temperature (˚C)	Restriction enzyme	Product size (bp)
Fok I	F: 5'- AGCTGGCCCTGGCACTGACTC TGCTCT -3'	62	*Fok* I	F: 266
	R: 5'- ATGGAAACACCTTGCTTCTTC TCCCTC -3'			f: 68+198
Bsm I	F: 5'- GGGAGACGTAGCAAAAGG -3'	56	*Bsm* I	B: 358
	R: 5'- AGAGGTCAAGGGTCACTG -3'			b: 192+166

Table 2. The genotype and allele frequencies of *Fok I* and *Bsm I* in VDR gene between patients with asthma and controls

Polymorphisms	Patients n = 101 (%)	Controls n = 206 (%)	OR (95% CI)	*P- Value*
Fok I				
Genotypes				
FF	24 (23.8)	66 (32.0)	1.00 (Ref)	
Ff	56 (55.4)	94 (45.6)	1.64 (0.92-2.90)	0.09
Ff	21 (20.8)	46 (22.3)	1.26 (0.63-2.52)	0.52
Alleles				
F	104 (51.5)	226 (54.9)	1.00 (Ref)	
F	98 (48.5)	186 (45.1)	1.15 (0.82-1.60)	0.43
Bsm I				
Genotypes				
Bb	74 (73.3)	167 (81.1)	1.00 (Ref)	
bB	25 (24.8)	35 (17.0)	1.61 (0.90-2.88)	0.11
BB	2 (2.0)	4 (1.9)	1.13 (0.20-6.30)	1.00
Alleles				
B	173 (85.6)	369 (89.6)	1.00 (Ref)	
B	29 (14.4)	43 (10.4)	1.44 (0.87-2.38)	0.16

By the DNA sequencing, we clearly knew that F, f, B, and b represented C, T, A, and G, respectively (Figure 1). The genotyping results by PCR-RFLP and DNA sequencing were 100% concordant. The genotype distributions were in Hardy–Weinberg equilibrium in each group studied. The frequencies of the FF, Ff, and ff genotypes of *Fok I* were 23.8 %, 55.4 %, and 20.8 % in cases, and 32.0 %, 45.6 %, and 22.3 % in controls, correspondingly. The frequencies of F and f alleles of *Fok I* were 51.5 % and 48.5 % in cases, and 54.9 % and 45.1 % in controls. The frequencies of the bb, bB, and BB genotypes of *Bsm I* were 73.3 %, 24.8 %, and 2.0 % in cases, and 81.1 %, 17.0 %, and 1.9 % in controls. The frequencies of b and B alleles of *Bsm I* were 85.6 % and 14.4 % in cases, and were 89.6 % and 10.4 % in controls. No significant differences were observed in the genotype and allele frequencies of the *Fok I* and *Bsm I* polymorphisms between the cases and controls (For *Fok I*: OR = 1.15, 95% CI: 0.82–1.60; for *Bsm I*: OR = 1.44, 95% CI: 0.87–2.38).

DISCUSSION

To the best of our knowledge, it is the first study to investigate the association between the *Fok I* and *Bsm I* polymorphisms of VDR gene and asthma in the Chinese Hans. We found no significant difference in the distribution of VDR polymorphisms between asthma patients and controls, which suggested that VDR polymorphisms may not significantly contribute to the susceptibility to asthma in the Chinese Hans.

The frequency of some certain alleles on *Fok I* and *Bsm I* revealed not significant different in diverse populations, but some were not. For example, the frequency of the F allele on *Fork I* was 54.9%, which was not significantly different in Japanese and Caucasians (53.0-60.7%).[20-23] However, the average frequency of the b allele on *Bsm I* was 89.6% in Asian populations as Chinese, Korean and Japanese (91.7%, 92.1%, and 87.4%, respectively),[24-26] and the frequency was 52.9-57.7% in Caucasians.[27-29] Taken together, these data suggested that the distribution of VDR gene frequencies might vary among the different ethnic groups, and therefore additional studies of VDR polymorphisms in different ethnic populations would be valuable.

Located on chromosome 12q in human[30], the vitamin D receptor *(*VDR*)* gene was cloned in 1988[31], consisting of 9 exons with at least 6 isoforms of exon 1 and spanning 60–70 kb of genomic sequence[32]. VDR is an important immune system regulator that interacts with target-cell nuclei to perform a variety of biologic functions, including calcium and phosphorous homeostasis, apoptosis, and cell differentiation.[31,33,34]

