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PAPER

The role of *Bsm*I and *Fok*I vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D in Brazilian patients with systemic lupus erythematosus

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Vitamin D deficiency has been described in systemic lupus erythematosus (SLE). *Bsm*I *VDR* (vitamin D receptor) gene polymorphism was associated with SLE in Asian patients. Studies in Brazilian populations have not been realized. A case-control study with 195 SLE patients and 201 healthy controls was conducted to investigate the influence of *Bsm*I and *Fok*I *VDR* gene polymorphisms on susceptibility to SLE. In addition, 25-hydroxyvitamin D [25(OH)D] was measured in SLE patients to evaluate possible associations with *VDR* polymorphic variants and clinical and laboratory expressions of disease. Genotyping was performed by RFLP-PCR. The measurement of 25(OH)D was performed by chemiluminescence. There was no statistically significant difference in genotype and allelic frequencies of *Bsm*I and *Fok*I polymorphisms between European-derived cases and controls. The mean serum levels of 25(OH)D were 25.51 ± 11.43 ng/ml in SLE patients. According to genotype distribution, 25(OH)D concentrations were significantly higher in patients carrying the *Fok*I f/f genotype compared with patients carrying the F/F genotype (31.6 ± 14.1 ng/ml versus 23.0 ± 9.2 ng/ml, $p = 0.004$), reinforcing its role in the functional activity of VDR. This feature may be considered in future clinical and experimental studies involving vitamin D measurements. Therefore, genetic-specific definitions of ideal levels of vitamin D in SLE need to be established in future studies. *Lupus* (2012) **21,** 43–52.

Key words: 25-hydroxyvitamin D; *Bsm*I polymorphism; *Fok*I polymorphism; genetics and immunology; systemic lupus erythematosus; vitamin D; vitamin D receptor

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease that involves many organs and systems. The etiology and pathogenesis of SLE remains poorly understood. Many observations suggest a role of genetic, immunologic, hormonal and environmental factors.[1] Abnormal immune system response allows the processing of self antigens by antigen presenting cells, hyperactivation of B and T cells and failure of immunoregulatory mechanisms.

Vitamin D and its associated receptor (vitamin D receptor – VDR) have immunomodulatory properties, and studies of a role for vitamin D deficiency in activation of the immune response have provided new insights into the function of this vitamin. It presents inhibitory effects on T cells, B cells and dendritic cells and alters the cytokine profile toward the Th2 phenotype tolerogenic.[2] These suppressive immunologic properties have led to investigation of a possible role in SLE pathogenic mechanisms.[3]

Studies have shown lower levels of vitamin D in SLE patients, suggesting that vitamin D deficiency may be a risk factor for SLE development.[4] However, vitamin D intake was not associated with risk of SLE in a large prospective cohort of women.[5] The majority of studies have documented an association between higher disease activity and lower vitamin D levels.[4] To date, the physiologic and clinical

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significance of vitamin D deficiency in SLE is still not entirely known.

The action of vitamin D depends on VDR that is synthesized by the *VDR* gene, located on chromosome 12 and composed of 9 exons.[6] It has emerged as a candidate for SLE susceptibility due to the role of vitamin D in the immune system. Four major polymorphisms (*Bsm*I, *Apa*I, *Taq*I and *Fok*I) have been described although their influences on VDR function are still unknown. Some studies have observed the occurrence of certain *VDR* gene polymorphic variants in SLE patients. A recent meta-analysis suggested that allele B from *Bsm*I *VDR* gene polymorphism had significant association with SLE in Asian patients.[7] In a single Chinese study, the *Fok*I polymorphism did not show association with SLE.[8] Furthermore, different studies described controversial associations of these allelic variants with specific clinical features in SLE patients.

Facing the lack of reports concerning the role of vitamin D and *VDR* gene allelic variants in SLE and few studies in Brazil, a case-control study was conducted to investigate whether the *Bsm*I and *Fok*I *VDR* gene polymorphisms could be susceptibility markers for SLE or the severity of SLE. In addition, 25-hydroxyvitamin [25(OH)D] was measured in SLE patients to evaluate possible associations with *VDR* gene polymorphic variants and clinical and laboratory expressions of disease.

Patients and methods

Study population

The study population was composed of 195 consecutive SLE patients identified as either European-derived or African-derived. This classification was based on physical appearance, as judged by the researcher at the time of blood collection, and data about the ethnicity of parents/grandparents reported by the participants. These classification criteria that are used in Brazil are well documented and have been assessed by our group in previous studies.[9] Also, a recent study assessing individual interethnic admixture and population substructure using a panel of 48-insertion–deletion ancestry-informative markers validated this classification in European-derived individuals from our geographic region.[10] SLE patients were followed at the Rheumatology Clinic of Hospital de Clínicas de Porto Alegre. Demographic, clinical and laboratory data about patients were collected from medical records. All patients fulfilled the American College of Rheumatology (ACR) revised criteria for the classification of SLE.[11] The control group was composed of 201 sex-matched European-derived healthy individuals from the same urban center. We did not include African-derived controls. The Ethics Committee of the Hospital de Clínicas de Porto Alegre approved the study protocol, and informed consent according to the Declaration of Helsinki was obtained from all participants.

Clinical and laboratory variables

The following variables were recorded during recruitment of patients using a standardized questionnaire: age, gender, phototype,[12] smoking status, alcohol consumption, body mass index (BMI), oral vitamin D supplementation and treatment with hydroxychloroquine, corticosteroids and immunosuppressive drugs. Clinical manifestations of SLE included the presence of photosensitivity, malar rash, discoid rash, oral or nasal ulcers, arthritis, serositis (pleuritis or pericarditis), nephritis and neurological disease, defined as seizures or psychosis. The laboratory evaluation included the presence of hematological disorders (hemolytic anemia, leukopenia, lymphopenia or thrombocytopenia), positive antinuclear antibody (ANA) (titer > 1:100), or other autoantibodies such as anti-dsDNA, anti-Sm, anti-RNP, anti-Ro/SS-A, anti-La/SS-B, anticardiolipin, lupus anticoagulant and false positive VDRL. The patients were also evaluated in regard to secondary antiphospholipid syndrome and Sjögren's syndrome, according to the classification criteria for both diseases.[13,14] The SLEDAI[15] and the SLICC damage index[16] were applied to each patient as a measurement of disease activity and cumulative damage, respectively.

