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Association of *Vascular Endothelial Growth Factor A* gene polymorphisms with susceptibility to Systemic lupus erythematosus in Iranian population

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Systemic lupus erythematosus (SLE) is an autoimmune, autoinflammatory disorder in which genetic factors have been implicated in the etiopathogenesis. Elevated levels of the vascular endothelial growth factor (VEGF) have been reported in patients with SLE. This study intended to evaluate the association of the *VEGFA* gene rs833061 and rs2010963 single nucleotide polymorphisms (SNPs) with the risk of SLE susceptibility in an Iranian population.

In this case-control study, 400 SLE patients and 400 age-, sex-, and ethnically-matched healthy controls were recruited. Genotyping of *VEGFA* gene rs833061 and rs2010963 polymorphisms in both SLE and control groups was done using real-time PCR allelic discrimination technique.

No significant difference between patient and control groups was detected in the alleles or genotypes of either rs833061 or rs2010963 SNPs. Moreover, the haplotypes were not associated with SLE susceptibility. However, rs833061 and rs2010963 polymorphisms were in linkage disequilibrium according to D' = 95 % but not according to r^2 = 42%. The associations between rs833061 (C vs. T: OR = 0.98, 95% CI = 0.80-1.20, *P* value = 0.87) and rs2010963 (C vs. G: OR = 0.89, 95% CI = 0.73 - 1.09, *P* value = 0.28) with risk of SLE were not significant. The clinical data of the patients, including anti-dsDNA (*P* value = 0.036), anti-SSA (*P* value = 0.039), and anti-SSAB (*P* value = 0.036), were associated with the genotypes of the *VEGFA* gene rs2010963 SNP.

It was recognized that *VEGFA* gene rs833061 and rs2010963 polymorphisms did not affect SLE susceptibility in the Iranian population.

Keywords: Systemic lupus erythematosus; VEGFA; Single nucleotide polymorphism

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disorder in which genetic, epigenetic, and environmental factors interplay determining disease susceptibility [1, 2]. The chronic inflammatory responses, immunopathogenic mediators, and several autoantibodies may result in an immune complex-associated vasculitis as well as endothelial damage [3, 4]. SLE is seen all over the world [5]. Epidemiological studies have indicated that the global incidence of SLE is between 4 and 7 in 100,000 per year [6, 7]. Most SLE patients are affected by the disease at a period of 15 to 40 years, and SLE is predominantly more common in women than men [5]. Despite a decrease in the mortality rate of SLE patients, They still experience an intense physical and psychological burden [8].

According to the suggested hypothesis, the pathogenesis of SLE may be initiated when vascular endothelial cells are damaged and activated [9]. It has been observed that the vascular endothelial growth factor (VEGF) is upregulated in SLE patients [10] and is involved in various biological functions, including endothelial cell proliferation and migration, angiogenesis, and vascular permeability [11]. It has further been established that an increased VEGF level is associated with the disease activity in SLE patients [3, 4]. On the other hand, vascular endothelial growth factor receptor 2 (VEGFR2) gene polymorphism has been reported to be involved in the pathogenesis of vascular diseases and may be involved in the endothelial function, and integrity [12]. In addition to the involvement of VEGF level in the disease activity of SLE patients, studies have also indicated that an abnormal VEGF level is associated with the clinical presentations of patients, including higher mean carotid intima media thickness and lupus nephritis [13, 14], platelet count [10], and pulmonary hypertension [15].

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The chromosome 6p.12 harbors the human *VEGFA* gene, and genetic single nucleotide polymorphisms (SNPs) in this gene that may cause functional impressions in the coding protein have previously been identified [16, 17]. Many studies have implied the role of *VEGFA* gene polymorphisms in different immunity disorders such as rheumatoid arthritis (RA) and SLE [18, 19]. Concerning this, the current study aimed to investigate the possible association of *VEGFA* gene rs833061 and rs2010963 SNPs with SLE susceptibility in the Iranian population.

Materials and Methods

Patients and controls

In this association study, 400 SLE patients who fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of SLE [20] and referred to the rheumatology clinic of Shariati Hospital, Tehran, Iran, were recruited. The baseline characteristics of the 400 SLE patients are provided in Table 1. Moreover, 400 healthy individuals who were age-, sex-, and ethnicity-matched with participants in the SLE group were selected as the control group. Healthy controls had no history of autoimmune or other disorders, either in themselves or in their first-degree relatives. This study gained approval from the Ethical Committee of Tehran University of Medical Sciences. Prior to blood sampling, written informed consent forms was signed by all SLE patients and healthy controls. For genotyping, of peripheral venous blood were collected from all study participants in EDTA-coated tubes using venipuncture.

