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ORIGINAL ARTICLE

Differential distribution of vitamin D receptor (*VDR*) gene variants and its expression in systemic lupus erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disorder that displays an important genetic background. Vitamin D_3 (VD_3) through its receptor (VDR) plays an important immunomodulatory role in autoimmune misbalance, being capable of modulating immune responses. Genetic alterations in *VDR* gene may contribute to an altered risk in SLE development and clinical manifestations. We investigated *VDR* SNPs (single nucleotide polymorphisms) frequencies in 128 SLE patients and 138 healthy controls (HC) and mRNA differential expression in 29 patients and 17 HC regarding SLE susceptibility as well as clinical features. We observed that rs11168268 G allele (OR = 1.55, $p = .01$) and G/G genotype (OR = 2.69, $p = .008$) were associated with increased SLE susceptibility. The rs2248098 G allele and A/G and G/G genotypes were associated to lower SLE susceptibility (OR = 0.66, $p = .01$; OR = 0.46, $p = .01$; OR = 0.44, $p = .02$, respectively). Regarding clinical features, we observed lower risk for: rs11168268 A/G genotype and nephritis (OR = 0.31, $p = .01$); rs4760648 T/T genotype and photosensitivity (OR = 0.24, $p = .02$); rs1540339 T/T genotype and antibody anti-dsDNA (OR = 0.19, $p = .015$); rs3890733 T/T genotype and serositis (OR = 0.10, $p = .01$). We identified a significant downregulation in *VDR* expression levels when compared patients and controls overall ($p = 1.04e^{-7}$), in *Cdx-2* A/G and G/G ($p = .008$ and $p = .014$, respectively) and in patients with nephritis ($p = .016$)

Our results suggested that *VDR* SNPs influence upon SLE susceptibility and in particular clinical features, acting on mRNA expression in SLE patients overall and the ones with nephritis.

KEYWORDS
genetic variants, nephritis, SLE, VDR

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder featured by different clinical manifestations (Mok & Lau, 2003). Indeed, SLE clinical heterogeneity led to the establishment of 11 criteria by the American College of Rheumatology (ACR), in which 4 are simultaneously needed for the disease's formal diagnosis (Hochberg, 1997; Tsokos, 2011). SLE hallmark is the over production of autoantibodies, which leads to deposition of antibody-containing immune complexes throughout the body featuring tissue and organ damage (Silva et al., 2013). Additionally, a common feature in most of autoimmune diseases is a strong sex bias and especially in SLE this discrepancy is increased towards woman in childbearing age (Yan et al., 2012). According to the Brazilian Society of Rheumatology, epidemiology data in Brazil, estimates around 65,000 people with lupus, the majority being women with 1 affected in every 1700 (Sociedade Brasileira de Reumatologia, 2020).

Several clinical complications are observed in patients with SLE, with emphasis on lupus nephritis (LN), one of the most severe manifestations of this disease that results in a glomerulonephritis caused by an inflammatory response to endogenous immunogenic chromatin (Anders et al., 2020).

As a multifactorial disease, SLE presents an active interplay from many altered genes, particularly the ones involved with immune response regulation, responsible for disease's establishment and maintenance (Iruretagoyena et al., 2015; Mok & Lau, 2003). The steroid hormone vitamin D (VD_3) has as primary function calcium homeostasis and bone metabolism (Veldurthy et al., 2016), however recent studies have been reported as a pleiotropic regulator of human physiology and immune system modulation (Di Rosa et al., 2011). In fact, VD_3 has emerged as a potent immunosuppressive hormone, interfering with T regulatory (Th) cell functions and modulation which may be a key mechanism in SLE's development (Kamen & Tangpricha, 2010). Besides that, SLE Brazilian patients present overall low levels of vitamin D (Eloi et al., 2017).

VD_3 exerts its actions through interaction with its specific receptor named Vitamin D Receptor (VDR), which is widely spread throughout several organs, tissues and noteworthy, in all immune cells (Wang et al., 2012). *VDR* is located on chromosome 12 (12q13.11) and encloses several single nucleotide polymorphisms (SNPs), which can modulate VDR levels and activity (Silva et al., 2013). The SNPs described in the *VDR* are mainly in the promoter regions close to the f and c sites of exon 1, between exons 2 and 9 and in the 3′UTR region (Figure 1). The most frequently *VDR* polymorphisms in the literature are: *Cdx2* (G > A), *FokI* (C > T), *BsmI* (A > G), *EcoRV* (G > A), *ApaI* (G > T) and *TaqI* (T > C) (Uitterlinden et al., 2004). Polymorphisms in the *VDR* gene can alter both gene function and expression, thus leading to altered VD action. Since vitamin D levels has already been associated to inflammatory diseases including SLE (Wöbke et al., 2014), attention in its role on disease's pathogenesis has dramatically grown.

Therefore, considering VD_3 a key regulator in immune system, we aimed to evaluated the TagSNPs: rs11168268, rs2248098, rs1540339, rs4760648 and rs3890733 and functional SNPs: rs2228570 (FokI) and rs11568820 (Cdx-2) (Figure 1a), in SLE Northeast Brazilian patients in order to understand their consequence in our studied subject group, since associations were previously described with different consequences in other populations (Table 1). We also assessed *VDR* mRNA levels in order to evaluated gene expression profile in these patients, according its clinical manifestations (LN, photosensitivity, antibody anti-dsDNA and serositis) and also comparing different genotypes from rs11568820 SNP (Cdx2).