Measurement of vitamin D

Blood samples were collected at the time of medical consultation, between 4 p.m. and 6 p.m., after at least four hours of fasting. To limit the effect of seasonal fluctuation of vitamin D photosynthesis, patients were recruited in the spring period.[17] All samples were frozen at -70°C and analyzed at the same time. Serum 25(OH)D levels were measured by chemiluminescence (LIASON – DiaSorin Inc., Stillwater/MN, CV 6% intra-assay). Measurement of 25-hydroxyvitamin D was performed only in patients because there was no possibility of collecting serum from controls. The blood of the controls had previously only been collected for DNA extraction.

Vitamin D deficiency was considered as levels of 25(OH)D < 20 ng/ml.[18] Normal range of 25(OH)D

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levels was defined as $\geq$30 ng/ml.[19] Serum levels between 20 and 30 ng/ml were classified as insufficiency status and <10 ng/ml as critically low levels of vitamin D.[20]

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood cells using a salting-out method.[21] DNA samples were stored at −20°C. Genotyping was performed as previously described.[22–25] To *Fok*I genotyping (rs10735810, recently merged with rs2228570), a polymerase chain reaction (PCR) amplification of VDR gene fragment was carried out to a total volume of 25 µl, containing genomic DNA, Taq polymerase buffer, $MgCl_2$, dNTP, specific primers and Taq DNA polymerase (Invitrogen Corporation, San Diego, CA, USA). The primers used were 5′-AGCTGGCCCTGGCACTGACTC TGCTCT-3′ and 5′- ATGGAAACACCTTGCTT CTTCTCCCTC-3′.[22] The cycling conditions 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 265 bp was cleaved by *Fok*I (FastDigest® *Bse*GI, Fermentas, Canada), according to the manufacturer's instructions. Two fragments of 169 bp and 96 bp, respectively, were obtained if the product was excisable. The fragments were visualized in 6% polyacrylamide gel stained with ethydium bromide under ultraviolet light. The polymorphism was then divided into three groups: excisable (f/f), unexcisable (F/F) and heterozygote (F/f). For *Bsm*I genotyping (rs1544410), a PCR was carried to a total volume of 50 µl, containing reagents described above. The primers used were 5′-CAACCAAGACTACAAG TACCGCGTCAGTGA-3′ and 5′- AACCAGC GGGAAGAGGTCAAGGG-3′.[23] The cycling conditions were set as follows: one cycle at 95°C for 3 min, 30 cycles at 95°C for 30 s, 56°C for 30 s, 72°C for 30 s, and a final cycle of extension at 72°C for 10 min.[26] The PCR product of 825 bp was cleaved by *Bsm*I (FastDigest®*Mva*1269I, Fermentas, Canada), according to the manufacturer's instructions. Two fragments of 650 bp and 175 bp, respectively, were obtained if the product was excisable. The fragments were visualized with the same conditions as described above. The polymorphism was then divided into three groups: excisable (b/b), unexcisable (B/B) and heterozygote (B/b).

Statistical analysis

A descriptive analysis of data through calculation of mean and standard deviation (SD) for quantitative variables was performed while the frequency and percentage were calculated for categorical variables. The median and interquartile range were calculated to quantitative variables with asymmetrical distribution. We used the chi-square test with continuity correction or Fisher's exact test to compare the frequencies of polymorphic variants. The odds ratio and 95% confidence interval were also calculated. For the comparison of clinical and laboratory variables with the frequencies of polymorphic variants, we used the chi-square test to compare qualitative variables and ANOVA (or Kruskal–Wallis) for quantitative variables, using Bonferroni correction to the level of statistical significance. The Hardy–Weinberg equilibrium test was performed in cases and controls using the chi-square test.

A multiple linear regression was used to evaluate association within genotypes with levels of 25(OH)D, adjusting for potential confounders[27] such as phototype, ethnicity, age, gender, smoking status, BMI, hydroxychloroquine use, corticosteroid use and vitamin D supplementation. A multiple logistic regression was used to evaluate factors associated with 25(OH)D deficiency, after this variable had been categorized as previously described. Odds ratio and 95% confidence intervals were estimated using logistic regression to determine disease characteristics associated with vitamin D deficiency, adjusting for the same confounding factors as described above.

Data were analyzed with SPSS software version 17.0 and a two-tailed value of $p < 0.05$ was taken to indicate statistical significance.

Results

One hundred and ninety-five SLE patients were included: 148 (75.9%) European-derived and 47 (24.1%) African-derived. One hundred and eighty-three (93.8%) were female and 12 (6.2%) were male. The average age of patients was 42.6 ± 13.9 years and the average age at diagnosis was 32.5 ± 13.7 years. Table 1 shows the frequencies of clinical and laboratory features of SLE patients. Secondary Sjögren's syndrome was found in 6.7% (13/195) and antiphospholipid syndrome in 7.2% (14/195). The median for SLEDAI and SLICC was 2 and 0, respectively. We observed that male patients had a higher frequency of nephritis (83.3% *vs.* 43.2%, $p = 0.016$), although the number of individuals in this group was

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relatively small. No other statistically significant differences were found between genders.

Of the 195 SLE patients included, 153 had complete *Fok*I and *Bsm*I genotyping and 25(OH)D measurement. Forty-two patients did not complete at least one of these variables due to problems during the implementation of techniques for genotyping and determination of vitamin D. The missing data were random and did not interfere in the final analysis, since when comparing these two groups (with and without all data), we did not find significant differences for clinical and laboratory features (data not shown).