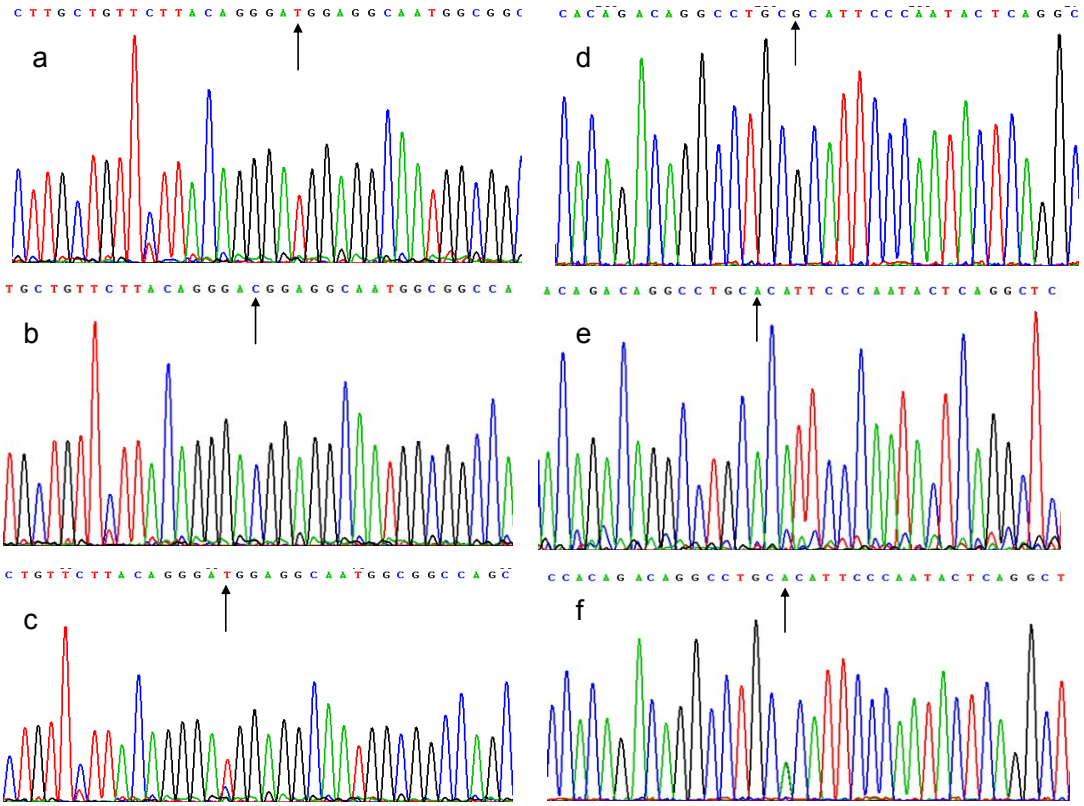

Figure 1. Sequencing of VDR gene *Fok I* and *Bsm I*

a: Sequencing of VDR gene *Fok I* homozygote genotype TT;
b: Sequencing of VDR gene *Fok I* homozygote genotype CC;
c: Sequencing of VDR gene *Fok I* heterozygote genotype CT;
d: Sequencing of VDR gene *Bsm I* homozygote genotype GG;
e: Sequencing of VDR gene *Bsm I* homozygote genotype AA;
f: Sequencing of VDR gene *Bsm I* heterozygote genotype AG.

Moreover, it adjusts the efficiency of RNA polymerase II-mediated transcription by specifically binding the active form of vitamin D 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$].[35-37] Vitamin D and vitamin D receptor (VDR) deficiency results in several immune system-mediated diseases, such as inflammatory bowel disease.[38]

Treatment with vitamin D can alter cytokine expression profiles, IgE production, and the pattern of airway eosinophilia during allergen sensitization in a murine model of pulmonary eosinophilic inflammation. So it is supposed that the vitamin D pathway may influence the development of allergy and asthma.[10] The hypothesis is supported by the linkage of asthma to a genomic region at the centromeric end of chromosome 12q which includes the VDR locus.[14,39,40] Importantly, Poon et al[16] reported significant associations between VDR polymorphisms and haplotypes and atopy susceptibility of asthma in a founder population from northeastern Quebec in Canada. In the meantime, Raby et al[15] reported an independent replication of association of genetic variation at the VDR locus with asthma and related phenotypes in African American and Hispanic populations.

Therefore, we postulated VDR gene polymorphisms might modulate the susceptibility to asthma in the Chinese Hans. The change of a single *Fok I* restriction site can lead to the ATG start cordon alteration in the second exon of the VDR, and the minute alternations of the *Bsm I* site can influence protein expression.[41] Thus the polymorphisms of both genes were included in the present study. However, no significant association was found between their polymorphisms and the risk of asthma in the current study. Our results were in agreement with Vollmert's report,[17] which showed no significant linkage/association with asthma or related

phenotypes in a family study. Nevertheless, our findings were different from Poon's and Raby's studies.[15,16]

Although it is difficult to decipher the reasons for these discrepancies, several possibilities should be considered. Firstly, it may be due to the genetic trait differences, VDR gene polymorphism is distinct in specific population, various ethnicity and geographic region. Secondly, asthma is a multi-factorial disease and different individual could be exposed to various environmental factors, and genetic susceptibility might have caused different results. Finally, the imperfect study design may be one of reasons, such as a limited sample size, non-random sampling and possibility of selection bias from the hospital-based case-control study.

Wittke et al found that 1, 25(OH) 2D3 had no effect on experimental asthma severity in spite of increased level of IgE and Th2 cytokines.[42] Their results supported our findings from a different point of view. Although the exact molecular mechanism of VDR in the development of asthma is not yet well known, its effect on Th cell development is of particular interest for the disease. VDR is an immunoregulatory switch and adjust Th1/Th2 balance, the function of VDR in immune-mediated diseases may dependent on levels of VDR in immune cells after stimuli.[5,43,44]

In conclusion, we found that VDR gene polymorphisms were not associated with the risk of asthma in the Chinese Hans. Nevertheless, further studies will be needed to explore the complicated interaction between environmental factors and VDR gene polymorphisms in the susceptibility of asthma, especially in ethnically diverse populations.

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