2 | MATERIAL AND METHODS

2.1 | Study design, location and subjects

We performed a case-control study to perform a genetic association analysis and an experimental study with a quantitative case-control approach to expression analysis. Genotyping patient group was composed by 128 females SLE patients (mean age 37.1 years ± 10.5) diagnosed according to the ACR (Hochberg, 1997). All patients were selected from the Division of Rheumatology from a hospital in the metropolitan region of Recife, Pernambuco, Brazil. Patients were classified according to the criteria of the American College of Rheumatology (ACR) (Hochberg, 1997) and SLICC (cumulative organic damage index (SLICC/ACR) or SLEDAI (disease activity index) according to patient status. For clinical and laboratory evaluation of SLE patients was collected the following data: photosensitivity, malar or discoid rashes, oral ulcers, serositis (pleuritis, pericarditis), arthritis, neuropsychiatric disorder (seizures, headache, psychosis), haematological alterations (haemolytic anaemia, leucopenia, lymphopenia, thrombocytopenia), presence of anti-double-strand DNA antibody (anti-ds-DNA), presence of antinuclear antibodies (ANA) and nephritic disorder. Nephritic disease was evaluated on laboratory parameters, specifically changes in urine summary and 24 h proteinuria, as follows: persistent proteinuria (>0.5 g/day or 3+) or abnormal cylindruria. The health control (HC) group consisted by 138 healthy females (mean age 33.5 years ± 13.4). The exclusion criteria were autoimmune, renal, chronic inflammatory disease or infection diseases. Subjects were chosen randomly in the population and matched for sex, age, ethnic group and same geographical area of the patients. Clinical and laboratorial characteristics are available at Table 2.

For assessing *VDR* gene expression levels we sampled 29 SLE patients (clinical and laboratorial confirmation) and 17 individuals as controls randomly selected. The inclusion and exclusion criteria were the same of genotyping study. SLE patients and healthy controls also denied any calcium or vitamin D replacement in the past two years. To evaluate the correlation of *VDR* mRNA levels and SLE activity, we assessed the SLEDAI mean to obtain the activity profile of SLE group.

2.2 | VDR association study

Genomic DNA was isolated from peripheral blood samples using DNA Wizard Genomic DNA Purification Kit (Promega, Madison, WI)

