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RESEARCH ARTICLE

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FOXP3 gene polymorphisms increase the risk of systemic lupus erythematosus in a Han Chinese population

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ABSTRACT

Background: FOXP3 is a transcription factor that regulates the development and function of Treg, playing an essential role in preventing autoimmune diseases. Variation in *FOXP3* can impair the function of Treg cells, thus destroying their inhibitory capacity and leading to autoimmune diseases. This paper investigated whether the three SNPs in the *FOXP3* gene (-3279 C/A, -924 A/G and -6054 del/ATT) are associated with systemic lupus erythematosus (SLE) susceptibility in the Han Chinese population.

Materials and methods: The study cohort comprised 122 SLE patients and 268 healthy controls. Genotyping was performed by polymerase chain reaction sequence-specific primer (PCR-SSP). Furthermore, we examined the potential clinical manifestations associated with *FOXP3* polymorphisms in SLE patients.

Results: The results showed that the -3279 (C > A) was significantly associated with the SLE risk in a homozygote (OR = 3.24, 95% CI = 1.23–8.52, p = .013, AA vs. CC), dominant (OR = 1.68, 95% CI = 1.07–2.65, p = .025, AC + AA vs. CC), recessive (OR = 2.90, 95% CI = 1.12–7.55, p = .023, AA vs. AC + CC) and allelic (OR = 1.72, 95% CI = 1.18–2.53, p = .005, A vs. C) models. In addition, -924 (A > G) was positively associated with SLE risk in the heterozygote (OR = 1.66, 95% CI = 1.04–2.66, p = .033, AG vs. AA) and dominant (OR = 1.59, 95% CI = 1.01–2.49, p = .042, AG + GG vs. AA) models, whereas -6054 (del > ATT) was not associated with SLE. Moreover, the immunological index analysis suggested that decreased complement C4 occurred more frequently in SLE patients carrying the minor allele (A) -3279 (C > A) than those not (p = .005).

Conclusions: We demonstrated that -3279 (C > A) and -924 (A > G) were associated with an increased risk of SLE and the immunological index, indicating that the *FOXP3* variation is potentially related to the occurrence and development of SLE.

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FOXP3; systemic lupus erythematosus; polymorphism; immune index

1. Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune condition characterized by developing autoantibodies targeting nuclear antigens and forming immune complexes [1]. These immune complexes, which can activate the complement system, impact various organ systems such as skin, eyes, kidneys, heart, joints, and central and peripheral nervous systems [2–5]. The production of autoantibodies results from multiple immunological dysregulations, including abnormalities in the regulation of B and T cells, breakdown of immune self-tolerance, and impaired clearance of apoptotic cells and immune complexes [6]. Notably, autoreactive $CD4^+$ T cells are involved in stimulating B cell differentiation, proliferation and maturation, leading to increased autoantibody production, which is pivotal in the initiation and progression of SLE [7,8].

A subset of T lymphocytes with suppressive functions is crucial in maintaining self-tolerance [9]. Specifically, $CD4^+CD25^+$ T cells significantly prevent autoimmune reactions and undesirable responses from auto-reactive T cells [10]. Besides, the transcription

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factor forkhead box P3 (FOXP3) is pivotal in governing the development and differentiation of $CD4^+CD25^+$ Tregs. Previous investigations involving promoter activity analysis have revealed that variations in the FOXP3 gene promoter region can impact promoter activities, decrease FOXP3 mRNA expression, and eventually reduce FOXP3 protein expression [11]. Then, these reduced FOXP3 expressions in Tregs alter the frequency of Tregs and reduce Tregs' suppressive function [12,13]. Studies have demonstrated a significant decrease in $CD4^+CD25^+$ Tregs in individuals with active SLE [14]. Another study suggests that the immune-suppressive activities of Tregs with attenuated Foxp3 expression were nearly abolished *in vitro* and *in vivo* [15]. Thus, we hypothesize that single nucleotide polymorphisms (SNPs) within the FOXP3 gene promoter region may contribute to changes in the quantity and function of Tregs and susceptibility to SLE.

