Research article

Vitamin D receptor gene \textit{BsmI} polymorphisms in Thai patients with systemic lupus erythematosus

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Abstract

The immunomodulatory role of 1,25-dihydroxyvitamin D3 is well known. An association between vitamin D receptor (VDR) gene \textit{BsmI} polymorphisms and systemic lupus erythematosus (SLE) has been reported. To examine the characteristics of VDR gene \textit{BsmI} polymorphisms in patients with SLE and the relationship of polymorphisms to the susceptibility and clinical manifestations of SLE, VDR genotypings of 101 Thai patients with SLE and 194 healthy controls were performed based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The relationship between VDR gene \textit{BsmI} polymorphisms and clinical manifestations of SLE was evaluated. The distribution of VDR genotyping in patients with SLE was 1.9% for BB (non-excisable allele homozygote), 21.78% for Bb (heterozygote), and 76.23% for bb (excisable allele homozygote). The distribution of VDR genotyping in the control group was 1.03% for BB, 15.98% for Bb, and 82.99% for bb. There was no statistically significant difference between the two groups ($p = 0.357$). The allelic distribution of B and b was similar within the groups ($p = 0.173$). The relationship between VDR genotype and clinical manifestation or laboratory profiles of SLE also cannot be statistically demonstrated. In conclusion, we cannot verify any association between VDR gene \textit{BsmI} polymorphism and SLE. A larger study examining other VDR gene polymorphisms is proposed.

Introduction

The importance of genetic influences on systemic lupus erythematosus (SLE) has been recognized through cumulative genetic epidemiologic studies. Many population-based studies have shown associations between the disease and alleles of immunologically relevant genes, including certain major histocompatibility complex (MHC) loci, \textit{Fcγ} receptor, and cytokines [1]. 1,25-dihydroxyvitamin D3 is thought to exert many of its action through interaction with a specific intracellular receptor. At the molecular level, 1,25-dihydroxyvitamin D3 inhibits the accumulation of mRNA for interleukin (IL)-2, interferon (IFN)-\textit{γ}, and granulocyte-macrophage colony-stimulating factor (GM-CSF). At the cellular level, the hormone interferes with T helper cell (Th) function, reducing Th induction of immunoglobulin production by B cells. When given \textit{in vivo}, 1,25-dihydroxyvitamin D3 has been particularly effective in prevention of autoimmune diseases such as experimental autoimmune encephalitis and murine lupus [2]. It has been demonstrated that patients with SLE have a lower level of 25-hydroxyvitamin D3 than do healthy controls [3]. In addition, high-dose 1,25-dihydroxyvitamin D3 and its analog may be useful therapeutic agents for psoriatic arthritis [4] and rheumatoid arthritis [5].

Polymorphism of the vitamin D receptor (VDR) gene was found to be associated with many diseases, including osteoporosis [6], hyperparathyroidism [7], and prostate cancer [8]. An association between VDR gene polymorphism and SLE in Japanese and Chinese patients has been reported with mixed results [9-11]. Although Asians are closely related ethnically, the genetic admixture in Japan or China is different from that of Thailand. Because a high prevalence and high clinical severity of SLE are also observed in the Thai population, we examined the characteristics of VDR gene \textit{BsmI} polymorphisms in Thai patients.

\textbf{bb} = excisable allele homozygote; \textbf{Bb} = heterozygote; \textbf{BB} = non-excisable allele homozygote; \textbf{HWE} = Hardy-Weinberg equilibrium; \textbf{IFN} = interferon; \textbf{IL} = interleukin; \textbf{SLE} = systemic lupus erythematosus; \textbf{Th} = T helper cell; \textbf{VDR} = vitamin D receptor.
a larger cohort of Thai patients with SLE and the relationship of polymorphisms to the susceptibility and clinical manifestations of SLE.

Materials and methods
This study was conducted in accordance with the principles embodied in the Declaration of Helsinki and was approved by the ethical committees of the Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. DNA from 101 patients with SLE was examined. All patients fulfilled the 1982 revised criteria for SLE [12]. All were females older than 15 years of age. They did not meet criteria for other autoimmune diseases. DNA from 194 unrelated healthy subjects served as controls. All healthy subjects were females older than 15 years of age. VDR genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Genomic DNA was extracted from peripheral white blood cells using standard phenol-chloroform method. PCR was carried out in a final reaction volume of 50 μl. Oligonucleotide primers designed to anneal to exon 7 (primer 1, 5’ CAACCAAGACTACTAACAATCCGCGTCAGTGA-3’) and intron 8 (primer 2, 5’ CAACCAAGACTACTAACAATCCGCGTCAGTGA-3’) were used to amplify 825 bp fragment, including the polymorphic BsmI site in intron 7 of the gene. The following reagents were added to a 200-μl ultramicrocentrifuge tube: 5 μl of 10 × buffer (100 mM Tris HCl pH 9.0, 500 mM KCl, and 1.0% Triton x-100), 2 μl of MgCl₂ (25 mM), 3 μl of deoxynucleotide triphosphate (2 mM each) (Promega, Madison, WI, USA), 0.5 μl of primer 1 (20 μM), 0.5 μl of primer 2 (20 μM), 2.5 units of Taq DNA polymerase (Promega), 300 ng of template DNA, and water to a final volume of 50 μl.