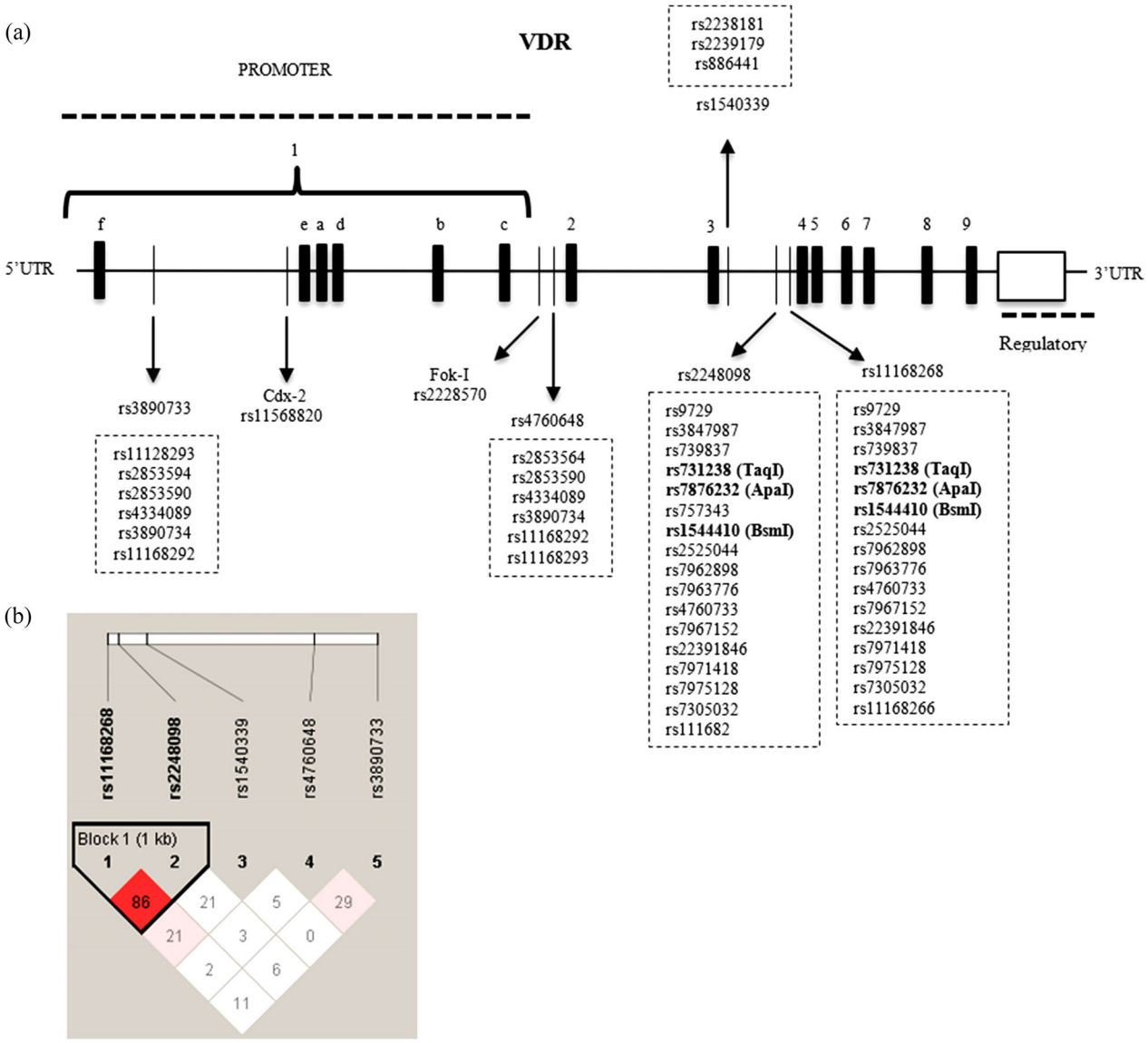

FIGURE 1 a) VDR gene schematic structure. The arrows indicate the position of all the assessed SNPs and the doted boxes indicate all the tagged SNPs. (b) Haplotype graphical representation. Linkage Disequilibrium (LD) plot among the studied polymorphisms, whereas in a *D*' values. The marked red box represents the LD shows (*D*' = 0.86) between the rs11168268 and rs2248098, demonstrating a strong linkage disequilibrium. Graph from Haploview Software

according to manufacturer's protocol. Polymorphisms were selected using the SNPBrowser software 4.0 (Applied Biosystems, Foster City, CA) and HapMap database (http://hapmap.ncbi.nlm.nih.gov/). We selected TagSNPs: rs11168268, rs2248098, rs1540339, rs4760648, rs3890733 (TagSNPs are representative SNPs in a gene region by linkage disequilibrium) (LD) (Stram, 2004) and rs2228579 (Fok1) and rs11568820 (Cdx-2), SNPs with functional impact. All selected SNPs presented at least 10% Minimum Allele Frequency (MAF) in CEU and YRI populations and covered most of *VDR* gene (Figure 1a).

Genotyping was evaluated by Taqman Probes® (Applied Biosystems, Foster City, CA) using the ABI7500 Real-Time PCR platform (Applied Biosystems, Foster City, CA). Allelic discrimination followed as recommended by the manufacturer and analysed using the SDS software 2.3 (Applied Biosystems, Foster City, CA).

2.3 | VDR gene expression study

RNA isolation was performed using the Qiagen Whole Blood RNAse kit, as described in manufacturer's instructions. The RNA integrity was performed by agarose gel electrophoresis and quantification by Nanodrop 2000 (Thermo Scientific USA). SuperScript III First-Strand Synthesis System for RT-PCR kit (Invitrogen, USA) was performed for cDNA synthesis using for each sample a standard input of 500 ng from total RNA for reaction of 20 μl of cDNA. Oligo(dT) was used as primers in all samples.

The mRNA levels were determined for the target gene *VDR* and the reference genes *GAPDH* and *β-Actin* was used for data normalization (*VDR*: Hs00172113_m1, *GAPDH*: Hs02758991_g1, *ACTB*: Hs 99999903_M1). Expression assays were performed on ABI 7500

TABLE 1 Most frequents SNPs assessed according populations

VDR SNP	Function	Author (reference)	Country (ethnicity)	Population (cases/controls)	Relevant results
rs1544410 (*BsmI*)	Located on intron 8 (A > G or B > b). Could affect mRNA stability and *VDR* gene expression. Could generate an alteration in the splice sites for mRNA transcription or a change in the intron regulatory elements of VDR.	Ozaki et al., 2000	Japan (Asian)	58/87	SLE and allele B ($p < .0001$) Nephritis and allele b ($p = .03$)
		Huang et al., 2002	Taiwan (Asian)	47/90	SLE and allele B ($p < .0001$)
		Luo et al., 2012	China (Han Chinese)	337/239	SLE and allele B ($p = .031$) Nephritis and allele B ($p = .027$)
rs2228570 (*FokI*)	Located on exon 2, generates a non-synonymous polymorphism with a change of C > T (also called F > f), resulting in a change of threonine to methionine. The presence of the restriction site FokI C allele (F allele), generates a new start codon (ATG) 9 bp after the common starting site, which translates to a shorter truncated VDR protein of 424 amino acids with more transactivation capacity as a transcription factor than the wild type full-length VDR A isoform (VDRA) with 427 amino acids.	Luo et al., 2011	China (Han Chinese)	271/130	SLE and allele F ($p = .001$)
		Carvalho et al., 2015	Portugal (Caucasian)	170/192	CT genotype and higher SLICC value ($p = .031$)
		Salimi et al., 2019	Southeast Iranian	1027/139	CT genotype and higher SLE susceptibility ($p = .02$)
rs7975232 (*ApaI*)	Located on intron 8 (A > C also called A > a), does not change the amino acid sequence of the VDR protein, therefore could affect mRNA stability and the gene expression of VDR;	Salimi et al., 2019	Southeast Iranian	1027/139	No association
rs731236 (*TaqI*)	Located on the exon 9 (C > T also called T > t) and generates a synonymous change of the isoleucine amino acid in the coding sequence, therefore it does not change the encoded protein, but it could influence the stability of the mRNA.	Carvalho et al., 2015	Portugal (Caucasian)	170/192	TT genotype and higher SLICC value ($p = .046$)
		Salimi et al., 2019	Southeast Iranian	1027/139	Tt genotype and higher SLE susceptibility ($p = .0002$)
rs11168268	TagSNP	Silva et al., 2013	Brazil (Southeast)	158/190	Cutaneous alterations ($p = .036$)