Although the FOXP3 gene polymorphism has been identified as a potential risk factor for SLE, the reported findings have been controversial [6,11,16–18]. For instance, one study indicated that the -3279 (C > A) variant in the FOXP3 gene is linked to SLE in the Brazilian population [6], while another study did not find an association between this variant and SLE susceptibility [17]. Additionally, variants such as -924 (A > G) and -6054 (del > ATT) within the FOXP3 promoter region have been strongly linked to various autoimmune diseases like multiple sclerosis (MS), rheumatoid arthritis [19,20], immune thrombocytopenia [21], ulcerative colitis [22] and vitiligo [23,24], but have not shown an association with SLE susceptibility due to limited available data [6]. It is important to note that these SNPs vary across racial and ethnic groups. Therefore, this study aimed to evaluate possible associations of three SNPs in the promoter of the *FOXP3* gene with SLE susceptibility. In addition, correlations between susceptible SNPs genotypes and immunologic markers were established to evaluate the influence of SNPs variation on clinical manifestations.

2. Materials and methods

2.1. Study sample

The case-control study involved 130 SLE patients and 280 healthy controls of northern Chinese Han origin from the Department of Rheumatology and Immunology, the First Affiliated Hospital of Henan University of Science and Technology. Demographic and clinical characteristics of patients with SLE were obtained, such as anti-nuclear antibody (ANA), anti-double-stranded DNA (anti-dsDNA) antibodies, erythrocyte sedimentation rate (ESR), hypersensitivity C-reactive protein (hs-CRP) and complement C3 and C4. All patients met the diagnostic criteria of SLE according to the SLE classification criteria revised by the 1997 American College of Rheumatology (ACR) [25]. Exclusion criteria: (1) associated with other autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, primary Sjogren's syndrome and argyria arthritis. (2) SLE patients with systemic infection. (3) Patients with cardiac, respiratory and renal diseases due to causes other than SLE [26]. (4) Patients with other malignant tumours, neurodegenerative diseases and mental diseases. (5) Pregnant or lactating women. Participants who were free of SLE or other autoimmune conditions were selected as healthy volunteers and paired based on age and gender with individuals diagnosed with SLE. This study was approved by the Ethics Committee of The First Affiliated Hospital of Henan University of Science and Technology (2023-03-K0049), and written consent was obtained from all participants. The study was conducted following the Declaration of Helsinki.

2.2. Sample collection and DNA extraction

Peripheral venous blood was collected from each individual into a tube containing 50 mM EDTA. Genomic DNA was extracted from the whole blood using the classic isopropyl alcohol precipitation method, which is particularly effective for isolating genomic DNA from larger blood samples compared to the adsorption column technique. An ultra-fine ultraviolet spectrophotometer determined DNA purity and concentration. The extracted DNA was stored in a refrigerator at −80 °C.

2.3. Genotyping

Genotyping of the three SNPs (-3279 C/A, -924 A/G and -6054 del/ATT) was performed using the polymerase chain reaction sequence-specific primers (PCR-SSPs). Table 1 reports the primer sequences for genotyping [27]. Gradient PCR was used to determine the optimal annealing temperature, and PCR was performed in a 20 µL volume containing 50 ng DNA, 0.8 µM each of forward and reverse primers, and 10 µL 2× MasterMix. The PCR protocol comprised an initial denaturation step at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s and extension at 72 °C for 40 s, with a final elongation of 72 °C for 5 min. The products of the PCR reactions were analysed through electrophoresis with 2%

Table 1. Primers used in the genotyping by PCR-SSP.

SNP	Allele	Forward primer	Reverse primer	Product size (bp)
−3279 (C > A)	A	5′-CTGGCTCTCTCCCCAACTGA-3′	5′-ACAGAGCCCATCATCAGACTCTCTA-3′	334
rs3761548	C	5′-TGGCTCTCTCCCCAACTGC-3′	5′-ACAGAGCCCATCATCAGACTCTCTA-3′	333
−924 (A > G)	A	5′-CCCAGCTCAAGAGACCCCA-3′	5′-GGGCTAGTGAGGAGGCTATTGTAAC-3′	442
s2232365	G	5′-CCAGCTCAAGAGACCCCG-3′	5′-GCTATTGTAACAGTCCTGGCAAGTG-3′	427
−6054 (del > ATT)	del	5′-ACCTTTAAGTCTTCTGCCATTTATTCTATTATTT-3′	5′-TGATTATCAGCGCACACACTCAT-3′	358
rs5902434	ATT	5′-CCTTTAAGTCTTCTGCCATTTATTCTATTATTA-3′	5′-TGATTATCAGCGCACACACTCAT-3′	356

agarose gels visualized by ethidium bromide staining at 0.5 mg/mL under an ultraviolet illuminator.