Table 1. Baseline characteristics and clinical manifestations of 400 SLE patients.

Characteristic	Value
Age	35.53±14.34
Age of onset	27.19±10.70
Sex; Female/Male	351 (87.75%)/ 49 (12.25%)
Serositis	67 (16.75%)
Malar rash	209 (52.25%)
Palmar Erythema	27 (6.75%)
Vascular Ulcers	29 (7.25%)
Discoid rash	44 (11%)
Mucosal lesion	153 (38.25 %)
Photosensitivity	231 (57.75%)
Arthritis	295 (73.75%)
Aseptic necrosis	22 (5.5%)
Muscle weakness	51 (12.75%)
Raised Muscle Enzyme	42 (10.5%)
Myositis in Muscle Biopsy	13 (3.25%)
Urine cast	60 (15%)
Hematuria	138 (34.5%)
Raised Creatinine	36 (9%)
Hypertension	67 (16.75%)
Pericarditis	29 (7.25%)
Cardiomyopathy	5 (1.25%)
Libman–Sacks endocarditis	2 (0.5%)
Valvular lesion	40 (10%)
Ischemic heart disease	7 (1.75%)

Characteristic	Value
Raynaud's phenomenon	58 (14.5%)
Thrombophlebitis	11 (2.75%)
Pleuritis	55 (13.75%)
Lupus pneumonitis	9 (2.25%)
Interstitial fibrosis	5 (1.25%)
Pulmonary hypertension	7 (1.75%)
Embolism	5 (1.25%)
Convulsion	40 (10%)
Psychosis	11 (2.75%)
Peripheral neuropathy	18 (4.5%)
Central involvement	22 (5.5%)
Raised liver enzyme	211 (52.75%)
Hepatitis	5 (1.25%)
sicca	2 (0.5%)
Retinitis	5 (1.25%)
Leukopenia	138 (34.5%)
Lymphopenia	124 (31 %)
Thrombocytopenia	98 (24.5%)
Anemia	111 (27.75%)
Hemolytic anemia	69 (17.25%)
Coombs test	60 (15%)
CRP	233 (58.25%)
FANA	372 (93%)
Anti-dsDNA	333 (83.25%)
Anti-SSA	13 (3.25%)
Anti-malaria	375 (93.75%)
Anti-SSB	9 (2.25%)
Anti- Phospholipid	31 (7.75%)
Anti-SSAB	13 (3.25%)
Anticardiolipin-IgG	22 (5.55%)
Anticardiolipin-IgM	13 (3.25%)
Lupus anticoagulant	7 (1.75%)
Anti-B2GP1 IgG	11 (2.75%)
Anti-B2GP1 IgM	5 (1.25%)
Increased complement level	242 (60%)
Musculoskeletal manifestations	309 (77.25%)

Characteristic	Value
Renal Manifestations	240 (60%)
Cardiac Manifestations	67 (16.75%)
Pulmonary Manifestations	37 (9.25%)
Neuropsychiatric Manifestations	71 (17.75%)
Hepatic Manifestations	211 (52.75%)
Ophthalmologic Manifestation	5 (1.25%)
Hematologic Manifestations	249 (62.25%)
Cutaneous manifestations	295 (73.75%)

CRP, C-reactive protein; FANA, Fluorescent antinuclear antibody; Anti-dsDNA, anti-double strand DNA; Anti-SSA, anti-sjögren's-syndrome-related antigen A; Anti-SSB, anti-sjögren's-syndrome-related antigen B; Anti-B2GP1, anti-β2 glycoprotein I antibody

Genotyping of *VEGFA* gene polymorphisms

The DNA was extracted from the peripheral blood of the study subjects using the phenol-chloroform DNA extraction approach. All study subjects were genotyped for *VEGFA* gene rs833061 and rs2010963 polymorphisms using the real-time allelic discrimination TaqMan method (Applied Biosystems, Foster City, USA). The amplification mixture, with a final volume of 10 µl, contained 5 µl TaqMan Master Mix containing Taq DNA polymerase and dNTPs (Applied Biosystems, Foster City, USA), 0.25 µl TaqMan Genotyping Assay mix containing primers and FAM or VIC labeled probes (Applied Biosystems, Foster City, USA), 4.5 µl of genomic DNA (20 - 30 ng/µl), and 0.25 µl of H_2O. Real-time allelic discrimination PCR was performed by StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, USA), based on the following conditions: initially 60 °C for 30 seconds, then 95 °C for 10 min, then 40 cycles of amplification (95 °C for 15 seconds and 60 °C for 1 min), and finally 60 °C for 30 seconds.