(Continues)

TABLE 1 (Continued)

VDR SNP	Function	Author (reference)	Country (ethnicity)	Population (cases/controls)	Relevant results
rs3890733	TagSNP	Silva et al., 2013	Brazil (Southeast)	158/190	Arthritis ($p = .001$)
rs2248098	TagSNP	Silva et al., 2013	Brazil (Southeast)	158/190	Immunological alterations ($p = .040$)
rs4760648	TagSNP	Silva et al., 2013	Brazil (Southeast)	158/190	Antibody anti-dsDNA ($p = .036$)

platform (Applied Biosystems, Foster City, CA, USA). Relative quantity (RQ) of *VDR* mRNA was measured by quantification cycle (Cq) values obtained for *VDR* and each of the endogenous reference genes from all samples. Then, the mean value for each gene in each group was used to calculate *VDR* mRNA levels using ΔCq as quantification method (Livak & Schmittgen, 2001). We performed all qPCR assays in technical triplicates.

2.4 | Statistical analysis

SNPStats tool (http://bioinfo.iconcologia.net/SNPstats) was used for calculate allele and genotype frequencies and Hardy–Weinberg equilibrium, and Fisher's exact test was used to the statistical significance of difference in allele and genotype frequencies. After Bonferroni's Correction, a $p < .071$ was considerate statistically significant for SNP association study. For haplotype associations and linkage disequilibrium analysis (LD) was used Haploview Software (version 4.2). The multivariate analysis logistic regression was performed to investigate the association between the qualitative variables and dependent variable binary: SLE risk and ACR clinical phenotypes. The open-source R Studio 4.1.2 (www.r-project.org) was used for all statistical analyses. The post hoc power analysis was performed in the G*Power 3.1.9.4 software and the results were included at Table 2.

The statistical tests applied to gene expression analyses were: Shapiro–Wilk, to verify the sample's distribution, and Student's *t*-test and one-way ANOVA for analysis of variance, considering as statistically significant in both $p < .05$ in a 95% confidence interval (95% CI).

3 | RESULTS

The *VDR* allelic and genotypic frequencies from all assessed SNPs were in Hardy–Weinberg equilibrium in SLE patients and HC, except for patients' group in rs2228570 (FokI). The frequencies distribution presented significantly differed between SLE patients and HC in three out of the seven assessed SNPs namely: rs11168268, rs2248098 and rs2228570 as shown in Table 3.

For the rs11168268 (A > G) SNP, the G allele (OR = 1.55, CI = 1.08–2.23, $p = .01$) and G/G genotype (OR = 2.69, CI = 1.24–6.01, $p = .008$) were associated to increased SLE susceptibility. In the other hand for rs2248098 (A > G) SNP, the G allele (OR = 0.66, CI = 0.46–0.94, $p = .01$) and A/G (OR = 0.46, CI = 0.24–0.86, $p = .01$) and G/G (OR = 0.44, CI = 0.20–0.93, $p = .02$) genotypes were associated to lower SLE susceptibility, as shown in Table 3. For the rs2228570 (C > T) SNP, the frequency of C allele, C/T and C/C genotypes was increased in controls when compared to patients (OR = 0.19, $p = 2.7 \times 10^{-16}$; OR = 0.14, $p = 1.55 \times 10^{-7}$; OR = 0.05, $p = 1.77 \times 10^{-13}$, respectively), as shown in Table 3. However, this last result regarding rs2228570 is biased once the patient's group did not present H-W equilibrium.

TABLE 2 Clinical and laboratorial features from the SLE patients studied

Clinical/laboratorial characteristics	*n* (%)
Photosensitivity	80 (62.5%)
Malar Rash	77 (60.16%)
Discoid Rash	22 (17.19%)
Oral ulcers	27 (21%)
Serositis	28 (21.88%)
Arthritis	92 (71.8%)
Neuropsychiatric disorder	11 (8.5%)
Nephritic disorder	65 (50.7%)
Haematological alterations	87 (67.9%)
Antinuclear factor positive (FAN)	128 (100%)
Antibody anti DNA (anti ds-DNA)	33 (25.7%)

For the SNPs rs4760648, rs1540339 and rs11568820, no significant difference in allelic and genotypic distribution was observed (Table 3).

