No Association of Vitamin D Receptor Gene Start Codon *Fok* I Polymorphisms in Chinese Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective***. To examine whether the start codon *Fok* I of the vitamin D receptor (VDR) gene polymorphism is a marker of susceptibility to or severity of systemic lupus erythematosus (SLE) in Chinese patients in Taiwan.

Methods. A control group of 90 healthy people and 52 patients with SLE were examined. Using polymerase chain reaction (PCR) based restriction analysis, we evaluated the relationship between *Fok* I polymorphisms and SLE, where an unexcisable length was determined to be 265 bp (FF), and the 2 fragments measuring 169 bp and 96 bp were determined to be excisable lengths (ff).

Results. For the genotype of VDR *Fok* I start codon polymorphism, there were no statistically significant differences between the 2 groups (chi-squared test, p = 0.07). Additionally, we did not detect any association of VDR genotype with the clinical and laboratory profiles in SLE patients.

Conclusion. Our results suggest that the vitamin D receptor *Fok* I start codon polymorphism is not related to patients with SLE in Taiwan. (J Rheumatol 2002;29:1211–3)

Key Indexing Terms:
VITAMIN D RECEPTOR POLYMORPHISM SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a systemic disease with a wide spectrum of clinical and laboratory manifestations. The etiology is still unknown. Recently researchers have revealed that complex cytokine networks including both type-1 T-helper cell (Th1) and Th2-type cytokines might be involved in the pathogenesis of SLE[1]. It has been reported that 1α,25–dihydroxy vitamin D3 ($1,25(OH)_2 D_3$) inhibited interferon (IFN-γ) secretion by Th1 cells in a dose dependent manner[2]. $1,25(OH)_2 D_3$ binds to a nuclear receptor termed vitamin D_3 receptor (VDR)[3].

The VDR genes have been used as genetic markers for the prediction of bone mass density in menopausal women[4]. The single nucleotide polymorphism (SNP) sites most frequently used were start codon (*Fok* I) and intron 8 (*Bsm* I) polymorphisms. In a study of postmenopausal Mexican-American women, Gross, *et al* found a marked association between the VDR gene start codon *Fok* I polymorphism and bone mineral density (BMD)[5]. Epidemiologic study results have confirmed the association between VDR gene polymorphisms and BMD in premenopausal black and white women[6]. However, studies with subjects of diverse ethnic backgrounds may yield discrepant results. Recently, VDR gene *Bsm* I polymorphisms have been used as genetic markers to determine their association with SLE[7,8]. We examined the polymorphism of the start codon *Fok* I of VDR gene to determine whether the polymorphism could serve as a marker of susceptibility to or severity of SLE in Chinese patients in Taiwan.

MATERIALS AND METHODS

Fifty-two patients had definite SLE according to the 1982 revised American College of Rheumatology (ACR) criteria[9]. In addition, 90 unrelated, healthy individuals living in central Taiwan served as control subjects. There were 49 female and 3 male patients with SLE enrolled in this study. Genomic DNA was prepared from peripheral blood using a genomic DNA isolation reagent kit (Genomaker, Taichung, Taiwan). Polymerase chain reaction (PCR) for VDR gene start codon polymorphisms were carried out to a total volume of 25 μl, containing genomic DNA (2–6 pmol of each primer); 1× Taq polymerase buffer (1.5 mM $MgCl_2$); and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA, USA). The primers of the vitamin D receptor gene were forward (5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3') and backward (5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3') according to the report by Harris, *et al*[6]. PCR amplification was performed using a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer). The cycling conditions for intron 8 polymorphism were set as follows: one cycle at 94°C for 5 min, 35 cycles at 94°C for 30 s, 58°C for 30 s, 72°C for 20 s, and a final cycle of extension at 72°C for 7 min.

The PCR product of the 265 bp band was mixed with 2 units of *Fok* I (Novel, Beverly, MA, USA). The reaction buffer was prepared according to the manufacturer's instructions. The restriction site was designed to be located at the recognizable allele of ATG to form an excision site. Two fragments of 169 bp and 96 bp, respectively, were present if the product was excisable. The reaction was incubated at 37°C overnight. Then, 10 μl of the product was loaded into 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was then divided into 3 groups: excisable (FF), unexcisable (ff), and heterozygote (Ff).

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Allelic frequency was expressed as a percentage of the total number of alleles. Results from control subjects and SLE patients were compared using the chi-squared test (2 × 2 contingency tables) for statistical significance. Fisher's exact test was used when the assumption of the chi-squared test was violated, when one cell had an expected count of < 1, or > 20% of the cells had an expected count of < 5. The distributions of the VDR start codon *Fok* I gene polymorphisms in each group were evaluated. $P < 0.05$ was considered statistically significant. Odds ratios (OR) were calculated from allelic frequency with 95% confidence interval (95% CI) for the polymorphism of the VDR gene.

