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SHORT COMMUNICATION

Lack of association of vitamin D receptor gene BsmI polymorphisms in patients with systemic lupus erythematosus

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Abstract Several data link vitamin D to many autoimmune diseases. Association between vitamin D receptor (VDR) gene BsmI polymorphisms and systemic lupus erythematosus has been reported. In this study, we examine whether the VDR gene BsmI polymorphisms are markers for susceptibility to or severity of SLE. This study incorporated 60 patients with SLE living in northeastern Iran. Three genotypes, BB, Bb and bb, were detected based on polymerase chain reaction–restriction fragment length polymorphism (PCR/RFLP). The distribution of VDR genotyping in patients with SLE was 23.3% for BB, 60% for Bb and 16.7% for bb and in the control group was 33.3% for BB, 46.7% for Bb and 20% for bb ($P = 0.334$). No association of VDR gene BsmI polymorphisms with SLE disease activity index (SLEDAI), SLE damage score and major organ involvement was detected. The result of this study did not show any association between VDR gene BsmI polymorphisms and SLE.

Keywords Vitamin D receptor · Polymorphisms · Systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus is a multifactorial autoimmune disorder characterized by the production of autoreactive T cells and autoantibodies against nuclear, cytoplasmic and cell surface antigens that may affect every organ system. Several studies have provided interesting insights into the complexity of the genetic interactions involved in SLE but have not yet resulted in the identification of specific factors [1].

Recent data showed vitamin D as an immunosuppressive hormone. It interferes with Th cell functions at the cellular level reducing Th induction of immunoglobulin production [2]. $1,25(OH)_2D3$ is thought to exert many of its actions through interaction with a specific intracellular receptor (VDR). It inhibits the accumulation of mRNA for interleukin (IL) 2, interferon (IFN) γ and granulocyte–macrophage colony-stimulating factor (GM-CSF) and also negatively regulates IL_{12} production by downregulation of NF-KB activation [3, 4]. An association between VDR gene polymorphism and SLE in Japanese and Chinese patients has been reported with diverse results [5–7].

We examine the polymorphisms of the VDR gene intron 8 (BsmI) to determine whether the VDR gene BsmI polymorphisms are markers for susceptibility to or severity of SLE in northeastern Iran.

Patients and methods

Sixty unrelated patients with definite SLE according to the 1982 revised American College of Rheumatology criteria were recruited sequentially from the northeastern Iran between November 2007 and June 2008 [8]. Mean age of the patients was 28 ± 9 years. The control group consisted

Table 1 Distribution of VDR genotyping according to SLEDAI

VDR genotyping	SLEDAI ≤ 10	SLEDAI = 11–20	SLEDAI > 20	Total	
	Number (%)	Number (%)	Number (%)	Number (%)	*P* value
BB	6 (42.9)	4 (28.6)	4 (28.6)	14 (100)	0.984
Bb	16 (44.4)	12 (33.3)	8 (22.2)	36 (100)	
bb	5 (50.0)	3 (30.0)	2 (20.0)	10 (100)	
Total	27 (45.0)	19 (31.7)	14 (23.3)	60 (100)	

of 45 unrelated healthy individuals with the mean age of 31 ± 8 years ($P = 0.147$). This study was approved by the Ethics Committee at the Mashhad University of Medical Sciences.

The main clinical manifestations they had were nephritis (48.3%), hematologic abnormalities (55%), neurologic involvements (20%), skin rash (83.3%), serositis (16.7%), arthritis (85%) and vasculitis (33.3%).

Genomic DNA was isolated from whole blood using DNA extraction kit (Biogene, Mashhad, Iran) by salting-out procedure. VDR gene sequences were amplified by the polymerase chain reaction (PCR) method followed by restriction fragment length polymorphism (RFLP) technique. An 813-bp region of the VDR gene was amplified using the following primers: [5].

Primer A (sense): 5′ AAC TTG CAT GAG GAG GAG CAT GTC 3′

Primer B (antisense):5′ GGA GAG GAG CCT GTG TCC CAT TAG 3′

The PCR product was mixed with 1 unit of BsmI (Mval 269 I, Fermentas, Canada) enzyme and the reaction buffer. Expected fragment sizes on agarose gel containing ethidium bromide were 477 bp for the 813 B allele and 318 bp for the 813 b allele. The polymorphism was then divided into three groups: excisable allele homozygote (bb), unexcisable allele homozygote (BB) and heterozygote (Bb).

The relationship between BsmI polymorphisms, SLE disease activity index (SLEDAI) and damage score according to SLICC/ACR SLE damage index was evaluated. We consider SLEDAI > 10 as active disease.

Results

The distribution of VDR genotyping in patients with SLE was 14(23.3%) for BB, 36(60%) for Bb, 10(16.7%) for bb and in the controls was 15(33.3%) for BB, 21(46.7%) for Bb and 9(20.0%) for bb. There was no statistically significant difference between two groups ($P = 0.334$). The allelic distribution of B and b was similar within the two groups ($P = 0.549$).

No association of VDR gene BsmI polymorphisms with disease activity index ($P = 0.984$) (Table 1), SLE damage score ($P = 0.504$) and major organ involvement ($P = 0.090$) was detected.