Regarding *VDR* polymorphisms and clinical and laboratorial characteristics we report association between following SNPs and clinical features: rs11168268 A/G genotype (OR = 0.31, CI = 0.11–0.8, $p = .01$) with lower nephritis susceptibility; rs4760648 T/T genotype (OR = 0.24, CI = 0.05–0.9, $p = .02$) with diminished photosensitivity; rs1540339 T/T genotype (OR = 0.19, CI = 0.04–0.78, $p = .015$) with less frequency of antibody anti-dsDNA and rs3890733 T/T genotype (OR = 0.10, CI = 0.002–0.81, $p = .01$) with lesser serositis development, as seen in Table 4. Multivariate analysis results are demonstrated at Table 5.

Haplotype analysis was performed to assess linkage disequilibrium among the tested TagSNPs. We observed LD between TagSNPs rs11168268 and rs2248048 (*Dt'* = 0.86) as shown in Figure 1b. Even though we identified a haplotype combination, no association was observed to SLE or its clinical features susceptibility (data not shown).

We performed a relative gene expression assay to evaluate the mRNA levels from *VDR* in SLE patients as well as in healthy controls. We observed that overall *VDR* gene expression was downregulated in patients (−10.51 FC, $p = 1.04e^{-7}$) when compared to HC (Figure 2a). We also analysed whether the Cdx-2 (rs11568820) genotypes (A/G-G/G) influence *VDR* gene expression in SLE patients. Our analyses indicated that the A/G and G/G genotypes decrease *VDR* mRNA levels (−9.6, $p = .008$ and −12.6 FC, $p = .014$, respectively) when compared to A/A genotype (Figure 2b).

When assessing *VDR* gene expression and altered risk for SLE clinical manifestations, we found a differential expression in patients with nephritis (−5.7 FC, $p = .016$; Figure 2c). We also found a differential expression in patients with skin alterations (+1.3 FC, $p = .587$), but the data comparison is not statistically significant. The differential expression reported with mRNA levels from *VDR* and clinical manifestations are showed in Figure 2a–c. Other clinical features were analysed, but they did not present enough sampling power to be included in the expression analyses.

4 | DISCUSSION

In present study, we observed an association between rs11168268 and rs2248098 and SLE development, where rs11168268 (A > G) SNP, the G allele and G/G genotype conferred risk for SLE; and rs2248098 (A > G) SNP, the G allele, as well the A/G and G/G genotypes conferring lower susceptibility to SLE. Interestingly, although these SNPs were not associated with SLE itself, it had already been previously reported as associated to clinical features in a southeast Brazilian population study (Silva et al., 2013).

In our study, a strong linkage disequilibrium was observed between rs11168268 and rs2248098. Interesting, Cavalcanti et al. (2016) also verified significant linkage disequilibrium between these same SNPs ($D' = 0.91$, $r^2 = .72$), corroborating our data ($D' = 0.86$).

VDR plays a key role regulating vitamin D pathway and its physiological importance in immune modulation relates it to several immune disorders, including SLE (Kamen et al., 2006; Wöbke et al., 2014). In our study the presence of C allele as well C/T and C/C genotypes from FokI SNP indicated a lower risk of SLE susceptibility. FokI display a cytosine to thymine change (C > T) creating a methionine codon three codons latter, which in turn, leads to a final protein with 424 amino acids (aa) shorter than the one with the T allele, with 427aa. In fact, the shorter variant (C) seems to interact more strongly to the transcription factor II B (TFIIB) compared to the longer one. Therefore, it seems that the VDR shorter protein may be more efficient than the longer one in activating vitamin D pathway (Dzhebir et al., 2016). Besides the statistical association, it is important to mention that in our population for this SNP, the patient's group was out of Hardy–Weinberg equilibrium.