RESULTS

The frequencies of the genotypes in the SLE group and control group are shown in Table 1. The distribution of the FF homozygote in the control group was 23.3%, that of the ff homozygote was 28.9%, and of the Ff heterozygote was 47.8%. In SLE patients, the distribution was 21.2% for the FF, 65.4% for the Ff, and 13.4% for the ff. Using the chi-squared test, the distribution of start codon *Fok* I polymorphism of VDR gene was compared, showing no significant differences between the healthy control group and the SLE patient group (chi-squared = 5.33, p = 0.07). The clinical manifestations and laboratory findings, given as ACR criteria, are shown in Table 2. We did not find any relationship of start codon *Fok* I polymorphism of VDR gene with clinical symptoms and laboratory profiles of SLE in Chinese patients.

DISCUSSION

SLE is an autoimmune disease of variable clinical presentation characterized by a remitting and relapsing course[10,11].

Table 1. Results of VDR genotyping in patients with SLE and healthy control subjects.

	VDR Genotype		
	FF	Ff	ff
SLE, n = 52 (%)	11 (21.2)	34 (65.4)	7 (13.4)
Control, n = 90 (%)	21 (23.3)	43 (47.8)	26 (28.9)

Chi-squared = 5.33; p = 0.07.

Table 2. Relationship between VDR genotype and clinical signs and findings in patients with SLE.

	FF, n = 11 (%)	Ff, n = 34 (%)	ff, n = 7 (%)	Total, n = 52 (%)
Malar rash	3 (27.3)	21 (61.8)	4 (57.1)	28 (53.8)
Discoid lupus	2 (18.2)	10 (29.4)	2 (28.6)	14 (26.9)
Photosensitivity	3 (27.3)	21 (61.8)	4 (57.1)	28 (53.8)
Mucosal ulcer	2 (18.2)	13 (38.2)	3 (42.9)	18 (34.6)
Arthritis	4 (36.4)	14 (41.2)	5 (71.4)	23 (44.2)
Serositis	2 (18.2)	8 (23.5)	2 (28.6)	12 (23.1)
Glomerulonephritis	4 (36.4)	17 (50)	4 (57.1)	25 (48.1)
Neuropsychiatric	1 (9.1)	6 (17.6)	2 (28.6)	9 (17.3)
Hematological	4 (36.4)	12 (35.3)	1 (14.3)	17 (32.7)
Immunology	7 (63.6)	24 (70.6)	4 (57.1)	35 (67.3)
ANA	11 (100)	34 (100)	7 (100)	52 (100)

ANA: antinuclear antibodies.

Genetic factors are believed to play an important role in the etiology of SLE with the possible involvement of several genes[12,13]. On the other hand, it is still controversial as to which cytokines play roles in the pathogenesis of SLE. However, there are results of several studies supporting a Thl/Th2-cytokine imbalance in SLE[1]. As we know, Th1 secrete IFN-γ and interleukin-2 (IL-2), while Th2 secrete IL-4 and IL-10. Recently, decreased production of IL-12 and Th1-type cytokines in patients with early stage SLE was reported, suggesting that an imbalance between IL-10 and IL-12 may play a primary role in Th1/Th2 imbalance[14].

Known to be an immunosuppressive hormone, $1,25(OH)_2D_3$ is reported to negatively regulate IL-12 production by downregulation of NF-κB activation and binding to the p40-κB sequence[15]. $1,25(OH)_2D_3$ is a secosteroid hormone that binds to the nuclear receptor VDR[3]. In addition, $1,25(OH)_2D_3$ directly inhibits IFN-γ secretion by Th1 clones, while it has little effect on IL-4 secretion by Th2 clones[2]. In mice, when given *in vivo*, $1,25(OH)_2D_3$ prevented the induction of spontaneous, induced autoimmune diseases, and inhibited Th1 induce IgG2a responses[16,17]. Vitamin D is known to regulate cell proliferation, calcium absorption from gut, cell differentiation, and may also influence androgen and estrogen activation. The action of vitamin D is dependent on the VDR.

Morrison, *et al* found that the VDR genotype at intron 8 *Bsm* I could be used to predict differences in bone density in healthy individuals[4]. Further, this allelic variant has also been reported to increase the risk of advanced prostate cancer[18], primary parathyroid tumor[19], colorectal cancer[20], and metastatic breast cancer[21]. Recently, VDR gene *Bsm* I polymorphisms were used as genetic markers to determine their association with SLE from our previous study, as well as in a Japanese report[7,8]. The theory was proposed, by which the BB genotype may promote Th1/Th2 cytokine imbalance through the repression of IL-2 gene transcription of negative regulation of IL-12 production. In a study of postmenopausal Mexican-American women, Gross, *et al* found a marked association between the VDR gene start codon *Fok* I polymorphism and BMD[5]. By using restriction endonuclease *Fok* I to identify a single base difference of the VDR gene start codon from individual to individual, we were able to determine the distribution of single nucleotide polymorphisms in the disease group. For the genotype of VDR *Fok* I start codon polymorphism, there were no statistically significant differences between the SLE patients and healthy control subjects (chi-squared test, p = 0.07). Additionally, we did not detect any association of VDR genotype with the clinical and laboratory profiles in SLE patients.

Although the VDR gene *Fok* I polymorphism was not an appropriate genetic marker in our present study, with the growing accumulation of mapped genes, the likelihood that these regions contain a candidate gene is growing. The

candidate genes might provide further analysis for tissue expression or clinical presentations in a variety of groups.

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