Statistical analysis

The chi-square test was used to survey the association of alleles, genotypes, dominant, and recessive models of inheritance with disease risk. Odds ratios (ORs) with 95% confidence intervals (95% CI) were also measured for each comparison. The Pearson's chi-square or Fisher exact test was used to evaluate the associations between genotype frequency and the clinical data in SLE subjects. The Hardy–Weinberg Equilibrium (HWE) was calculated for the control group in each SNP. For the analysis of allele, genotype, and haplotype frequencies, the SHEsis online tool (http://shesis.bio-x.cn/SHEsis.html) [21] was used. *P* values less than 0.05 were considered significant in all tests.

Results

The SLE group was composed of 351 (87.75%) females and 49 (12.25%) males, while the control group was consisted of 344 (86%) females and 56 (14%) males; hence, the groups were gender-matched (*P* value>0.05). Furthermore, the average age of SLE patients and healthy individuals was 35.53±14.34 and 36.44±13.51, respectively, representing age-matched groups (*P* value > 0.05). The distribution of the genotypes of both SNPs did not indicate a deviation from HWE (Table 2).

Table 2. Allele and genotype frequencies of the *VEGF* gene rs833061 and rs2010963 SNPs in SLE patients and healthy controls.

dbSNP	Frequency	
	SLE patients (N = 400)	Controls (N = 400)
	rs833061	
C	319 (39.9%)	322 (40.25%)
T	481 (60.1%)	478 (59.75%)
CC	55 (13.75%)	63 (15.6%)
TC	209 (52.25%)	196 (49.1%)
TT	136 (34%)	141 (35.2%)

Table 2. Allele and genotype frequencies of the *VEGF* gene rs833061 and rs2010963 SNPs in SLE patients and healthy controls.

dbSNP	Frequency			
	SLE patients (N = 400)		Controls (N = 400)	
HWE for controls = 0.707				
Association Test				
	Model		OR	95 % CI	***P*** value
Allele	C vs. T		0.98	0.80-1.20	0.87
Codominant	CC vs. TT		0.90	0.58-1.39	0.65
Codominant	TC vs. TT		1.10	0.81-1.50	0.52
Dominant	CC+TC vs. TT		1.05	0.78-1.41	0.71
Recessive	CC vs. TC+TT		0.85	0.57-1.26	0.42
rs2010963				
C	320 (40%)		341 (42.62%)	
G	480 (60%)		459 (57.37%)	
CC	73 (18.25%)		74 (18.5%)	
GC	174 (43.5%)		193 (48.25%)	
GG	153 (38.25%)		133 (33.25%)	
HWE for controls = 0.786				
Association Test				
	Model		OR	95 % CI	***P*** value
Allele	C vs. G		0.89	0.73 - 1.09	0.28
Codominant	CC vs. GG		0.85	0.49-1.49	0.58
Codominant	GC vs. GG		0.78	0.57-1.06	0.12
Dominant	CC+GC vs. GG		0.80	0.60-1.07	0.14
Recessive	CC vs. GC+GG		0.98	0.68-1.40	0.92

For rs833061, the T allele was considered as the major allele (according to the NCBI SNP tool; https://www.ncbi.nlm.nih.gov/snp/). The minor C allele was less frequent in SLE patients than in healthy subjects (39.9% vs. 40.25%); however, this association was not significant (OR = 0.98, 95% CI = 0.80 - 1.20, *P* value = 0.87). Moreover, the difference in the prevalence of both CC (OR = 0.90, 95% CI = 0.58-1.39, *P* value = 0.65) and TC (OR = 1.10, 95% CI = 0.81-1.50, *P* value = 0.52) genotypes did not show a statistical difference. Finally, no statistically significant difference was observed in the dominant (CC + TC vs. TT) or recessive (CC vs. TC + TT) comparisons (OR = 1.05, 95% CI = 0.78-1.41, *P* value = 0.71 and OR = 0.85, 95% CI = 0.57-1.26, *P* value = 0.42, respectively).