Discussion

One of the most recent agents found to be associated with autoimmunity is vitamin D. Vitamin D has several immunomodulatory effects and thus may play a role in the course of autoimmune diseases and known to be an immunosuppressive hormone [4]. 1,25-Dihydroxyvitamin D3 inhibits the amassing of mRNA for interleukin-12 and interferon (IFN)-γ while IL-10 and transforming growth factor ß production is enhanced, resulting in inhibition of T cell activation and further tilts the T cell response toward Th2 dominance [9]. Although in lupus Th2-type cytokine is predominant, both Th1 and Th2 cytokine may be involved in the pathogenesis of lupus. In patients with early stage SLE, decreased production of IL_{12} and Th_1-type cytokines was reported [6, 7].

Vitamin D deficiency correlates with many autoimmune diseases as well as multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis and lupus [2, 4, 10]. 1,25-Dihydroxyvitamin D3 was shown to be therapeutically effective in animal model of lupus [11].

It has been demonstrated that VDR gene BsmI polymorphisms are genetic markers of SLE [2, 5–7, 10]. A study in Japan of 58 patients with SLE found that BB genotype might trigger the development of SLE and that the bb genotype was associated with lupus nephritis [5]. Another similar study in Taiwan with the population of 47 patients with SLE also found an increased distribution of VDR BB genotype in SLE, but indicated no association between the frequency of VDR allelic variations and clinical manifestations or laboratory profiles [6]. Sakulpipatsin et al. did not show any association between VDR gene BsmI polymorphism and SLE in 101 Thai patients with lupus [2].

In the present study, no statistically significant difference was detected in VDR gene BsmI polymorphisms between patients and healthy subjects living in northeastern Iran. Table 2 shows the comparison between the distributions of VDR gene BsmI polymorphisms in four studies. Furthermore, we could not statistically demonstrate the

Table 2 The comparison of VDR genotypes between four studies

Studies	BB	bb	Bb	Total		*P* value
	Number (%)	Number (%)	Number (%)	Number		
Ozaki (2000)	9 (15.5)	24 (41.4)	25 (43.1)	58	Patients	0.0001
	5 (5.7)	72 (82.8)	10 (11.5)	87	Controls	
Huang (2002)	2 (4.3)	12 (25.5)	33 (70.2)	47	Patients	0.0001
	3 (3.3)	78 (86.7)	9 (10)	90	Controls	
Sakulpipatsin (2006)	2 (1.9)	77 (76.23)	22 (21.76)	101	Patients	0.357
	2 (1.03)	161 (82.99)	31 (15.98)	194	Controls	
Abbasi (2009)	14 (23.3)	10 (16.7)	36 (60)	60	Patients	0.334
	15 (33.3)	9 (20.0)	21 (46.7)	45	Controls	

P value <0.05 was considered statistically significant

relationship between VDR genotype and organ involvement or laboratory profiles, SLE disease activity index and SLE damage score. It is generally agreed that the difference in the distribution of intron 8 VDR polymorphism between studies with inconsistent findings may be due to ethnic factors, other SLE cohorts are necessary to confirm these findings in other population.

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References

1. Sanchez E, Sabio JM, Callejas JL, de Ramon E, Garcia-Portales R, Garcia-Hernandez FJ (2006) Association study of genetic variants of pro-inflammatory chemokine and cytokine genes in systemic lupus erythematosus. BMC Med Genet 23:48
2. Sakulpipatsin W, Verasertniyom O, Nantiru KJ, Totemchokchyakam K, Lertsrisatit P, Janwityanujit S (2006) Vitamin D receptor gene Bsm1 polymorphisms in thai patients with systemic lupus erythematosus. Arthritis Res Ther 8: R48. doi:10.1186/ar1910
3. D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, Sinigaglia F, Panina-Bordignon P (1998) Inhibition of IL-12 production by 1, 25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of P40 gene. J Clin Invest 101:252–262
4. Arnson Y, Amital H, Shoenfeld Y (2007) Vitamin D and autoimmunity: new aetiological and therapeutic considerations. Ann Rheum Dis 66:1137–1142
5. Ozaki Y, Nomura S, Nagahana M, Yoshimura C, Kagawa H, Fukuhara S (2000) Vitamin-D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. Nephron 85:86–91
6. Huang CM, Wu MC, Wu JK, Tsai FJ (2002) Association of vitamin D receptor gene Bsm1 polymorphisms in Chinese patients with systemic lupus erythematosus. Lupus 11:31–34
7. Huang CM, Wu MC, Wu JY, Tsai FJ (2002) No association of vitamin D receptor gene start codon fok 1 polymorphisms in Chinese patients with systemic lupus erythematosus. J Rheumatol 29:1211–1213
8. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25:1271–1277
9. Lemire JM, Archer DC, Beck L, Spiegeburg HL (1995) Immunosuppressive actions of 1, 25-Dihydroxy vitamin D3: preferential inhibition of Th1 functions. J Nutr 125(6suppl):1704S–1708S
10. Muller K, Kriegbaum NJ, Baslund B, Sorensen OH, Thymann M, Bentzen K (1995) Vitamin D3 metabolism in patients with rheumatic diseases: low serum levels of 25-hydroxyvitamin D3 in patients with systemic lupus erythematosus. Clin Rheumatol 14:397–400
11. Lemire JM, Ince A, Takashima M (1992) 1, 25-dihydroxy vitamin D3 attenuates the expression of experimental murine lupus of MRL/1mice. Autoimmunity 12:143–148