The frequencies of *Bsm*I and *Fok*I *VDR* gene polymorphisms were studied in European-derived SLE patients and healthy controls (Table 2). The genotypic frequencies were in Hardy–Weinberg equilibrium in both cases and controls. Among 121 European-derived SLE patients, 20 (16.5%) were B/B, 61 (50.4%) were B/b and 40 (33.1%) were b/b. When compared with 201 European-derived healthy controls [31 (15.4%), 97 (48.3%) and 73 (36.3%), respectively], no statistically significant difference was observed for *Bsm*I genotype distribution ($p = 0.836$). Among 131 European-derived SLE patients, 64 (48.9%) were genotyped as F/F, 56 (42.7%) as F/f and 11 (8.4%) as f/f. When compared with 198 European-derived healthy controls [86 (43.4%), 94 (47.5%) and 18 (9.1%), respectively], there was no statistically significant difference in *Fok*I genotype distribution ($p = 0.626$). In addition, no statistically significant difference was found in the frequencies of *Bsm*I and *Fok*I between European- and African-derived SLE patients (Table 2) (p = 0.248 and $p = 0.775$, respectively). Allelic frequencies of alleles B, b, F and f were studied in European-derived SLE patients and compared with European-derived controls and African-derived patients. No significant differences were observed (Table 2). Associations between

Table 1 Demographic, clinical, and laboratory features of SLE patients

Patients' features	*Whole* (n = *195*)	*European-derived* (n = *148*)	*African-derived* (n = *47*)	p *value*[a]
Female (%)	183 (93.8)	138 (93.2)	45 (95.7)	0.734
Age (years ± SD)	42.6 ± 13.9	42.3 ± 14.0	43.5 ± 13.7	0.607
Age at diagnosis (years ± SD)	32.5 ± 13.7	32.7 ± 13.7	33.9 ± 13.6	0.425
Disease duration (years)[b]	9.2 (3.4–15.2)	9.7 (3.4–15.7)	8.2 (3.3–14.8)	0.600
Malar rash (%)	120 (61.5)	94 (63.5)	26 (55.3)	0.404
Discoid rash (%)	27 (13.8)	16 (10.8)	11 (23.4)	0.053
Photosensitivity (%)	153 (78.5)	123 (83.1)	30 (63.8)	**0.009**
Oral ulcers (%)	78 (40.0)	62 (41.9)	16 (34.0)	0.432
Arthritis (%)	165 (84.6)	127 (85.8)	38 (80.9)	0.556
Serositis (%)	55 (28.2)	40 (27.0)	15 (31.9)	0.644
Nephritis (%)	89 (45.6)	65 (43.9)	24 (51.1)	0.491
Neurologic disorders (%)	24 (12.3)	19 (12.8)	5 (10.6)	0.885
Hematologic disorders (%)	151 (77.4)	111 (75.0)	40 (85.1)	0.214
Hemolytic anemia (%)	59 (30.3)	45 (30.4)	14 (29.8)	1.000
Leuko/lymphopenia (%)	124 (63.6)	90 (60.8)	34 (72.3)	0.209
Thrombocytopenia (%)	50 (25.6)	35 (23.6)	15 (31.9)	0.348
Immunologic disorders (%)	142 (72.8)	110 (74.3)	32 (68.1)	0.516
Anti-dsDNA (%)	107 (54.9)	83 (56.1)	24 (51.1)	0.664
Anti-Sm (%)	31 (15.9)	20 (13.7)	11 (23.4)	0.178
Anticardiolipin (%)	59 (30.3)	44 (29.9)	15 (31.9)	0.940
Lupic anticoagulant (%)	21 (10.8)	20 (13.6)	1 (02.1)	0.053
False positive VDRL (%)	14 (07,2)	10 (06.8)	4 (08.5)	0.747
ANA (%)	193 (99.0)	146 (99.3)	47 (100)	1.000
Anti-Ro/SSA (%)	87 (44.6)	62 (42.5)	25 (53.2)	0.264
Anti-La/SSB (%)	28 (14.4)	18 (12.3)	10 (21.3)	0.202
Anti-RNP (%)	56 (28.7)	41 (28.1)	15 (31.9)	0.750
Sjögren (%)	13 (06,7)	12 (08.2)	1 (02.1)	0.194
Antiphospholipid syndrome (%)	14 (07.2)	11 (07.4)	3 (06.4)	1.000
SLEDAI[b]	2 (0–4)	2 (0–4)	2 (0–5)	0.625
SLICC[b]	0 (0–1)	0 (0–1)	0 (0–1)	0.814

[a]Chi-square test for qualitative variables and Mann–Whitney for asymmetric quantitative variables or Student's *t*-test for symmetric quantitative variables.
[b]Median (interquartile range).
SD: standard deviation, SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, SLICC: Systemic Lupus International Collaborating Clinics.

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Table 2 *Bsm*I and *Fok*I genotypic and allelic frequency in European-derived SLE patients, European-derived controls and African-derived SLE patients

	European-derived patients (%)	*European-derived controls (%)*	*African-derived patients (%)*
*Bsm*I genotype	$n=121$	$n=201$	$n=42$
B/B	20 (16.5)	31 (15.4)	03 (07.1)
B/b	61 (50.4)	97 (48.3)	21 (50.0)
b/b	40 (33.1)	73 (36.3)	18 (42.9)
p value[a]	0.836		0.248
*Bsm*I alleles	$2n=242$	$2n=402$	$2n=84$
B	101 (41.7)	159 (39.6)	27 (32.1)
b	141 (58.3)	243 (60.4)	57 (68.9)
p value[a]	0.643		0.155
*Fok*I genotype	$n=131$	$n=198$	$n=42$
F/F	64 (48.9)	86 (43.4)	19 (45.2)
F/f	56 (42.7)	94 (47.5)	18 (42.9)
F/f	11 (08.4)	18 (09.1)	05 (11.9)
p value[a]	0.626		0.775
*Fok*I alleles	$2n=262$	$2n=396$	$2n=84$
F	184 (70.2)	266 (67.2)	56 (66.7)
f	078 (29.8)	130 (32.8)	28 (33.3)
p value[a]	0.459		0.631

[a]Chi-square test to compare European-derived patients and controls and European- and African-derived patients.
B: allele B, b: allele b, F: allele F, f: allele f.