For the rs2010963 SNP of the *VEGFA* gene, the minor C allele was less frequent in SLE patients; nonetheless, this association was not significant (OR = 0.89, 95% CI = 0.73 - 1.09, *P* value = 0.28). Moreover, the CC (OR = 0.85, 95% CI = 0.49-1.49, *P* value = 0.58) and GC (OR = 0.78, 95% CI = 0.57-1.06, *P* value = 0.12) genotypes had almost similar frequencies between patient and control groups. As well, the dominant (CC+GC vs. GG) and recessive (CC vs. GC+GG) comparisons resulted in no statistically significant differences (OR = 0.80, 95% CI = 0.60 - 1.07, *P* value = 0.14 and OR = 0.98, 95% CI = 0.68-1.40, *P* value = 0.92, respectively).

With respect to haplotype frequencies, according to the rs833061 (C/T) and rs2010963 (C/G) order of polymor-

phisms, four haplotypes (CC, CG, TC, and TG) were identified, none of which was significantly associated with SLE risk (Table 3).

The linkage disequilibrium (LD) analysis indicated that rs833061 and rs2010963 were in LD according to D' = 95 %, but not according to r^2 = 42% (Figure 1).

As seen in Table 4, none of the clinical presentations of the SLE subjects was significantly associated with the frequency of the genotypes for the rs833061 SNP. However, the clinical data of the SLE patients, including anti-dsDNA (*P* value < 0.001), anti-SSA (*P* value = 0.001), and anti-SSAB (*P* value = 0.001) was significantly associated with the genotypes of the rs2010963 SNP.

Table 3. Overall haplotype associations of the single-nucleotide polymorphisms according to Haploview.

Block 1 Haplotypes			Frequencies			
Row	rs833061	rs2010963	Hap Freq. (Case) N (%)	Hap Freq. (Control) N (%)	OR* (95% CI*)	*P* value
1	C	C	1.26 (0.4%)	4.88 (0.9%)	-	-
2	C	G	135.74 (39%)	217 (41%)	0.91 (0.69-1.20)	0.51
3	T	C	136.74 (39.3%)	211 (39.8%)	0.96 (0.73-1.27)	0.82
4	T	G	74.26 (21.3%)	96.88 (61.83%)	1.20 (0.85-1.69)	0.28

*OR; odds ratio, CI; 95% confidence interval for difference between Hap.freq case – control