2.4. Statistical analysis

Five inheritance models were tested for each SNP, including the heterozygote, homozygote, dominant, recessive and allele models. The Hardy–Weinberg equilibrium (HWE) was evaluated in the control group using Pearson's Chi-square test, with an HWE analysis with a p value exceeding .05, suggesting that the population under study is representative [28]. Besides, the Chi-square test was employed to compare the distribution frequencies of genotypes and alleles, with a significance level set at $p < .05$ [29], aiming to evaluate the association between *FOXP3* polymorphisms (-3279 C/A, -924 A/G and -6054 del/ATT) and SLE risk [30]. Furthermore, gender-based subgroup analysis was performed to investigate the potential gender-specific associations of FOXP3 polymorphisms with SLE susceptibility. The calculated linkage disequilibrium (LD) was between paired SNPs and expressed as D', where $D' = 1$ corresponds to complete LD, while $D' = 0$ indicates no LD. The haplotype analysis and LD testing was based on the online SHEsis (http://analysis.bio-x.cn/myAnalysis.php). SNP–SNP interactions were studied using the generalized multifactor dimensionality reduction (MDR) model with three SNPs [31], and functional annotation was assessed by the Encyclopedia of DNA Elements tool HaploReg v4.2 [32] to predict the potential SNP functions. The risk associated with individual genotypes or alleles was calculated as the odds ratio (OR) with 95% confidence intervals (95% CIs). For all analyses, $p < .05$ was considered statistically significant, and all data statistics used SPSS 26.0 (IBM SPSS Inc., Armonk, NY).

3. Results

3.1. Patient characteristics

Eight (6.15%) patients were not included in the study due to loss of follow-up, and 12 (4.29%) controls were excluded because they did not match the age and gender ratio of the cases. Consequently, 122 (93.85%) patients and 268 (95.71%) controls were included in the final data analyses. Table 2 reports the demographic and immunological indicators of all patients. Among the patients, there were 110 females (90.2%) and 12 males (9.8%), the sex ratio was 9:1 (female:male), and the average age was 36.5 ± 12.8. In the controls, the proportion of females and males was almost the same, i.e. 90.3/9.7. Around 44.3% of patients were positive for anti-ds-DNA antibody, 91.8% with ANA, 36.9% patients with decreased complement C3, 64.4% patients with decreased complement C4, 46.7% patients with increased ESR and 16.4% patients with increased hs-CRP.

Table 2. Demographic characteristics of patients with systemic lupus erythematosus.

Characteristics		SLE, *n* (%)
Gender (female:male)		9:1
Age (years)		36.5 ± 12.8
Anti-dsDNA Ab	Negative	68 (55.7%)
	Positive	54 (44.3%)
AnA	Negative	10 (8.2%)
	Positive	112 (91.8%)
C3	Normal	73 (59.8%)
	Decreased	45 (36.9%)
C4	Normal	42 (35.6%)
	Decreased	76 (64.4%)
hs-CRP	Increased	20 (16.4%)
	Normal	102 (83.6%)
ESR	Increased	57 (46.7%)
	Normal	65 (53.3%)

Anti-dsDNA: anti-double-stranded DNA; AnA: anti-nucleosome antibody; C3: complement 3; C4: Complement 4; hs-CRP: hypersensitive C-reactive protein; ESR: erythrocyte sedimentation rate.
Data are expressed as mean, standard deviation and percentage (%).

3.2. Hardy–Weinberg's equilibrium analysis

The three SNPs of FOXP3, including -3279 (C > A), -924 (A > G) and -6054 (del > ATT), were genotyped in 122 patients and 268 healthy controls to evaluate association with SLE susceptibility. Gradient PCR was used to determine the optimal annealing temperature. Finally, we set the annealing temperature to 61 °C and found that annealing at elevated temperatures can cause a decrease in yield or the absence of the desired product, while annealing at excessively low temperatures may

