

Associations between vitamin D receptor gene polymorphisms and chronic spontaneous urticaria in Chinese Han population

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Abstract

Introduction: Previous studies found that vitamin D receptor (VDR) TaqI, BsmI, FokI and Apal gene polymorphisms are associated with several inflammatory diseases. However, the relationship between VDR gene polymorphisms and chronic spontaneous urticaria (CSU) is not clear.

Aim: The purpose of our study was to explore the relationship between the polymorphism of VDR and the incidence of chronic spontaneous urticaria in the Chinese Han population. Meanwhile, the vitamin D levels in patients with chronic spontaneous urticaria were also detected and the effects of VDR gene polymorphism on vitamin D levels were detected.

Material and methods: The genotypes of four VDR polymorphisms (TaqI, BsmI, Apal, and FokI) were studied using allele-specific PCR analysis in 90 CSU patients and 90 healthy controls.

Results: Compared to the control group, the mutant allele (C) of FokI were more common in patients with CSU (57.2% vs. 45%, $p = 0.020$, odds ratio (OR) = 0.612, 95% confidence interval (CI): 0.403–0.928). We found that serum vitamin D levels were significantly lower in CSU patients than in healthy controls ($p = 0.023$). However, the effect of VDR gene polymorphism on vitamin D levels was not found in patients of CSU.

Conclusions: We first reported the effect of VDR gene FokI (rs2228570) polymorphism on the incidence of chronic spontaneous urticaria in the Chinese Han population.

Key words: urticaria, vitamin D receptor, gene polymorphisms.

Introduction

Urticaria is a common disorder characterized by the activation and release of histamine of mast cells. The prevalence of the disease is approximately 20% in the general population [1]. In the end, about 20% of the cases will become chronic spontaneous urticaria (CSU) [2], whose signs and symptoms recur daily, or almost daily, lasting for more than 6 weeks without any identifiable cause [3].

It is well known that vitamin D is a secosteroid with mineral homeostasis and bone metabolism mechanism. In addition, since vitamin D has been shown to have potential immunoregulatory activity, regulating multiple immune cells through vitamin D may have clinical significance in determining susceptibility to autoimmune diseases. Recently, the role of vitamin D in a variety of chronic diseases such as malignant tumours, autoimmune diseases, infectious diseases, and allergic diseases,

including asthma and atopic dermatitis, has been a hot research topic [4–8].

Previous epidemiological and laboratory studies have shown that vitamin D deficiency is associated with a variety of diseases, including autoimmune diseases, tuberculosis and chronic spontaneous urticaria (CSU) [9]. Vitamin D activity is mediated through its active form, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), combined to the vitamin D receptor (VDR), which further results in conformational change and leads to dimerization of the retinoid X receptor.

The dimeric complex interacts with vitamin D response elements in target genes involved in cell division, cell adhesion, and other functions, leading to changes in gene activity [10]. The stimulation of VDR and retinoid acid receptors at the cellular level can inhibit cell proliferation and angiogenesis, and induce cell differentiation and apoptosis [11]. In virtue of vitamin D activity is mediated by the VDR, it is

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helpful to elucidate the role of vitamin D in the aetiology of CSU by analysing the changes of genetic variations of VDR. The relationship between four famous human VDR, FokI, BsmI, ApaI, TaqI gene polymorphisms and disease risk were widely studied previously. However, data on the relationship between VDR gene polymorphisms and Chinese CSU patients is still lacking.

Aim

In this study, we conducted a case-control study involving 180 individuals to explore the role of the VDR gene polymorphisms (FokI, BsmI, ApaI, TaqI) in Chinese Han CSU patients, aiming to interpret whether the VDR gene polymorphisms could affect CSU susceptibility.

Material and methods

Subjects

This study included 90 patients with chronic spontaneous urticaria and 90 healthy controls from the Chinese Han population living in and near Nanjing. This prospective study was conducted at the Institute of Dermatology, Chinese Academy of Medical Sciences from August 2016 to October 2016. All patients conform to the diagnostic criteria for CSU [2] and are aged more than 18 years old. Demographic and clinical data of the CSU patients were collected. Written informed consent was obtained from each patient before participating in this study. The study was approved by the clinical research ethics committee of the Institute of Dermatology, Chinese Academy of Medical Sciences.

Estimation of serum 25(OH)D levels

Serum 25(OH) D levels were measured using a commercial ELISA kit according to the manufacturer's instructions (EUROIMMUN Medizinische Labordiagnostika AG).