VDR polymorphisms and clinical and laboratory features of SLE patients described in Table 1 were investigated. Both allelic and genotypic distributions were considered. After applying the Bonferroni correction, no results achieved statistical significance (data not shown).

The 25(OH)D levels were measured in 181 SLE patients and a mean of 25.51 ± 11.43 ng/ml was found. European-derived patients ($n=134$) had levels of 25.9 ± 11.43 ng/ml, and African-derived ($n=47$) had 24.3 ± 11.44 ng/ml ($p=0.384$). When patients were classified according to vitamin D status, the following distribution was observed: 55 (30.4%) had normal, 63 (34.8%) insufficient, 52 (28.7%) deficient and 11 (6.1%) critically low serum levels. Fifty-six percent of patients with deficiency were taking at least 800 IU of vitamin D per day. According to genotype distribution, 25(OH)D level was significantly higher in patients with f/f genotype when compared with F/F (31.6 ± 14.1 ng/ml *vs.* 23.0 ± 9.2 ng/ml, $p=0.004$), even after adjusting for confounding variables (Tables 3 and 4). Vitamin D levels were not different when *Bsm*I genotypes were considered (Table 3).

Vitamin D deficiency did not present association with any demographic, clinical and laboratory features in SLE patients (Table 5). Secondary antiphospholipid syndrome, Sjögren's syndrome, SLEDAI disease activity index and SLICC damage index were similar in patients with or without levels of 25(OH)D lower than 20 ng/ml. All data were adjusted for confounding variables.

Table 3 Vitamin D levels according to *Bsm*I and *Fok*I genotypic frequency in SLE patients

Genotype	*25(OH)D measurement*
B/B ($n=22$)	25.5 ± 09.4
B/b ($n=81$)	23.7 ± 12.1
b/b ($n=54$)	26.7 ± 11.1
p value[a]	0.322
F/F ($n=77$)	23.0 ± 09.2
F/f ($n=67$)	25.2 ± 12.5
f/f ($n=15$)	31.6 ± 14.1
p value[a]	**0.025**

B: allele B, b: allele b, F: allele F, f: allele f.
[a]ANOVA test.

Table 4 Model of multiple linear regression[a] for possible factors associated with serum levels of 25(OH)D in SLE patients ($n=181$)

Variable FokI[b]	*B*	*Standard error*	*Beta*	*95% CI B*	p *value*
F/f	2.07	1.91	0.092	−1.71–5.86	0.281
f/f	9.09	3.14	0.243	2.88–15.30	**0.004**

[a]Co-variables that were included in the model: phototype, ethnicity, age, gender, smoking status, BMI, hydroxychloroquine use, current corticosteroids use and vitamin D supplementation.
[b]Both categories are compared with F/F genotype.
CI: confidence interval.

Discussion

The pathogenesis of SLE is complex and probably involves multiple factors. Target tissue damage could be caused by pathogenic autoantibodies and immune complexes. Abnormalities in the immune system permit the processing of self antigen by antigen-presenting cells, persistence and hyperactivation of B and T cells and failure to maintain immunoregulatory networks. Several cells involved in the immune system express VDR and key vitamin D metabolizing enzymes, which could explain the suppressive effects of vitamin D on immunity.

Most biological activities of vitamin D are mediated by the VDR. Genetic variation on the *VDR* gene could lead to significant receptor dysfunction, which could affect calcium metabolism, cell proliferation and the immune response. Polymorphisms in *VDR* gene have been associated with health outcomes involving low bone density, cardiovascular disease, cancers, autoimmunity and infections, but

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Table 5 Clinical and laboratory features in SLE patients according with vitamin D deficiency