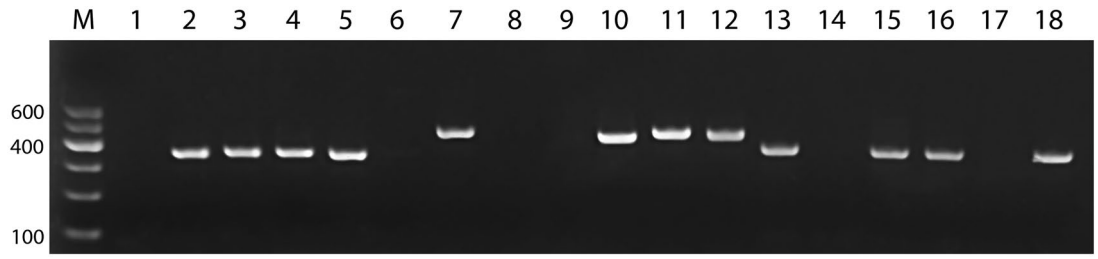


Figure 1. Typical band patterns of PCR-SSP. The band pattern for rs3761548 (C > A), lanes 1 and 2: CC genotype; lanes 3 and 4: AC genotype; lanes 5 and 6: AA genotype; the band pattern for rs2232365 (A > G), lanes 7 and 8: AA genotype; lanes 9 and 10: GG genotype; lanes 11 and 12: AG genotype. The band pattern for rs5902434 (del > ATT), lanes 13 and 14: homozygous del genotype; lanes 15 and 16: heterozygous del/ATT genotype; lanes 17 and 18: homozygous ATT genotype. Lane M shows a 600 bp DNA marker.

Table 3. Distribution of genotype and allele frequencies of the FOXP3 polymorphisms in SLE patients and controls.

SNP	Genotype	SLE, *n* (%)	Controls, *n* (%)	*p* Value	*p*/HWE
−3279 (C > A)	CC	76 (62.3)	197 (73.5)	.022	.47
rs3761548	AC	36 (29.5)	63 (23.5)		
	AA	10 (8.2)	8 (3.0)		
	C	188 (77.0)	457 (85.3)	.005	
	A	56 (23.0)	79 (14.7)		
−924 (A > G)	AA	40 (32.8)	117 (43.6)	.102	.66
rs2232365	AG	66 (54.1)	116 (43.3)		
	GG	16 (13.1)	35 (13.1)		
	A	146 (59.8)	350 (65.3)	.142	
	G	98 (40.2)	186 (34.7)		
−6054	del/del	40 (32.8)	114 (42.5)	.174	.11
(del > ATT)	del/ATT	60 (49.2)	109 (40.7)		
rs5902434	ATT/ATT	22 (18.0)	45 (16.8)		
	del	140 (57.4)	337 (63.0)	.144	
	ATT	104 (42.6)	199 (37.0)		

HWE: Hardy–Weinberg equilibrium.

lead to non-specific amplification. Figure 1 illustrates representative photographs where ambiguous genotypes were repeated. Notably, 10% of the samples were randomly selected and sequenced by Sangon Biotech (Shanghai, China) to ensure that results were consistent with genotyping. The genotype frequencies of FOXP3 in the healthy controls followed HWE ($p > .05$).

3.3. Genotype and allele frequencies distribution of the FOXP3 polymorphisms in SLE patients and controls

Tables 3 and 4 present the genotype and allele frequency distributions of FOXP3 polymorphisms in patients and controls and their association with SLE risk. The genotype and allele frequencies of FOXP3 -3279 (C > A) differed significantly between patients and controls ($p = .02$; $p = .005$; Table 3). Besides, the susceptible A allele frequency increased in patients compared to controls (23.0% vs. 14.7%; Table 4). In the dominant model, the presence of the AC + AA genotype in cases was significantly higher than in controls (37.7% vs. 26.5%, $p = .025$). When the recessive model was evaluated, the frequency of AA in cases was significantly lower than in controls (8.2% vs. 3.0%, $p = .023$). Therefore, -3279 (C > A) was significantly associated with the SLE risk in homozygotes (OR = 3.24, 95% CI = 1.23–8.52, $p = .013$, AA vs. CC), dominant (OR = 1.68, 95% CI = 1.07–2.65, $p = .025$, AC + AA vs. CC), recessive (OR = 2.90, 95% CI = 1.12–7.55, $p = .023$, AA vs. AC + CC) and allelic (OR = 1.72, 95% CI = 1.18–2.53, $p = .006$, A vs. C) models.

As for FOXP3 -924 (A > G), no statistical differences were observed in the distribution of the FOXP3 genotype and the allele frequency between patients with SLE and healthy controls. However, the frequency of the susceptible AG genotype increased in patients compared to controls (54.1% vs. 43.3%; Table 4) in the heterozygote model (OR = 1.66, 95% CI = 1.04–2.66, $p = .033$, AG vs. AA). In the dominant model, the

Table 4. Association analysis of the *FOXP3* polymorphisms with susceptibility to SLE.