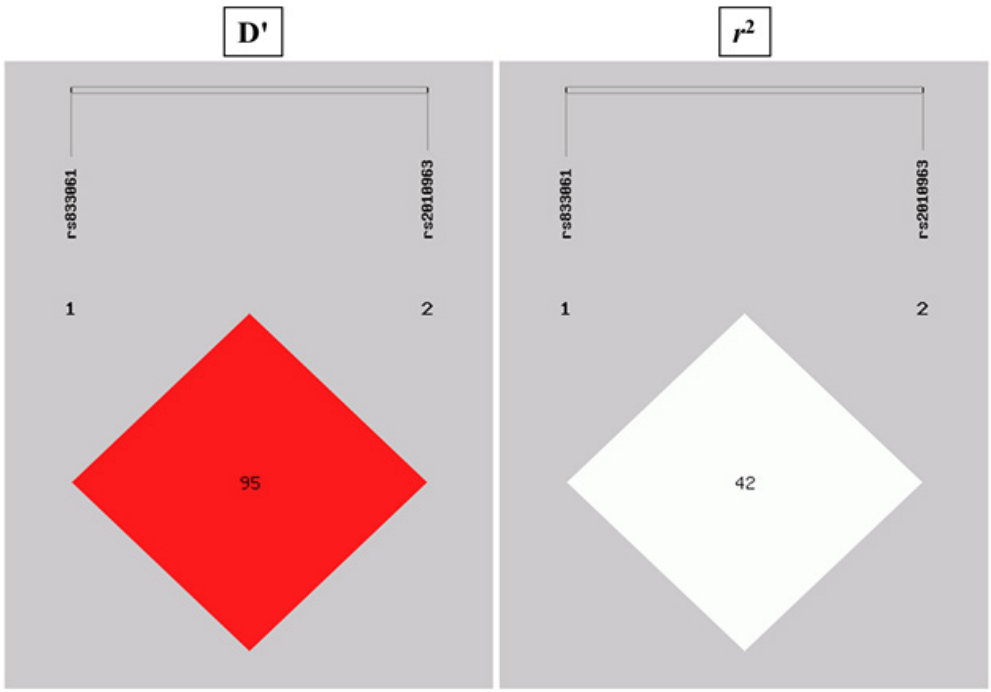

Figure 1. Linkage disequilibrium (LD) test of *VEGF* gene rs833061 and rs2010963 polymorphisms in systemic lupus erythematosus (SLE) patients. The left and right diagrams represent the D′ and r^2 values, respectively. The values within each square, ranging from 0% to 100%, exhibit the score of the related LD measurement (D′ or r^2). The greater the score, the higher the possibility of two alleles of different positions to be inherited simultaneously (linkage disequilibrium). Thereupon, the D′ or r^2 score confers the possibility, by which two alleles might impress the SLE risk.

Table 4. . Association of VEGF gene rs833061 and rs2010963 SNPs with clinicopathological manifestations in SLE patients.

Characteristic	rs833061			*P* value	rs2010963			*P* value
	CC	CT	TT		CC	CG	GG	
Sex; Female/Male	21 (5.25%)/2 (0.5%)	82 (20.5%)/5 (1.25%)	248 (62%)/42 (10.5%)	0.103	65 (16.25%)/30 (7.5%)	141 (35.25%)/20 (5%)	145 (36.25%)/34 (8.5%)	0.301
Serositis	7 (1.81%)	36 (9.04%)	17 (4.22%)	0.641	10 (2.38%)	21.43 (5.36%)	26.19 (6.55%)	0.295
Malar rash	31 (7.83%)	113 (28.31%)	55 (13.86%)	0.453	29 (7.14%)	90 (22.62%)	79 (19.64%)	0.202
Palmar Erythema	2 (0.6%)	12 (3.01%)	7 (1.81%)	0.708	5 (1.19%)	7 (1.79%)	10 (2.38%)	-
Vascular Ulcers	2 (0.6%)	29 (7.23%)	10 (2.41%)	0.356	5 (1.19%)	14 (3.57%)	21 (5.36%)	0.204
Discoid rash	10 (2.41%)	22 (5.42%)	12 (3.01%)	0.515	5 (1.19%)	17 (4.17%)	24 (5.95%)	0.185
Mucosal lesion	24 (6.02%)	51 (12.56%)	48 (12.05%)	0.127	31 (7.47%)	57 (14.29%)	36 (8.93%)	0.0604
Photosensitivity	19 (4.81%)	123 (30.72%)	65 (16.27%)	0.209	74 (18.45%)	169 (42.26%)	157 (39.29%)	0.127
Arthritis	43 (10.84%)	166 (41.57%)	104 (25.9%)	0.672	54 (14.29%)	14 (35.12%)	117 (29.17%)	0.192
Aseptic necrosis	0 (0%)	7 (1.8%)	5 (1.2%)	0.567	5 (1.19%)	2 (0.6%)	5 (1.19%)	0.170
Muscle weakness	10 (2.41%)	17 (4.22%)	17 (4.22%)	0.376	10 (2.38%)	17 (4.17%)	17 (4.17%)	0.318
Raised Muscle Enzyme	0 (0%)	12 (3.01%)	7 (1.81%)	0.485	2 (0.6%)	12 (2.98%)	5 (1.19%)	0.212
Myositis in Muscle Biopsy	0 (0%)	7 (1.81%)	5 (1.2%)	0.567	0 (0%)	10 (2.38%)	2 (0.6%)	0.109
Urine cast	14 (3.61%)	19 (4.82%)	17 (4.22%)	0.139	12 (2.98%)	14 (3.57%)	26 (6.55%)	0.144
Hematuria	22 (5.42%)	84 (21.08%)	51 (12.65%)	0.719	31 (7.47%)	57 (14.29%)	69 (17.26%)	0.189
Raised Creatinine	5 (1.2%)	27 (6.63%)	10 (2.41%)	0.559	5 (1.19%)	19 (4.76%)	19 (4.76%)	0.277
Hypertension	10 (2.41%)	41 (10.24%)	12 (3.01%)	0.347	7 (1.79%)	36 (8.93%)	24 (5.95%)	0.165
Pericarditis	2 (0.6%)	19 (4.82%)	10 (2.41%)	0.619	5 (1.19%)	10 (2.38%)	14 (3.57%)	0.279
Cardiomyopathy	2 (0.6%)	5 (1.2%)	0 (0%)	0.409	0 (0%)	2 (0.6%)	5 (1.19%)	-
Libman–Sacks endocarditis	0 (0%)	2 (0.6%)	2 (0.6%)	0.744	2 (0.6%)	0 (0%)	2 (0.6%)	0.451
Valvular lesion	10 (2.41%)	12 (3.01%)	10 (2.41%)	0.230	5 (1.19%)	7 (1.79%)	19 (4.76%)	0.112
Ischemic heart disease	2 (0.6%)	5 (1.2%)	0 (0%)	0.409	0 (0%)	5 (1.19%)	5 (1.19%)	0.255
Raynaud's phenomenon	9.63 (2.41%)	29 (7.23%)	7 (1.81%)	0.290	5 (1.19%)	19 (4.76%)	26 (6.55%)	0.165
Thrombophlebitis	0 (0%)	5 (1.2%)	5 (1.2%)	0.545	2 (0.6%)	2 (0.6%)	5 (1.19%)	0.291
Pleuritis	7 (1.81%)	29 (7.23%)	7 (1.81%)	0.368	5 (1.19%)	19 (4.76%)	19 (4.76%)	0.277
Lupus pneumonitis	2 (0.6%)	7 (1.81%)	2 (0.6%)	0.646	0 (0%)	7 (1.79%)	5 (1.19%)	0.221
Interstitial fibrosis	2 (0.6%)	5 (1.2%)	0 (0%)	0.409	0 (0%)	5 (1.19%)	2 (0.6%)	0.246
Pulmonary hypertension	0 (0%)	5 (1.2%)	2 (0.6%)	0.625	2 (0.6%)	2 (0.6%)	2 (0.6%)	0.295