The cycling condition was set as follows: one cycle at 95°C for 3 minutes, 30 cycles at 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. One final cycle of the extension was performed at 72°C for 10 minutes.

One microliter of the PCR product was digested at 65°C for 1 hour in the final volume of 10 μl with 5 units of restriction enzyme BsmI (New England Biolabs Inc., Ipswich, MA, USA) in 1 × buffer. The digested samples were fractionated by electrophoresis in a 1.5% agarose gel. Restriction fragments were detected by staining with ethidium bromide, and genotypes were determined by comparing the restriction length polymorphism band patterns with a 100 bp DNA ladder run on the same gel. The presence of the BsmI restriction site generated 175 bp and 650 bp fragments, whereas the absence of this site yielded an 825 bp fragment.

The genotypes were classified as excisable allele homozygote (bb), non-excisable allele homozygote (BB), and heterozygote (Bb).

Statistical analysis
Analyses were performed with Epi Info™ 2002 Results from patients with SLE and control subjects were compared using the χ² test for statistical significance. Hardy-Weinberg equilibrium (HWE) was determined by Pearson’s χ² goodness-of-fit test.

Results
The distribution of VDR genotyping in patients with SLE was 1.9% for BB, 21.78% for Bb, and 76.23% for bb. The distribution of VDR genotyping in the control group was 1.03% for BB, 15.98% for Bb, and 82.99% for bb. There was no statistically significant difference between the two groups (p = 0.357) (Table 1). The genotype frequencies were consistent with HWE in patients and controls (χ² = 0.08, p = 0.77 and χ² = 0.14, p = 0.71, respectively). The allelic distribution of B and b was similar within the two groups (p = 0.173) (Table 2). The relationship between VDR genotype and clinical manifestation or laboratory profiles of SLE cannot be statistically demonstrated (Table 3).

Discussion
Most tissues in the body, including heart, stomach, pancreas, bone, skin, gonads, and activated T and B lymphocytes, have the nuclear receptor for 1,25-dihydroxyvitamin D3 (VDR). Thus, it is not surprising that 1,25-dihydroxyvitamin D3 has a multitude of biologic effects that are non-calcemic in nature [13]. Recent research shows that the biologic action of vitamin D extends well beyond the classic function to include effects on immunity, muscle and vasculature, reproduction, and the growth and differentiation of many cell types [14]. 1,25-Dihy-
droxyvitamin D3 directly inhibits synthesis and secretion of IL-2 [15,16] and IFN-γ [17,18] and also inhibits immunoglobulin production [19]. Genomic actions of 1,25-dihydroxyvitamin D3 are mediated through its nuclear receptor (VDR). The VDR regulates gene transcription by binding to the hexameric core binding motif in promoter region of target genes, VDR element (VDRE) [17]. Extensive studies focused on this VDR gene in various phenotypes have revealed the association between VDR polymorphism and many non-skeletal diseases [20].

Although SLE has features consistent with Th2-type cytokine predominance, both Th1 and Th2 cytokines may be involved in the pathogenesis of SLE [21]. Mononuclear cells of patients with SLE have defects in IL-2 signal transduction and decreased production of IFN-γ [22]. IFN-γ, tumor necrosis factor (TNF)-α, and IL-1 are the most important adhesion molecules inducing cytokine, and they increase in autoimmune renal disease, particularly in Mrl/lpr-Fas and NZB/W mice [23].