DNA extraction and genotyping

Genomic DNA was extracted using the MiniBEST Universal Genomic DNA Extraction Kit Ver. 5.0 (Takara, Japan) according to the manufacturer's protocol. To detect a specific allele, the DNA of 180 samples was used for allele-specific PCR analysis according to a previously reported protocol [12]. All the sequences were available from NCBI and used for primer design. All primers were designed by CT Bioscience Co. Ltd. (Jiangsu, China). The primer sequences are listed in Table 1. SYBR Green was used as the detection system for all of the SNP-specific primers for (FokI, BsmI, ApaI, TaqI). A reaction mixture of 10 µl was used, which contained 5 µl SYBR Green PCR master mix (CT Bioscience, China), 0.5 µM each primer, 10–30 ng of DNA, and water to a volume of 10 µl. The conditions of quantitative PCR were as follows: 10 min at 94°C, followed by 45 cycles of 20 s at 94°C and 30 s

at 60°C. Melting curve analysis: 5 s at 95°C with an increase of 4.4°C/s, 1 min at 60°C with an increase of 2.2°C/s, continuous at 95°C with an increase of 0.11°C/s, and the last step of 30 sec at 40°C. All quantitative PCRs of the genotypes were carried out using the Light Cycler 480 II PCR system (Roche, Germany).

We declare that all methods were performed in accordance with the relevant guidelines and regulations.

Statistical analysis

All calculations were performed using SPSS 19.0 software for Windows (Chicago, IL, USA). Continuous variables are presented as the mean ± standard deviation or median (interquartile range). Categorical variables are described as percentages. The Hardy-Weinberg equilibrium rule for the four polymorphic loci of the VDR gene was evaluated using the χ^2 test. Linkage disequilibrium (LD) and haplotype analyses were done using the online software SHEsis [13]. The differences in allele and genotype frequencies between cases and controls were assessed by χ^2 tests (Fisher's exact test was applied if the expected frequency was less than 5). Comparison of the average 25(OH)D level between groups was performed using Student's *t* test. *P*-values less than 0.05 were considered statistically significant.

Results

Characteristics of the study sample

Table 2 shows the general characteristics of cases and controls in this study. The patients and the control group were matched by age and sex (mean age of CSU patients: 34.87 ±11.69 years with 35 men and 55 women; mean age of controls: 35.72 ±10.18 years, with 39 men and 51 women).

Table 1. The primer sequences

Primer ID	Sequence	SNP
1544410-A	CCACAGACAGGCTGCsA	rs1544410
1544410-G	CCACAGACAGGCTGCsG	rs1544410
1544410-COM	CCAGTTCACGCAAGAGCAGA	rs1544410
731236-C	AGGACGCCGCGTGATsC	rs731236
731236-T	AGGACGCCGCGTGATsT	rs731236
731236-COM	TACGTCTGCAGTGTGTTGGA	rs731236
2228570-A	GCTTGCTGTTCTTACAGGGA _s A	rs2228570
2228570-C	GCCGCCATTGCCTCCG	rs2228570
2228570-G	GCTTGCTGTTCTTACAGGGA _s G	rs2228570
2228570-T	GCCGCCATTGCCTCCA	rs2228570
2228570-COM	GGCACTGACTCTGGCTCTG	rs2228570
7975232-A	TGGGATTGAGCAGTGAGGT	rs7975232
7975232-C	TGGGATTGAGCAGTGAGGG	rs7975232
7975232-COM	GAAGGCACAGGAGCTCTCAG	rs7975232

Table 2. General characteristics of cases and controls in this study

Parameter	CSU patients (n = 90)	Healthy controls (n = 90)	P-value
Age [years]	34.87 ±11.69	35.72 ±10.18	0.364
Sex (male/female)	35/55	39/51	0.276
Duration of disease [months]	14.21 ±2.35		
MPV [fl]	8.37 ±1.40	8.44 ±1.42	0.384
ASST(% positive)	53.3		
25(OH)D levels [ng/ml]	18.08 ±5.97	20.31 ±3.43	0.023

Genotype and allele frequencies

All four genotyped SNPs were in Hardy-Weinberg equilibrium both for patients with CSU and for controls (all $p > 0.05$). Compared with the control group, the mutant allele(C) of FokI were more prevalent in CSU patients (57.2% vs. 45%, $p = 0.020$, odds ratio (OR) = 0.612, 95% confidence interval (CI): 0.403–0.928). But, there were no allelic or genotypic differences between CSU patients and controls for BsmI, Apal, and TaqI (all $p > 0.05$, Table 3).