Clinical features	*Whole* (n = 181)	*25(OH)D* < 20 ng/ml (n = 63)	*25(OH)D* ≥ 20 ng/ml (n = 118)	*Not adjusted OR (95% CI)*	*Adjusted OR (95% CI)*	p *value*[a]
European-derived (%)	134 (74.0)	44 (69.8)	90 (76.3)	0.72 (0.36–1.43)	0.82 (0.40–1.76)	0.589
Female (%)	170 (93.9)	58 (92.1)	112 (94.9)	0.62 (0.18–2.12)	0.59 (0.17–2.05)	0.409
Age (years)[b]	42.6 ± 13.9	41.7 ± 14.9	43.0 ± 13.4	0.99 (0.97–1.02)	0.99 (0.97–1.02)	0.527
Age at diagnosis (years)[b]	32.3 ± 13.5	30.6 ± 12.9	33.2 ± 13.8	0.98 (0.96–1.01)	0.96 (0.92–1.01)	0.117
Disease duration (years)[c]	9.2 (3.4–15.2)	10.9 (3.6–18.4)	8.2 (3.3–14.5)	1.03 (0.97–1.07)	1.04 (0.99–1.08)	0.117
Smoking (%)	61 (33.7)	21 (33.3)	40 (33.9)	0.98 (0.51–1.86)	0.92 (0.46–1.81)	0.802
BMI (kg/m^2)[b]	26.9 ± 5.3	27.3 ± 6.0	26.7 ± 5.0	1.02 (0.97–1.08)	1.03 (0.97–1.09)	0.385
Vitamin D intake (IU)	800 (0–800)	800 (0–800)	800 (0–800)	1.00 (0.99–1.00)	1.00 (0.99–1.00)	0.894
Hydroxychloroquine (%)	176 (97.2)	61 (96.8)	115 (97.5)	0.80 (0.13–4.89)	0.91 (0.14–5.84)	0.919
Corticosteroids – current dose (mg/day)[c]	0 (0–10.0)	0 (0–17.5)	0 (0–10.0)	1.00 (0.98–1.03)	1.00 (0.98–1.03)	0.815
Corticosteroids – cumulative dose (mg/day)[c,d]	3.15 (0–15.0)	5 (0–15.0)	2.5 (0–15.0)	1.00 (0.98–1.02)	0.99 (0.96–1.03)	0.657
Immunosuppressants (%)[e]	122 (67.4)	40 (63.5)	82 (69.5)	0.76 (0.40–1.46)	0.62 (0.29–1.30)	0.207
Malar rash (%)	111 (61.3)	35 (55.6)	76 (64.4)	0.70 (0.37–1.29)	0.67 (035–1.29)	0.230
Discoid rash (%)	24 (13.3)	9 (14.3)	15 (12.7)	1.14 (0.47–2.79)	1.23 (0.49–3.05)	0.660
Photosensitivity (%)	143 (79.0)	48 (76.2)	95 (80.5)	0.78 (0.37–1.62)	0.74 (0.35–1.59)	0.442
Oral ulcers (%)	72 (39.8)	24 (38.1)	48 (40.7)	0.90 (0.48–1.68)	0.82 (0.43–1.59)	0.563
Arthritis (%)	152 (84.0)	56 (88.9)	96 (81.4)	1.83 (0.74–4.56)	1.89 (0.75–4.8)	0.180
Serositis (%)	51 (28.2)	23 (36.5)	28 (23.7)	1.85 (0.95–3.60)	1.70 (0.85–3.41)	0.136
Nephritis (%)	83 (45.9)	25 (39.7)	58 (49.2)	0.68 (0.37–1.27)	0.57 (0.29–1.13)	0.109
Neurologic disorders (%)	22 (12.2)	7 (11.1)	15 (12.7)	0.86 (0.33–2.23)	0.72 (026–2.01)	0.536
Hematologic disorders (%)	140 (77.3)	49 (77.8)	91 (77.1)	1.04 (0.50–2.16)	1.01 (0.48–2.14)	0.975
Immunologic disorders (%)	134 (74.0)	51 (81.0)	83 (70.3)	1.79 (0.85–3.77)	2.00 (091–4.36)	0.085
ANA (%)	179 (99.4)	62 (98.4)	117 (99.2)	–	–	–
Sjögren syndrome (%)	11 (6.1)	2 (3.2)	9 (7.6)	0.40 (0.08–1.92)	0.41 (0.83–1.98)	0.264
Antiphospholipid syndrome (%)	14 (7.7)	7 (11.1)	7 (5.9)	1.98 (0.66–5.93)	2.13 (0.65–6.98)	0.214
SLEDAI[c]	2 (0–4)	2 (0–5)	2 (0–4)	1.09 (0.96–1.10)	1.03 (095–1.10)	0.485
SLICC[c]	0 (0–1)	0 (0–2)	0 (0–1)	1.18 (0.92–1.52)	1.19 (0.96–1.54)	0.196

[a]Logistic regression adjusted to phototype, ethnicity, age, gender, smoking status, BMI, hydroxychloroquine use, current corticosteroids use and vitamin D supplementation.
[b]Mean ± standard deviation (SD).
[c]Median (interquartile range).
[d]Mean daily dose of corticosteroids used in the last 12 months.
[e]Current use of mycophenolate mofetil, cyclophosphamide, azathioprine, methotrexate, cyclosporin and rituximab.
BMI: body mass index, CI: confidence interval, OR: odds ratio, SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, SLICC: Systemic Lupus International Collaborating Clinics, IU: international units.

the effects of these polymorphisms on VDR protein expression and function need more clarification.[28] The *Bsm*I, *Apa*I and *Taq*I polymorphisms show strong linkage disequilibrium with each other. The *Bsm*I and *Apa*I polymorphisms are located in intron 8 and the *Taq*I polymorphism is located at exon 9, a region that was suggested by some authors to be related to mRNA stability.[23] Other studies suggest that a polymorphism in the *Fok*I located inside a start codon creates an alternative start site leading to a protein with a different length. The short protein (allele F) seems to be more active than the long variant (allele f) in terms of its transactivation activity as a transcription factor. However, this effect seems to be gene and cell specific.[29,30] An in vitro study[31]provided evidences that alleles F and f lead to clearly different effects in the immune system. Compared with allele f, the allele F showed a higher transcriptional activity of immune specific transcription factors, as well as on the more functional level of proliferation and cytokine synthesis by immune cells. Still, the clinical impact of the *Fok*I *VDR* polymorphism remains unclear.[32]

Some authors have studied the association of *Bsm*I and *Fok*I *VDR* polymorphisms with SLE development, but results were limited. Studies of Japanese ($n = 58$)[24] and Chinese ($n = 47$)[33] patients reported an association of the B/B genotype with disease, whereas studies of Thai ($n = 101$)[26] and Iranian ($n = 60$)[31] patients did not reproduce these results. In addition, b/b genotype was associated with renal disease in a Japanese study.[24] A Chinese study ($n = 52$) examining the *Fok*I *VDR* polymorphism, did not identify an association of *Fok*I polymorphism in patients with SLE compared with controls.[8] Our study has the largest number of

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SLE patients studied so far. We did not find associations between *Bsm*I and *Fok*I polymorphisms and SLE susceptibility or clinical and laboratory expressions of disease. These results could be explained by ethnic factors, since the influence of *Bsm*I on Asian SLE patients was also different from Thai and Iranian patients. The *Fok*I polymorphism seems not to be associated with SLE in different populations. Another interesting finding was the similar distribution of allele f between European- and African-derived patients, agreeing with the results of another study in Brazil.[34] These data are different from the literature, where the allele f is found predominantly in Caucasian and Asian people.[35] This could be due to the high degree of genetic admixture of our African-derived population.[36] .