Characteristic	rs833061			*P* value	rs2010963			*P* value
	CC	CT	TT		CC	CG	GG	
Embolism	0 (0%)	2 (0.6%)	5 (1.2%)	0.423	2 (0.6%)	7 (1.79%)	0 (0%)	0.125
Convulsion	7 (1.81%)	12 (3.01%)	14 (3.61%)	0.380	5 (1.19%)	14 (3.57%)	14 (3.57%)	0.331
Psychosis	0 (0%)	7 (1.81%)	7 (1.81%)	0.464	2 (0.6%)	12 (2.98%)	0 (0%)	0.0502
Peripheral neuropathy	2 (0.6%)	2 (0.6%)	0 (0%)	0.327	0 (0%)	2 (0.6%)	2 (0.6%)	0.229
Central Nervous system involvement	7 (1.81%)	12 (3.01%)	12 (3.01%)	0.442	12 (2.98%)	10 (2.38%)	10 (2.38%)	0.078
Raised liver enzyme	26 (6.63%)	65 (16.27%)	51 (12.65%)	0.283	26 (6.55%)	55 (13.69%)	60 (14.88%)	0.295
Hepatitis	2 (0.6%)	7 (1.8%)	0 (0%)	0.394	0 (0%)	2 (0.6%)	7 (1.79%)	0.148
sicca	0 (0%)	2 (0.6%)	2 (0.6%)	0.825	71 (17.86%)	168 (42.26%)	157 (39.29%)	0.108
Retinitis	2 (0.6%)	5 (1.2%)	0 (0%)	0.409	0 (0%)	0 (0%)	7 (1.79%)	0.058
Leukopenia	19 (4.82%)	58 (14.45%)	43 (10.84%)	0.540	29 (7.14%)	50 (12.5%)	48 (11.9%)	0.233
Lymphopenia	24 (3.61%)	46 (11.44%)	34 (8.43%)	0.618	19 (4.76%)	38 (9.52%)	45 (11.31%)	0.271
Thrombocytopenia	19 (4.82%)	39 (9.64%)	34 (8.43%)	0.238	21 (5.36%)	26 (6.55%)	43 (10.71%)	0.083
Anemia	19 (4.82%)	51 (12.65%)	41 (10.24%)	0.418	29 (7.14%)	40 (10.12%)	48 (11.9%)	0.132
Hemolytic anemia	5 (1.2%)	24 (6.02%)	14 (3.61%)	0.695	7 (1.79%)	17 (4.17%)	19 (4.76%)	0.325
Coombs test	5 (1.2%)	19 (4.82%)	12 (3.01%)	0.717	7 (1.79%)	14 (3.57%)	14 (3.57%)	0.342
CRP	17 (4.22%)	96 (24.1%)	51 (12.65%)	0.439	33 (8.33%)	69 (17.26%)	60 (14.88%)	0.278
FANA	55 (13.86%)	212 (53.01%)	128 (31.93%)	0.567	71 (17.86%)	167 (41.67%)	157 (39.29%)	0.214
Anti-dsDNA	48 (12.05%)	183 (45.78%)	108 (27.11%)	0.710	69 (17.26%)	133 (33.33%)	138 (34.52%)	< 0.001
Anti-SSA	0 (0%)	14 (3.61%)	5 (1.2%)	0.394	5 (1.19%)	14 (3.57%)	0 (0%)	0.001
Anti-SSB	0 (0%)	10 (2.41%)	2 (0.6%)	0.449	2 (0.6%)	10 (2.38%)	0 (0%)	0.084
Anti-malaria	55 (13.86%)	214 (53.61%)	125 (31.33%)	0.380	71 (17.86%)	167 (41.67%)	157 (39.29%)	0.214
Anti- Phospholipid	2 (0.6%)	31 (7.83%)	7 (1.81%)	0.200	5 (1.19%)	14 (3.57%)	21 (5.36%)	0.204
Anti-SSAB	0 (0%)	14 (3.61%)	5 (1.2%)	0.394	5 (1.19%)	14 (3.57%)	0 (0%)	0.001
Anticardiolipin-IgG	2 (0.06%)	22 (5.42%)	5 (1.2%)	0.354	5 (1.19%)	10 (2.38%)	14 (3.57%)	0.279
Anticardiolipin-IgM	0 (0%)	5 (1.2%)	2 (0.6%)	0.625	2 (0.6%)	2 (0.6%)	2 (0.6%)	0.430
Lupus anticoagulant	0 (0%)	10 (2.41%)	0 (0%)	0.232	0 (0%)	7 (1.79%)	2 (0.6%)	0.171
Anti-B2GP1 IgG	0 (0%)	12 (3.01%)	2 (0.6%)	0.352	0 (0%)	5 (1.79%)	10 (2.38%)	0.145
Anti-B2GP1 IgM	0 (0%)	5 (1.2%)	2 (0.6%)	0.625	0 (0%)	2 (0.6%)	5 (1.19%)	0.230
Increased complement level	34 (0.43%)	133 (33.13%)	77 (19.28%)	0.714	40 (10.11%)	100 (25%)	105 (26.19%)	0.227
Musculoskeletal manifestations	46 (11.45%)	166 (41.57%)	106 (26.51%)	0.588	60 (14.88%)	140 (35.12%)	119 (29.76%)	0.212