Recently, VDR gene BsmI polymorphisms have been used as genetic markers to determine their association with SLE [9-11]. A Japanese study of 58 patients with SLE found that the BB genotype might trigger the development of SLE and that the bb genotype was associated with lupus nephritis [9]. A Taiwanese study [10] of 47 Chinese patients with SLE also found an increased distribution of the VDR BB genotype in SLE but indicated no association between the frequency of VDR allelic variations and clinical manifestations or laboratory profiles. In our study, the BB genotype is low in both 194 healthy controls and 101 patients with SLE. However, this is in accordance with previous findings in the Thai population [24]. Thailand is geographically situated in an area between China and India. This genetic admixture may influence the distribution of VDR gene polymorphism. We cannot demonstrate

| BB % (ratio) n = 2 | Bb % (ratio) n = 22 | bb % (ratio) n = 77 | Total % (ratio) |
|-------------------|-------------------|-------------------|-----------------|
| Malar rash        | 50 (1/2)          | 54.54 (12/22)     | 54.54 (42/77)   | 54.45 (55/101) |
| Discoid rash      | 0 (0/0)           | 27.27 (6/22)      | 31.16 (24/77)   | 29.70 (30/101) |
| Photosensitivity  | 50 (1/2)          | 31.81 (7/22)      | 38.96 (30/77)   | 37.62 (38/101) |
| Oral ulcer        | 50 (1/2)          | 31.81 (7/22)      | 36.36 (28/77)   | 35.64 (36/101) |
| Arthritis         | 100 (2/2)         | 77.27 (17/22)     | 70.12 (54/77)   | 72.27 (73/101) |
| Serositis         | 50 (1/2)          | 18.18 (4/22)      | 9.09 (7/77)     | 11.88 (12/101) |
| - Pericardial effusion | 0 (0/0)   | 13.63 (3/22)      | 7.79 (6/77)     | 8.91 (9/101)   |
| - Pleural effusion | 50 (1/2)          | 9.09 (2/22)       | 6.49 (5/77)     | 7.92 (8/101)   |
| Renal disorder    | 50 (1/2)          | 68.18 (15/22)     | 64.93 (50/77)   | 65.34 (66/101) |
| Neurologic disorder | 0 (0/0)     | 9.09 (2/22)       | 20.77 (16/77)   | 17.82 (18/101) |
| - Seizure          | 0 (0/0)           | 9.09 (2/22)       | 15.58 (12/77)   | 13.86 (14/101) |
| - Psychosis        | 0 (0/0)           | 0 (0/22)          | 9.09 (7/77)     | 6.93 (7/101)   |
| Hematologic disorder |             |                   |                 |                |
| - Leukopenia       | 50 (1/2)          | 40.90 (9/22)      | 44.15 (34/77)   | 43.56 (44/101) |
| - Thrombocytopenia | 0 (0/0)           | 9.09 (2/22)       | 18.18 (14/77)   | 15.84 (16/101) |
| Immunologic disorder |             |                   |                 |                |
| - Anti-DNA         | 0 (0/0)           | 71.42 (15/21)     | 56.66 (34/60)   | 60.49 (49/81)  |
| - Anti-Sm          | 0 (0/0)           | 57.14 (8/14)      | 32.60 (15/46)   | 37.09 (23/62)  |
| ANA                | 100 (2/2)         | 100 (22/22)       | 98.68 (75/76)   | 99 (99/100)    |
| - Homogenous pattern | 50 (1/2)   | 50 (11/22)        | 48.68 (37/76)   | 49 (49/100)    |
| - Rim pattern      | 0 (0/0)           | 40.90 (9/22)      | 44.73 (34/76)   | 33 (33/100)    |
| - Nucleolar pattern | 0 (0/0)     | 0 (0/22)          | 3.94 (3/76)     | 3 (3/100)      |
| - Speckle pattern  | 50 (1/2)          | 45.45 (10/22)     | 59.21 (45/76)   | 56 (56/100)    |

ANA = anti-nuclear antibodies; bb = excisable allele homozygote; Bb = heterozygote; BB = non-excizable allele homozygote; SLE, systemic lupus erythematosus; Sm = Smith; VDR, vitamin D receptor.
any association between VDR gene BsmI polymorphism and SLE. We further examined the relationship between VDR genotype and the individual clinical manifestation or laboratory profiles of SLE, which also cannot be statistically demonstrated.

Conclusion
It was apparent that compared with the genotype distribution of the VDR gene reported in previous studies [9-11], the genotype frequencies in Thais were different. Because our study includes a larger number of patients and controls than any previous study, we conclude that there is no association between VDR gene BsmI polymorphisms and SLE, at least in Thai patients. We propose that other VDR gene polymorphisms be examined.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
WS conceived of the study, and participated in its design, coordination and acquisition of data. OV carried out the molecular genetic study and performed statistical analysis. KN and KT participated in coordination and interpretation of data. PL participated in acquisition of data and helped in drafting and revising the manuscript. SJ have been involved in drafting and revising the manuscript. SJ have been involved in drafting and revising the manuscript for important intellectual content and have given final approval of the version to be published. All authors read and approved the final manuscript.

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