A recent systematic review described two studies that showed significant relationship between the *Fok*I polymorphism and 25(OH)D serum levels.[37] Orton et al. found 25(OH)D concentrations of 25.8 ± 2.2 ng/ml in homozygous genotype coding for the shorter VDR variant compared with 33.3 ± 1.6 ng/ml in heterozygous and homozygous genotypes coding for the longer VDR variant ($p = 0.005$).[38] Smolders et al. showed statistically significant lower concentrations of 25(OH)D associated with F/F genotype compared with f/f genotype.[39] Both studies involved patients with multiple sclerosis (MS). However, two other studies did not evidence significant difference: neither Engelman et al. studying Hispanics and African-American patients from the Insulin Resistance Atherosclerosis Family Study[40] nor Wjst et al. studying patients from the German Asthma Family Study[41] observed associations between vitamin D levels and *Fok*I and *Bsm*I polymorphisms. Other studies, not included in this systematic review, reported controversial findings. Abrams et al. published in 2005 a significant association of F/F genotype with lower 25(OH)D serum concentrations when compared with f/f genotype (26.2 ± 1.2 ng/ml *vs.* 32.4 ± 1.6 ng/ml, $p = 0.009$), in healthy adolescents in the USA.[42] Abbas et al., studying patients with breast cancer, did not reproduce these results.[43] In SLE patients, we are reporting for the first time that the carriers of F/F genotype have significant lower 25(OH)D concentrations than the carriers of f/f genotype (23.0 ± 9.2 ng/ml versus 31.6 ± 14.1, $p = 0.004$). Although *Fok*I polymorphism did not have significant association with SLE, it could have important consequences for vitamin D metabolism in SLE patients. Previous studies suggest that 1,25-dihidroxyvitamin D [1,25$(OH)_2$D], an active form of vitamin D, has negative control over its own levels and the levels of its precursor [25(OH)D)] through the VDR.[44–46] Allele f may be associated with VDR dysfunction allowing increased synthesis of 25(OH)D, which would explain the observation of higher vitamin D levels in individuals with f/f genotype. However, other described genetic factors influencing production, elimination and transportation of vitamin D may influence 25(OH)D concentration and confound our finding.

The fact that we did not determine 25-hydroxyvitamin D levels in the control group was a limitation of our study. Consequently, it was not possible to compare the influence of *Fok*I polymorphism on vitamin D levels detected in patients with data from healthy controls. However, a previous study published by Abrams et al. had shown that this polymorphic variant influences the levels of vitamin D in healthy people.[42] The importance of our study was to demonstrate that this effect is found in SLE patients too, which has very important implications when we studied the vitamin D levels in this disease.

Vitamin D deficiency has been associated with SLE in studies with different populations. The first study that demonstrated low levels of vitamin D among SLE patients was published in 1979.[47] Since then, other studies have confirmed that the majority of SLE patients have decreased levels of vitamin D.[20,48,49] Kamen et al., comparing 123 SLE patients and 240 age- and sex-matched controls from the USA, noticed a trend toward lower 25(OH)D levels in cases (21.6 ± 12.9 ng/ml *vs.* 27.4 ± 15.7 ng/ml), which was significant in European-derived ($p = 0.04$) but not in African-derived individuals ($p = 0.36$). Overall, 67% of patients had vitamin D deficiency, and the average dose of 25(OH)D was significantly lower in African-derived, compared with European-derived (15.9 ± 9.4 ng/ml *vs.* 31.3 ± 14.9 ng/ml, $p < 0.01$).[20] Although there is evidence of a relationship between vitamin D deficiency and SLE, vitamin D intake was not associated with risk of SLE in a large prospective cohort of females.[5] The role of vitamin D in clinical and laboratory features of SLE has been demonstrated, specially associating low levels of vitamin D with activity and damage indexes, fatigue index and cardiovascular disease, but results are controversial in different populations studied.[50–56] In São Paulo, Brazil, a study evaluated 36 patients with SLE and 26 healthy controls. Patients were divided into two groups regarding the values of activity index (SLEDAI): Group 1 with mean SLEDAI of 22 (14–27) and Group 2 with mean SLEDAI of 1.7 (0–3). Levels of 25(OH)D were significantly lower in Group 1 compared with Group 2 or healthy controls (17.4 ± 12.5 ng/ml *vs.* 44.6 ± 14.5 *vs.* 37.8 ± 13.7, respectively, $p < 0.001$). There was an increased

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prevalence of vitamin D deficiency in SLE patients, especially those with active disease.[52] Our study found no significant association between vitamin D deficiency and clinical and laboratory manifestations of SLE. SLEDAI activity index, SLICC damage index, Sjögren's syndrome and antiphospholipid syndrome did not have association with vitamin D status. Factors such as season, age, ethnicity, sun exposition, use of sunscreens, smoking status, certain drugs, skin pigmentation, limited amount of vitamin D obtained from diet and recently described genetic factors may influence results in different studies and require careful consideration when we evaluate serum levels of vitamin D.[27]

Vitamin D deficiency in our population was observed in 34.8% of all patients and the mean level of 25(OH)D was 25.51 ± 11.43 ng/ml. This result was not considered surprising. Other studies in our region showed a high prevalence of vitamin D deficiency in specific groups of people. Scalco et al. detected 25(OH)D levels < 20 ng/ml in 85.6% of 98 subjects living in two non-profit homes for old people.[57] Premaor et al., studying resident physicians, found mean levels of 25(OH)D of 17.9 ± 8.0 ng/ml and 57.4% presented serum 25(OH)D levels below 20 ng/ml.[58] Our patients may have had a lower prevalence of vitamin D deficiency due to frequent use of oral vitamin D supplementation. Even so, more than 50% of patients with vitamin D deficiency were taking at least 800 IU of vitamin D per day. It has been suggested that SLE patients need larger doses of vitamin D to achieve normal serum levels. Current recommendations about vitamin D supplementation in SLE patients are being discussed, but there is still no consensus,[19,59,60] and our findings should be considered in order to determine optimal doses of vitamin D replacement.