Characteristic	rs833061			*P* value	rs2010963			*P* value
	CC	CT	TT		CC	CG	GG	
Renal Manifestations	31 (7.83%)	125 (31.33%)	82 (20.48%)	0.614	50 (12.5%)	102 (25.6%)	83 (20.83%)	0.138
Cardiac Manifestations	12 (3.01%)	34 (8.43%)	17 (4.22%)	0.563	7 (1.79%)	21 (3.36%)	33 (8.33%)	0.126
Pulmonary Manifestations	7 (1.81%)	46 (11.45%)	17 (4.22%)	0.408	10 (2.38%)	38 (9.52%)	24 (5.95%)	0.182
Neuropsychiatric Manifestations	12 (3.01%)	31 (7.83%)	24 (6.02%)	0.566	14 (3.57%)	31 (7.47%)	21 (5.36%)	0.262
Hepatic Manifestations	27 (3.63%)	65 (16.27%)	51 (12.65%)	0.283	26 (6.55%)	55 (13.69%)	60 (14.88%)	0.295
Ophthalmologic Manifestation	2 (0.6%)	5 (1.2%)	0 (0%)	0.409	0 (0%)	0 (0%)	7 (1.79%)	0.058
Hematologic Manifestations	34 (8.43%)	111 (27.71%)	72 (18.07%)	0.584	45 (11.31%)	83 (20.83%)	93 (23.21%)	0.157
Cutaneous manifestations	39 (9.64%)	169 (42.17%)	92 (22%)	0.494	45 (11.31%)	133 (33.33%)	119 (29.76%)	0.128

Discussion

SLE is defined as a multisystemic autoimmune disorder, and autoantibodies developed against several nuclear antigens have been implicated in its pathogenesis [22]. It has been reported that a systemic chronic inflammatory condition in SLE leads to damage in vascular endothelial cells, which in turn culminates in a marked promotion of angiogenic factors synthesis. These factors are involved in vascular permeability, vascular growth, and inflammatory response, resulting in a disruption of vascular network and impairment of several internal organs, which finally represents with complications like kidney and skin dysfunctions [23].