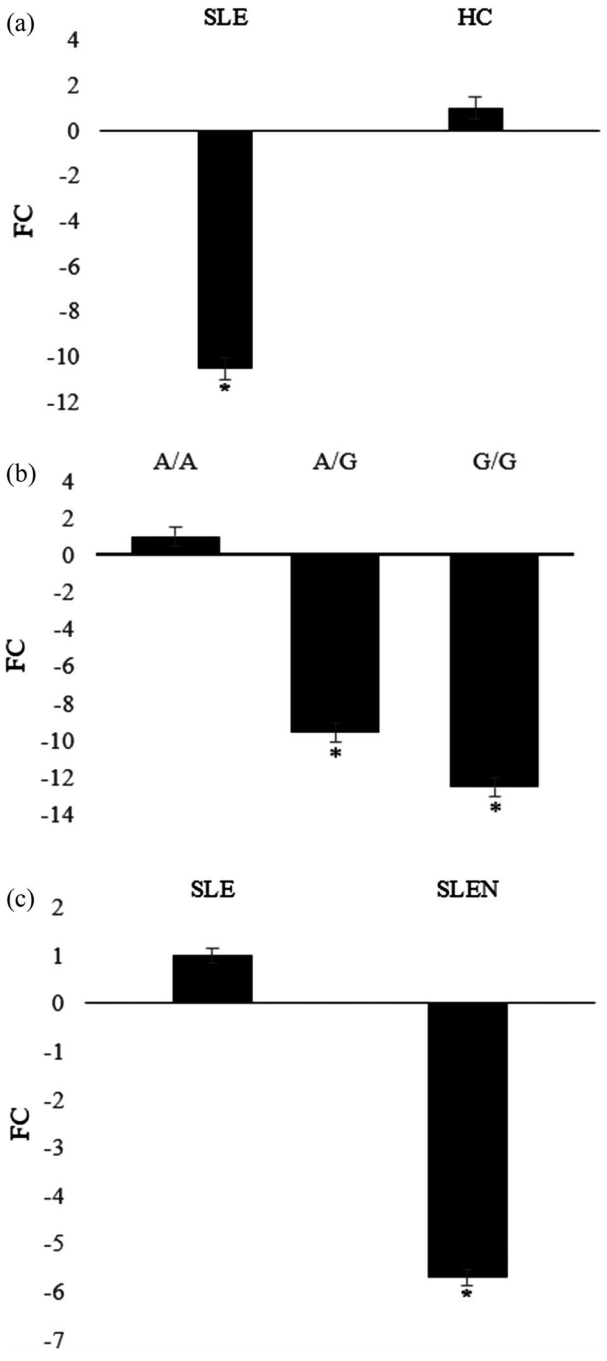

FIGURE 2 a) VDR expression graph comparing SLE patients and HC. (b) VDR expression graph of SLE patients among from Cdx-2 SNP genotypes (A/A $n = 6$; A/G $n = 10$; G/G $n = 5$). (c) VDR expression graph of SLE patients or SLE nephritis (SLEN). The results were normalized using *GAPDH* and *ACTB* as endogenous references. SLE: patients with Systemic lupus erythematosus ($n = 29$); HC: Healthy controls ($n = 17$); SLEN: patients with SLE and nephritis ($n = 12$); FC: fold-change. $^{*}p < .05$

Two previous *VDR* association studies were performed in Brazilian populations. The first one, by Monticielo et al. (2012), was performed in a south Brazilian population and included, amongst others, the two most studied *VDR* SNPs: *BsmI* and *FokI*. However, the authors did not find statistically significant differences in genotype and allelic

TABLE 3 Allelic and genotypic VDR SNPs and TagSNPs frequencies from all assessed SLE patients and HC

SNP ID	HC	SLE patients	OR (95%CI)	*p* Value
rs3890733	**N = 138**	**N = 127**		
Allele				
C	194 (70%)	169 (67%)	1.00	
T	82 (30%)	85 (33%)	1.18 (0.81–1.74)	.39
Genotype				
CC	69 (50%)	63 (49.6%)	1.00	
CT	56 (40.6%)	43 (33.9%)	0.84 (0.48–1.46)	.84
TT	13 (9.4%)	21 (16.5%)	1.76 (0.76–4.17)	.17
rs11568820	**N =109**	**N = 115**		
Allele				
G	124 (57%)	132 (57%)		
A	94 (43%)	98 (43%)	0.97 (0.66–1.44)	.92
Genotype				
GG	33 (30.3%)	40 (34.8%)		
AG	58 (53.2%)	52 (45.2%)	0.74 (0.39–1.39)	.36
AA	18 (16.5%)	23 (20%)	1.05 (0.45–2.45)	1.00
rs2228570	**N = 108**	**N = 107**		
Allele[a]				
T	71 (33%)	154 (72%)	1.00	
C	**145 (67%)**	**60 (28%)**	**0.19 (0.12–0.30)**	**2.7×10^{-16}***
Genotype[a]				
TT	12 (11.1%)	60 (56.1%)	1.00	
CT	**47 (43.5%)**	**34 (31.8%)**	**0.14 (0.06 - 0.32)**	**1.55×10^{-7}***
CC	**49 (45.5%)**	**13 (12.2%)**	**0.05 (0.02–0.13)**	**1.77×10^{-13}***
rs4760648	**N = 138**	**N = 127**		
Allele				
C	151 (55%)	132 (52%)	1.00	
T	125 (45%)	122 (48%)	1.11 (0.78–1.59)	.54
Genotype				
CC	37 (26.8%)	31 (24.4%)	1.00	
CT	77 (55.8%)	70 (55.1%)	1.08 (0.58–2.0)	.88
TT	24 (17.4%)	26 (20.5%)	1.29 (0.58–2.86)	.57
rs1540339	**N = 138**	**N = 128**		
Allele				
C	193 (70%)	176 (69%)	1.00	
T	83 (30%)	80 (31%)	1.05 (0.71–1.55)	.77
Genotype				
CC	63 (45.6%)	62 (48.4%)	1.00	
CT	67 (48.5%)	52 (40.6%)	0.78 (0.46–1.34)	.37
TT	8 (5.8%)	14 (10.9%)	1.77 (0.63–5.24)	.25

(Continues)

TABLE 3 (Continued)