In conclusion, our data clearly indicated that the *Fok*I and *Bsm*I *VDR* polymorphisms are not risk factors for SLE, supporting the idea that *VDR* polymorphisms influence SLE development according to the genetic background of the studied population. Vitamin D deficiency seems not to have a direct effect on clinical and laboratory features of SLE patients in our population. Nevertheless, our study is the first report that describes *Fok*I genotypes as an important factor in 25(OH)D concentration among SLE patients. It is important to take this finding into consideration in future clinical and experimental studies aiming to evaluate 25(OH)D levels. If genotypes are related to vitamin D status, serum concentrations of 25(OH)D required to reduce disease outcomes should be individualized for each patient and new guidelines about vitamin D supplementation may have to include this new genetic information. Therefore, genetic-specific definitions of ideal levels of vitamin D in SLE need to be established in future studies.

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Conflict of interest statement

None declared.

References

1 Manson JJ, Isenberg DA. The pathogenesis of systemic lupus erythematosus. *Neth J Med* 2003; 61: 343–346.
2 Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. *J Mol Med* 2010; 88: 441–450.
3 Kamen D, Aranow C. Vitamin D in systemic lupus erythematosus. *Curr Opin Rheumatol* 2008; 20: 532–537.
4 Kamen DL, Aranow C. The link between vitamin D deficiency and systemic lupus erythematosus. *Curr Rheumatol Rep* 2008; 10: 273–280.
5 Costenbader KH, Feskanich D, Holmes M, Karlson EW, Benito-Garcia E. Vitamin D intake and risks of systemic lupus erythematosus and rheumatoid arthritis in women. *Ann Rheum Dis* 2008; 67: 530–535.
6 Miyamoto K, Kesterson RA, Yamamoto H, *et al*. Structural organization of the human vitamin D receptor chromosomal gene and its promoter. *Mol Endocrinol* 1997; 11: 1165–1179.
7 Lee YH, Bae SC, Choi SJ, Ji JD, Song GG. Associations between vitamin D receptor polymorphisms and susceptibility to rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis. *Mol Biol Rep* 2011; 38: 3643–51.
8 Huang CM, Wu MC, Wu JY, Tsai FJ. No association of vitamin D receptor gene start codon fok 1 polymorphisms in Chinese patients with systemic lupus erythematosus. *J Rheumatol* 2002; 29: 1211–1213.
9 Monticielo OA, Chies JA, Mucenic T, *et al*. Mannose-binding lectin gene polymorphisms in Brazilian patients with systemic lupus erythematosus. *Lupus* 2010; 19: 280–287.
10 Santos NP, Ribeiro-Rodrigues EM, Ribeiro-Dos-Santos AK, *et al*. Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. *Hum Mutat* 2010; 31: 184–190.
11 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
12 Astner S, Anderson RR. Skin phototypes 2003. *J Invest Dermatol* 2004; 122: xxx–xxxi.
13 Vitali C, Bombardieri S, Jonsson R, *et al*. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American–European Consensus Group. *Ann Rheum Dis* 2002; 61: 554–558.