It has been reported that the *VEGF* gene plays a role in the normal function of lung and kidney and confers a surviving mediator in the neuronal cells [24]. SNPs in angiogenesis-regulating genes may impress the response toward angiogenic factors and, thereupon, modulate the proneness, initiation, and perpetuation of angiogenesis-associated disorders. Multiple SNPs have been discovered in the upstream and 5'-untranslated regions (5'-UTR) of the *VEGF* gene due to the existence of highly polymorphic sites [16, 25]. These polymorphisms may affect protein expression or specific disease status [17]. Moreover, SNPs in the *VEGF* and *VEGFR2* genes have been associated with the development of angiogenesis-dependent diseases [26]. The *VEGF* level has been reported to be highly heritable [27], and its polymorphisms include at least three SNPs that have been observed to impress the expression of *VEGF* mRNA. It has been observed that the SNPs at the −2549 (rs35569394) position located in the promoter region and the −634G/C (rs2010963) SNP found in the 5′-UTR are associated with overexpression of *VEGF* [28]. Furthermore, the 936C/T (rs3025039) SNP located in the 3′-UTR was associated with a significantly higher VEGF level in serum [28]. Tang et al. tried to perform a meta-analysis to disclose the association between the *VEGF* gene 634G/C (rs2010963) polymorphism and VEGF serum levels with SLE proneness. They found that the VEGF level was associated with increased SLE risk as well as with active SLE risk. They further observed that the VEGF level was associated with the development of lupus nephritis risk in SLE patients [29].

With respect to the studies implying the role of VEGF in the modulation of angiogenesis and endothelial cell proliferation and function, a limited number of studies have surveyed the possible association of *VEGF* gene polymorphisms with SLE susceptibility as well as its clinical presentations. In line with this, a study tried to investigate the association of the *VEGF* gene G1612A (rs10434) polymorphism with lupus-related neuropsychiatric manifestations in SLE patients. It was found that the AA genotype of rs10434 was significantly more prevalent in SLE patients with neuropsychiatric manifestations than in the subjects without this manifestation; hence, the AA genotype conferred a susceptibility risk of developing neuropsychiatric manifestations in SLE patients [19]. Moreover, it was found that the C allele *VEGF* gene rs2010963 SNP was associated with the development of SLE, while the G allele of this polymorphism conferred a protective effect in SLE susceptibility. Moreover, G allele and GG genotypes of rs1570360 were associated with an increased risk of lupus nephritis development [30]. Conversely, rs2010963 SNP was not identified in a recent meta-analysis to be associated with SLE susceptibility [29]. In addition, a meta-analysis of an rs2010963 association with chronic immune-mediated inflammatory diseases resulted in no significant association [31]. In the current study, the association of rs833061 and rs2010963 SNPs with SLE susceptibility were analyzed, and it was observed that none of the alleles, genotypes, or haplotypes of these polymorphisms were involved in the disease risk. This was the first study to evaluate the association of rs833061 SNP with SLE susceptibility.

Nonetheless, neither the rs833061 nor the rs2010963 polymorphism was associated with RA susceptibility in a previous study by the authors in the Iranian population [18]. Moreover, the data testified to a relation between the genotypes of the *VEGFA* gene rs2010963 SNP and the clinical data of SLE subjects, such as anti-dsDNA, anti-SSA, and anti-SSAB. However, the authors' previous study indicated an association between the genotypes of the *VEGFA* gene rs833061 SNP and rheumatoid factor (RF) in RA patients.

Conclusion

Considering all the facts, the present study intended to investigate the association of *VEGFA* gene polymorphisms, including rs833061 and rs2010963, in SLE susceptibility in the Iranian population. It was observed that none of the alleles, genotypes, and haplotypes of these SNPs were associated with the risk of SLE development, despite an LD according to only D′ value. Although rs2010963 has not been associated with SLE in previous studies or in other populations, this was the first report of an rs833061 association in SLE that needs to be further investigated in different ethnicities.

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Conflict of Interest

The authors declare no conflicts of interest.

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