SNP ID	HC	SLE patients	OR (95%CI)	*p* Value
rs2248098	**N = 138**	**N = 127**		
Allele[a]				
A	127 (46%)	143 (56%)	1.00	
G	**149 (54%)**	**111 (44%)**	**0.66 (0.46–0.94)**	**.01***
Genotype[a]				
AA	26 (18.8%)	43 (33.9%)	1.00	
AG	**75 (54.4%)**	**57 (44.9%)**	**0.46 (0.24–0.86)**	**.01***
GG	**37 (26.8%)**	**27 (21.3%)**	**0.44 (0.20–0.93)**	**.02***
rs11168268	**N = 138**	**N = 127**		
Allele[a]				
A	176 (64%)	135 (53%)	1.00	
G	**100 (36%)**	**119 (47%)**	**1.55 (1.08–2.23)**	**.01***
Genotype[a]				
AA	54 (39.1%)	41 (32.3%)	1.00	
AG	68 (49.3%)	53 (41.7%)	1.02 (0.57–1.83)	1.00
GG	**16 (11.6%)**	**33 (26%)**	**2.69 (1.24–6.01)**	**.008***

*$p < .05$; Values in bold are the results with association (significant *p*). The SNPs used in the study are in italics.
[a]Power > 0.8.

frequencies between SLE patients and healthy individuals. The other study performed by our research group in a southeast Brazilian cohort and even though we did not find any association to SLE itself, we reported association to cutaneous manifestations, arthritis, immunological alterations and antibody anti-dsDNA (Silva et al., 2013).

In relation to *VDR* SNPs and clinical manifestations, the study found statistically significant association with antibody anti-dsDNA (rs1540339), photosensitivity (rs4760648), nephritis (rs11168268) and serositis (rs3890733).

In our study, the presence of T/T genotype of rs1540339 SNP is associated with lower frequency of antibody anti-dsDNA presence. Corroborating with our findings, anti-dsDNA is an important marker to evaluate the disease activity in SLE patients. Studies have shown that SLE patients present vitamin D deficiency when compared to the general population. SLE patients with VD_3 deficiency presents increased disease's activity and raised anti-dsDNA levels, which strengthen VD_3 role as an immune modulator in autoimmune diseases (Mok et al., 2012).

Our results showed that the presence of rs4760648 T/T genotype confers a lower susceptibility to photosensitivity development and are in agreement with Silva et al (2013) that identified in southeast Brazilian population the same association. Photosensitivity is an important clinical manifestation in SLE patients and contributes to poor life quality of these individuals (Klein et al., 2011). Lesions caused by photosensitivity in SLE patients are characterized by increased epidermal apoptosis and infiltrate of inflammatory cells like dendritic cells in the dermis (Kim & Chong, 2013). The immunoregulation promoted by VD_3 in immune cells recruitment and cytokine liberation may play a crucial role in SLE patient response to lesions caused by ultraviolet (UV) exposure (Correa-Rodríguez et al., 2021).

The most frequent and severe clinical finding in SLE patients is LN (Tang et al., 2017). LN is an important condition and major risk factor for morbidity and mortality in SLE patients (Almaani et al., 2017). In our study, we found a significant association between LN and the A/G genotype of rs11168269 SNP. Corroborating with our findings, the TagSNP rs11168269 tags the *BsmI*, already reported as associated to LN lower susceptibility. Located on intron 8, *BsmI* represents the change of adenine for guanine (A > G), also called for B > b (BB, Bb and bb genotypes). The SNP *BsmI* may affect mRNA stability and *VDR* gene expression, altering in the splice sites in mRNA transcription or a change in intronic regulatory elements of VDR (Luo et al., 2011; Luo et al., 2012). The LN pathogenesis is not completely elucidated. Low levels of Vitamin D may play a role in SLE progression and nephritis development. On the other hand, VD_3 supplementation may prevent renal involvement by lessening proteinuria risk, a frequent condition in LN patients (Yu et al., 2019).

We also found an association between rs3890733 T/T genotype and lower susceptibility to serositis. Serositis is an inflammation of serous membranes and a significant cause of morbidity in SLE patients (Liang et al., 2017). Located at promoter region, rs3090733 is a TagSNP that tags another six SNPs by linkage disequilibrium. The rs4334089 is tagged by rs3090733 and its variant genotype A/A is associated to lower risk to upper respiratory infection (URI) development. It is hypothesized that the presence of this variant would improve the inflammatory response performed by the VD_3/VDR complex (Jolliffe et al., 2018). Although the literature lacks association studies correlating *VDR* and clinical features in SLE such as serositis, a study conducted by Luo et al. (2012), with SLE patients from Chinese population and *VDR* SNPs found a relation between *ApaI* and *BsmI* polymorphisms with serositis and also an increased risk to SLE development considering combined genotype Aa-bb.

TABLE 4 Genotypes from VDR SNVs and TagSNVs associated with SLE clinical manifestations

SNV	Clinical Feature	Genotype	OR	95%CI	*p* Value
rs1540339 (C > T)	Anti-dsDNA	TT	0.19	0.04–0.78	.015
rs3890733 (C > T)	Serositis	TT	0.10	0.002–0.81	.01
rs11168268 (A > G)	Nephritis	AG	0.31	0.11–0.8	.01
rs4760648 (C > T)	Photosensitivity	TT	0.24	0.05–0.9	.02

TABLE 5 Multivariate analysis using as dependent variables SLE susceptibility and ACR clinical phenotypes and as independent variables seven the SNPs analysed