Linkage disequilibrium and haplotype analysis

Linkage disequilibrium (LD) analysis showed that BsmI, Apal, and TaqI were in a powerful LD with each other (BsmI/Apal, $D' = 1$, $r^2 = 0.172$; Apal/TaqI, $D' = 1$, $r^2 = 0.191$; BsmI/TaqI, $D' = 1$, $r^2 = 0.899$), but not with FokI (Figure 1). Finally, only AAC, GCT, and GAT haplotypes were calculated among the seven haplotypes formed by BsmI, Apal, and TaqI in all study subjects (Table 4), for their frequencies were more than 3% both in CSU patients and in the control group. However, there was no significant difference in AAC, GCT and GAT haplotypes between patients with CSU and controls ($p = 0.463$, $p = 0.238$, $p = 0.087$, respectively).

Impact of polymorphisms of the VDR gene on serum 25(OH)D levels

To investigate the effect of VDR polymorphisms on serum 25(OH)D levels with CSU, we compared serum 25(OH)D levels of patients carrying VDR polymorphisms of different genotypes (Table 5). However, no significant difference was found between genotypes FokI, BsmI, Apal, and TaqI in terms of serum 25(OH)D levels in patients of CSU.

Discussion

CSU is a common allergic dermatosis that often requires long-term drug treatment and severe patients suffer a poor quality of life. Although the pathogenesis of CSU is not completely clear, the activation and release of histamine by basophils and mast cells is an important cause of this disease.

Allergic diseases are complicated, and are regulated by the interaction of heredity and environmental factors. In recent years, genetic research on allergic diseases has

become more advanced, particularly asthma. Similar to allergic diseases such as asthma, the genetic mechanism may be related to the pathogenesis of CSU. A large sample study showed that the prevalence of first-degree relatives of CSU patients was higher than that of the general population. Recent genetic studies have been designed to determine the genetic susceptibility of CSU. Up to now, polymorphisms of genes such as FcεRIα, FcεRIβ, FcεRIγ, HNMT, TNF-α, TGF-β1, PTPN22, CCR2, CCR5, HLA-A*33 and HLADRBI*04 have been found to be associated with chronic urticaria [14–19].

Lately, genetic association studies have confirmed the association between genetic polymorphisms of VDR and allergic diseases such as asthma, atopic dermatitis [20, 21]. These findings indicate that polymorphisms of VDR are probably associated with the risk of CSU. However, no study has directly addressed this issue. In conclusion, this is the first initial study on the risk of the impact of VDR SNPs FokI (rs2228570) polymorphism in CSU. Compared with the control group, the mutant allele(C) and genotype(CC,TC+CC) of FokI were more prevalent in CSU patients.

The VDR gene is located on the long arm of the chromosome 12q12-q14, and several single nucleotide polymorphisms (SNP) genes have been identified, which may affect the risk of disease. The FokI (rs2228570) polymorphism is located at the exon-2 of VDR, and the change of the ACG codon leads to the extra initiation of the start codon. Apal (rs7975232), TaqI (rs731236) and BsmI (rs1544410) polymorphisms are located in the 3' untranslated region (UTR), which participate in adjusting the stability of VDR mRNA. Due to its important function, FokI is the most studied VDR gene polymorphism. The VDR protein encoded by the FokI C allele lacks three amino acids. Compared with the T allele coding, this leads to the enhancement of VDR transcriptional activity [11].

Conclusions

In our study, we found that the level of serum 25(OH)D in CSU patients decreased significantly compared with the control group, which was consistent with previous studies [22]. We found that FokI (rs2228570) polymorphism have an effect on the risk of CSU. The sample size of our study was relatively small, so it might not detect the association

Table 3. Distributions of VDR (FokI, BsmI, ApaI, and TaqI) gene polymorphisms in patients with CSU (*n* = 90) and controls (*n* = 90)