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14 Miyakis S, Lockshin MD, Atsumi T, *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
15 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630–640.
16 Gladman D, Ginzler E, Goldsmith C, *et al*. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996; 39: 363–369.
17 Premaor MO, Alves GV, Crossetti LB, Furlanetto TW. Hyperparathyroidism secondary to hypovitaminosis D in hypoalbuminemic is less intense than in normoalbuminemic patients: a prevalence study in medical inpatients in southern Brazil. *Endocrine* 2004; 24: 47–53.
18 Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357: 266–281.
19 Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006; 84: 18–28.
20 Kamen DL, Cooper GS, Bouali H, Shaftman SR, Hollis BW, Gilkeson GS. Vitamin D deficiency in systemic lupus erythematosus. *Autoimmun Rev* 2006; 5: 114–117.
21 Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; 11: 5444.
22 Harris SS, Eccleshall TR, Gross C, Dawson-Hughes B, Feldman D. The vitamin D receptor start codon polymorphism (FokI) and bone mineral density in premenopausal American black and white women. *J Bone Miner Res* 1997; 12: 1043–1048.
23 Morrison NA, Qi JC, Tokita A, *et al*. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994; 20 367: 284–287.
24 Ozaki Y, Nomura S, Nagahama M, Yoshimura C, Kagawa H, Fukuhara S. Vitamin-D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. *Nephron* 2000; 85: 86–91.
25 Mory DB, Rocco ER, Miranda WL, Kasamatsu T, Crispim F, Dib SA. Prevalence of vitamin D receptor gene polymorphisms FokI and BsmI in Brazilian individuals with type 1 diabetes and their relation to beta-cell autoimmunity and to remaining beta-cell function. *Hum Immunol* 2009; 70: 447–451.
26 Sakulpipatsin W, Verasertniyom O, Nantiruj K, Totemchokchyakarn K, Lertsrisatit P, Janwityanujit S. Vitamin D receptor gene BsmI polymorphisms in Thai patients with systemic lupus erythematosus. *Arthritis Res Ther* 2006; 8: R48.
27 Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005; 135: 317–322.
28 Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta* 2006; 371: 1–12.
29 Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene* 2004; 338: 143–156.
30 Colin EM, Weel AE, Uitterlinden AG, *et al*. Consequences of vitamin D receptor gene polymorphisms for growth inhibition of cultured human peripheral blood mononuclear cells by 1, 25-dihydroxyvitamin D3. *Clin Endocrinol (Oxford)* 2000; 52: 211–216.
31 Abbasi M, Rezaieyazdi Z, Afshari JT, Hatef M, Sahebari M, Saadati N. Lack of association of vitamin D receptor gene BsmI polymorphisms in patients with systemic lupus erythematosus. *Rheumatol Int* 2010; 30: 1537–1539.
32 Van Etten E, Verlinden L, Giulietti A, *et al*. The vitamin D receptor gene FokI polymorphism: functional impact on the immune system. *Eur J Immunol* 2007; 37: 395–405.
33 Huang CM, Wu MC, Wu JY, Tsai FJ. Association of vitamin D receptor gene BsmI polymorphisms in Chinese patients with systemic lupus erythematosus. *Lupus* 2002; 11: 31–34.
34 Rezende VB, Barbosa F Jr, Montenegro MF, Sandrim VC, Gerlach RF, Tanus-Santos JE. An interethnic comparison of the distribution of vitamin D receptor genotypes and haplotypes. *Clin Chim Acta* 2007; 384: 155–159.
35 Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. *Epidemiol Rev* 2000; 22: 203–217.
36 Bortolini MC, Da Silva WAJWA, De Guerra DC, *et al*. African-derived South American populations: a history of symmetrical and asymmetrical matings according to sex revealed by bi- and uni-parental genetic markers. *Am J Hum Biol* 1999; 11: 551–563.
37 McGrath JJ, Saha S, Burne TH, Eyles DW. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. *J Steroid Biochem Mol Biol* 2010; 121: 471–477.
38 Orton SM, Morris AP, Herrera BM, *et al*. Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. *Am J Clin Nutr* 2008; 88: 441–447.
39 Smolders J, Damoiseaux J, Menheere P, Tervaert JW, Hupperts R. Fok-I vitamin D receptor gene polymorphism (rs10735810) and vitamin D metabolism in multiple sclerosis. *J Neuroimmunol* 2009; 207: 117–121.
40 Engelman CD, Fingerlin TE, Langefeld CD, *et al*. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. *J Clin Endocrinol Metab* 2008; 93: 3381–3388.
41 Wjst M, Altmuller J, Faus-Kessler T, Braig C, Bahnweg M, Andre E. Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signalling pathway. *Respir Res* 2006; 7: 60.
42 Abrams SA, Griffin IJ, Hawthorne KM, *et al*. Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. *J Bone Miner Res* 2005; 20: 945–953.
43 Abbas S, Nieters A, Linseisen J, *et al*. Vitamin D receptor gene polymorphisms and haplotypes and postmenopausal breast cancer risk. *Breast Cancer Res* 2008; 10: R31.
44 Takeyama K, Kitanaka S, Sato T, Kobori M, Yanagisawa J, Kato S. 25-Hydroxyvitamin D3 1alpha-hydroxylase and vitamin D synthesis. *Science* 1997 19; 277: 1827–1830.
45 Theodoropoulos C, Demers C, Delvin E, Menard D, Gascon-Barre M. Calcitriol regulates the expression of the genes encoding the three key vitamin D3 hydroxylases and the drug-metabolizing enzyme CYP3A4 in the human fetal intestine. *Clin Endocrinol (Oxford)* 2003; 58: 489–499.
46 Theodoropoulos C, Demers C, Petit JL, Gascon-Barre M. High sensitivity of rat hepatic vitamin D3-25 hydroxylase CYP27A to 1,25-dihydroxyvitamin D3 administration. *Am J Physiol Endocrinol Metab* 2003; 284: E138–E147.
47 O'Regan S, Chesney RW, Hamstra A, Eisman JA, O'Gorman AM, Deluca HF. Reduced serum 1,25-(OH)2 vitamin D3 levels in prednisone-treated adolescents with systemic lupus erythematosus. *Acta Paediatr Scand* 1979; 68: 109–111.
48 Damanhouri LH. Vitamin D deficiency in Saudi patients with systemic lupus erythematosus. *Saudi Med J* 2009; 30: 1291–1295.
49 Toloza SM, Cole DE, Gladman DD, Ibanez D, Urowitz MB. Vitamin D insufficiency in a large female SLE cohort. *Lupus* 2010; 19: 13–19.
50 Amital H, Szekanecz Z, Szucs G, *et al*. Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: is it time to routinely supplement patients with SLE with vitamin D? *Ann Rheum Dis* 2010; 69: 1155–1157.
51 Ruiz-Irastorza G, Gordo S, Olivares N, Egurbide MV, Aguirre C. Changes in vitamin D levels in patients with systemic lupus erythematosus: effects on fatigue, disease activity, and damage. *Arthritis Care Res (Hoboken)* 2010; 62: 1160–1165.
52 Borba VZ, Vieira JG, Kasamatsu T, Radominski SC, Sato EI, Lazaretti-Castro M. Vitamin D deficiency in patients with active systemic lupus erythematosus. *Osteoporos Int* 2009; 20: 427–433.
53 Thudi A, Yin S, Wandstrat AE, Li QZ, Olsen NJ. Vitamin D levels and disease status in Texas patients with systemic lupus erythematosus. *Am J Med Sci* 2008; 335: 99–104.

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54 Wu PW, Rhew EY, Dyer AR, *et al*. 25-hydroxyvitamin D and cardiovascular risk factors in women with systemic lupus erythematosus. *Arthritis Rheum* 2009; 61: 1387–1395.
55 Kim HA, Sung JM, Jeon JY, Yoon JM, Suh CH. Vitamin D may not be a good marker of disease activity in Korean patients with systemic lupus erythematosus. *Rheumatol Int* 2011; 31: 1189–94.
56 Szodoray P, Tarr T, Bazso A, Poor G, Szegedi G, Kiss E. The immunopathological role of vitamin D in patients with SLE: data from a single centre registry in Hungary. *Scand J Rheumatol* 2011; 40: 122–126.
57 Scalco R, Premaor MO, Froehlich PE, Furlanetto TW. High prevalence of hypovitaminosis D and secondary hyperparathyroidism in elders living in nonprofit homes in South Brazil. *Endocrine* 2008; 33: 95–100.
58 Premaor MO, Paludo P, Manica D, *et al*. Hypovitaminosis D and secondary hyperparathyroidism in resident physicians of a general hospital in southern Brazil. *J Endocrinol Invest* 2008; 31: 991–995.
59 Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004; 80(Suppl. 6): 1678S–1688S.
60 Kamen DL. Vitamin D in lupus – new kid on the block? *Bull NYU Hosp Jt Dis* 68: 218–222.

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