SNP	Genotype	Dependent variables	Exponential value	*p* Value
rs2228570	C/T	SLE susceptibility	3.06×10^{5}	6.92×10^{-11}
	T/T	SLE susceptibility	4.45×10^{4}	.00584
rs4760648	C/T	Serositis	0.086	.009
		Neurological alterations	0.179	.0316
	T/T		0.082	.0123
rs11168268	A/G	Antibody anti-dsDNA	0.121	.0208
rs1540339	C/T	Discoid rash	−3.663	.0313
		Photosensitivity	−6.495	.0408
		Nephritis	0.204	.0402
		Antibody anti-dsDNA	0.184	.0276
rs3890733	C/T	Neurological alterations	0.1705	.0214
		Antibody anti-dsDNA	0.121	.014
rs11568820	G/G	Arthritis	−32.402	.0014
	A/G	Nephritis	0.149	.045

Complex diseases as SLE presents several variants in specific genes, as VDR, which provides diverse clinical phenotypes, raising a challenge in identifying genetic variations associated simultaneously with correlated traits. Multivariate analysis should be done to detect independent predictors of different clinical phenotypes. The multivariate analysis performed in the present study (Table 5) found statistically significant association with SLE susceptibility and rs2228570 SNP (FokI). The analysis was also performed using ACR clinical characteristics directly related to the accumulation of immune complexes such as photosensitivity (rs1540339), serositis (rs4760648), neurological alterations (rs4760648, rs3890733), antibody anti-dsDNA (rs11168268, rs1540339, rs3890733), discoid rash (rs1540339), nephritis (rs1540339, rs11568820/Cdx-2) and arthritis (rs11568820/Cdx-2). Inflammatory process is one of the most important roles on SLE's pathogenesis, and vitamin D levels has already been associated to its modulation (Iruretagoyena et al., 2015; Wöbke et al., 2014). In addition, a recent study shows vitamin D levels are associated with SLE activity and DNA damage growth (Correa-Rodríguez et al., 2021).

Accessing *VDR* expression levels, we found a downregulation (−10.51 FC) in SLE patients comparing with HC group. VD_3/VDR complex plays an important role in immune cells as monocytes, macrophages, dendritic, T and B cells (Wang et al., 2012). SLE patients are deficient or insufficient in vitamin D levels compared with healthy controls, where 1,25(OH)2 D_3 serum levels and *VDR* mRNA expression in peripheral blood were decreased in SLE patients and it could inhibit the activation of $CD4^+$ T cells and suppress the immune response in SLE (Xiao et al., 2016). VD_3 inhibits the action of activated B cells and induces their apoptosis. B cells, on the other hand, express mRNAs for proteins involved in VD_3 activity, including *VDR*, which consequently is regulated by vitamin D levels (Chen et al., 2007), in concordance with our results.

In addition, when *VDR* gene expression was analysed in SLE patients according rs11568820 (Cdx-2), a downregulation was observed in patients with the genotype G/A and G/G, when compared to A/A genotype, indicating that, the G allele decreases *VDR* mRNA levels in these individuals. The Cdx-2 polymorphism is located at *VDR* promoter region and consists in change of adenine to guanine, potentiating the binding strength between VDR and its transcriptional complex (Ralston & Rossant, 2008; Savory et al., 2009).

When assessing *VDR* expression and risk for SLE clinical manifestations our analyses showed that patients with skin alterations as malar rash, discoid rash and photosensitivity presents an upregulation of *VDR* mRNA levels (+1.3 FC), however this data was not statistically significant. Individuals with SLE show increased cutaneous manifestations in response to UV light exposure that induces apoptosis with subsequent

immune complexes formation, justifying inflammation and skin lesions (Bijl & Kallenberg, 2006).

We also report a downregulation of *VDR* expression in patients that present nephritis (−5.7 FC). Interestingly, several studies report significant associations of *VDR* SNPs with LN (Luo et al., 2012, Emerah & El-Shal, 2013; Mostowska et al, 2013; Ozaki et al, 2000). Being ours the first one to bring up an expression data from nephritis in SLE patients, our results agree with the fact that $1,25(OH)_2D_3$ upregulates *VDR* gene expression in kidney cells (Andress, 2006; Healy et al., 2003; Healy et al., 2005) and since SLE patients with nephritic disorders have significantly lower vitamin D levels (Sumethkul et al., 2013), it justifies the *VDR* downregulation detection. The kidney is one of the main organs processing pro-forms of inactive vitamin D into active forms (1,25a-OH vitamin D) (Veldurthy et al., 2016) when its function is impaired, it may influence upon vitamin D levels, contributing to deficiency. Therefore, due to our sample limitation we suggest that further studies needed to be performed in other population to better elucidate the VDR role in LN.

Our results support *VDR* polymorphisms and mRNA expression levels associated to SLE and some clinical features, particularly nephritis. We also assessed in SLE patients according *Cdx-2* genotype, which indicated a downregulation when compared to healthy individuals. To the better of our knowledge, this was the first and only study to evaluate almost the completed *VDR* gene SNPs by linkage disequilibrium. Our main limitation is the relatively small sample size from SLE patients' group and not following the functional analysis from the associations detected, falling into the main gap of all genetic association studies. Thus, these findings reinforce the VDR key role in SLE and its clinical features.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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