VDR gene	Patients with CSU <i>n</i> (%)	Controls <i>n</i> (%)	OR (95% CI)	χ^2	<i>P</i> -value
FokI (rs2228570):					
TT	19 (21.1)	28 (31.1)		5.232	0.073
TC	39 (43.3)	43 (47.8)			
CC	32 (35.6)	19 (21.1)			
T	77 (42.8)	99 (55)		5.380	
C	103 (57.2)	81 (45)	0.612 (0.403–0.928)		0.020
BsmI (rs1544410):					
GG	83 (92.2)	79 (87.8)		2.468	0.308
GA	6 (6.7)	11 (12.2)			
AA	1 (1.1)	0 (0)			
G	172 (95.5)	169 (93.9)			
A	8 (4.4)	11 (6.1)	1.399 (0.549–3.565)	0.5	0.479
ApaI (rs7975232):					
CC	50 (55.6)	56 (62.2)		0.892	0.640
CA	32 (35.6)	28 (31.1)			
AA	8 (8.9)	6 (6.7)			
C	132 (73.3)	140 (77.8)			
A	48 (26.7)	40 (22.2)	0.786 (0.485–1.273)	0.963	0.327
TaqI (rs731236):					
TT	83 (92.2)	77 (85.6)		3.685	0.144
TC	6 (6.7)	13 (14.4)			
CC	1 (1.1)	0 (0)			
T	172 (95.6)	167 (92.8)			
C	8 (4.4)	13 (7.2)	1.674 (0.676–4.142)	1.264	0.261

Table 4. Distribution of haplotypes formed by the VDR (BsmI, ApaI, and TaqI) in patients with CSU (*n* = 90) and controls (*n* = 90)

Haplotypes	GCT	GAT	AAC	GAC	GCC	AAT	ACT
Controls, <i>n</i> (%)	140 (77.8)	27 (15)	11 (6.1)	2 (1.1)	0 (0)	0 (0)	0 (0)
CSU patients, <i>n</i> (%)	132 (73.3)	40 (22.2)	8 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)

Table 5. Serum 25(OH)D levels of patients with CSU (*n* = 90) carrying different genotypes of VDR polymorphisms

VDR	1	2	Genotypes (<i>n</i>)			<i>F</i>	<i>P</i> -value
			11	12	22		
BsmI	G	A	17.94 ±5.84 (83)	18.47 ±7.51 (6)	28.11 (1)	1.46	0.238
TaqI	T	C	17.94 ±5.84 (83)	18.47 ±7.51 (6)	28.11 (1)	1.46	0.238
FokI	C	T	18.12 ±6.32 (32)	17.88 ±6.22 (39)	18.45 ±5.08 (19)	0.058	0.944
ApaI	A	C	17.99 ±8.98 (8)	18.39 ±6.06 (32)	17.90 ±5.48 (50)	0.063	0.939

between other VDR SNPs and CSU risk. However, there was no significant difference between genotypes FokI, BsmI, ApaI, and TaqI in terms of serum 25(OH)D levels in patients with CSU. As far as we know, we have reported for

the first time that the polymorphism of the VDR gene FokI (rs2228570) may contribute to the increased risk of chronic spontaneous urticaria in the Chinese Han population. However, more multicentre studies of the larger sample

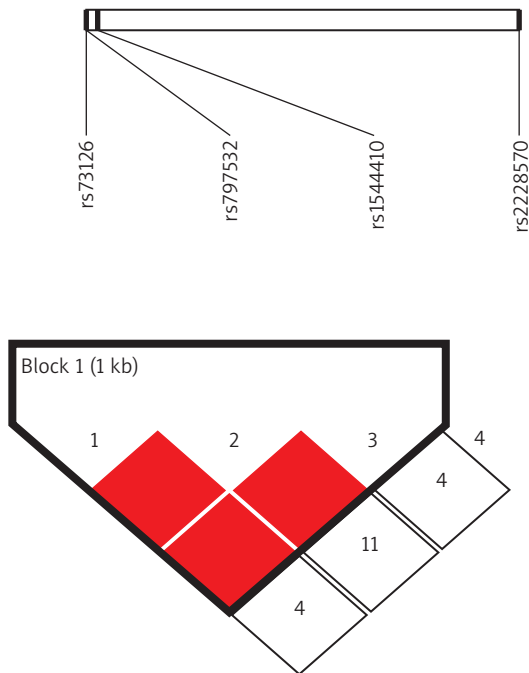


Figure 1. Linkage disequilibrium plot (obtained from SHEsis software) between VDR (Fok I, Bsm I, Apa I, and Taq I) genes. Linkage disequilibrium blocks are framed in black. Each square plots a D' value between a pair of polymorphic loci. The markers (rs731236, rs7975232, and rs1544410) represent VDR (Taq I, Apa I, and Bsm I), respectively

size are needed to confirm these associations. Besides, further functional studies are needed to clarify the mechanism of FokI polymorphism affecting chronic urticaria, and the effect of VDR gene polymorphism on the risk of CSU.

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Conflict of interest

The authors declare no conflict of interest.

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