			Case/control (122/268)	
SNP	Genetic model	Genotype	OR (95% CI)	*p* Value
−3279 (C > A)	Heterozygote	AC vs. CC	1.48 (0.91–2.41)	.113
rs3761548	Homozygote	AA vs. CC	3.24 (1.23–8.52)	**.013**
	Dominant	AC + AA vs. CC	1.68 (1.07–2.65)	**.025**
	Recessive	AA vs. AC + CC	2.90 (1.12–7.55)	**.023**
	Allele	A vs. C	1.72 (1.18–2.53)	**.005**
−924 (A > G)	Heterozygote	AG vs. AA	1.66 (1.04–2.66)	**.033**
rs2232365	Homozygote	GG vs. AA	1.34 (0.67–2.67)	.410
	Dominant	AG + GG vs. AA	1.59 (1.01–2.49)	**.042**
	Recessive	GG vs. AG + AA	1.00 (0.53–1.90)	.988
	Allele	G vs. A	1.26 (0.93–1.73)	.142
−6054 (del > ATT)	Heterozygote	del/ATT vs. del/del	1.57 (0.97–2.53)	.064
rs5902434	Homozygote	ATT/ATT vs. del/del	1.39 (0.75–2.60)	.297
	Dominant	del/ATT + ATT/ATT vs. del/del	1.52 (0.97–2.38)	.068
	Recessive	ATT/ATT vs. del/ATT + del/del	1.09 (0.62–1.91)	.763
	Allele	ATT vs. del	1.26 (0.92–1.71)	.144

n: the number of patients/controls; OR: odds ratio; CI: confidence interval; *p* values < 0.05 were considered as statistically significant.

presence of the AG + GG genotype was higher in patients with SLE when compared to controls (67.2%) versus (56.4%) respectively, suggesting that carrying the AG + GG genotype affects the susceptibility to develop SLE (OR = 1.59, 95% CI = 1.01–2.49, p = .042).

Regarding the -6054 (del > ATT) variant, there was no significant difference in the distribution of the FOXP3 genotype and allele frequency between cases and controls (p = .07; p = .14). No significant association between -6054 (del > ATT) and SLE risk was found in the five genetic models.

3.4. Analyses stratified by gender

It is acknowledged that SLE primarily affects women. To comprehensively investigate the influence of gender on our research findings, a subgroup analysis was performed based on gender to investigate potential gender-specific associations of FOXP3 polymorphisms with susceptibility to SLE. The results demonstrated that only -3279 (C > A) was significantly associated with the SLE risk in females in homozygotes (OR = 3.65, 95% CI = 1.12–11.87, p = .073, AA vs. CC), dominant (OR = 1.78, 95% CI = 1.09–2.91, p = .020, AC + AA vs. CC), recessive (OR = 3.22, 95% CI = 1.00–10.39, p = .039, AA vs. AC + CC) and allelic (OR = 1.80, 95% CI = 1.18–2.74, p = .006, A vs. C) models (Table 5).

3.5. Association of FOXP3 haplotype with SLE risk

Linkage disequilibrium analysis was performed for the three SNPs, as depicted in Figure 2, which revealed a strong LD among -3279 C/A, -924 A/G and -6054 del/ATT (D' > 0.6). In the association study of *FOXP3* haplotype, the frequency of the -3279 A/-924G/-6054ATT haplotype was significantly higher in cases (18.7% vs. 10.8%, OR = 1.91, 95% CI = 1.25–2.92; p = .002, Table 6), which increased the risk of SLE. In addition, the frequency of -3279 C/-924A/-6054ATT in controls was higher than in the SLE (1.1% vs. 8.2%, OR = 0.13, 95% CI = 0.04–0.44; p < .001), suggesting that it was negatively associated with the risk of SLE and had a protective effect against the disease. There was a significant reduction in the frequency of -3279 C/-924G/-6054del in patients compared to controls (0.4% vs. 6.5%, OR = 0.06, 95% CI = 0.01–0.44; p < .001).

Table 5. Subgroup analysis based on gender.

			Case/control (110/242)	
SNP	Genetic model	Genotype	OR (95% CI)	p Value
−3279 (C > A)	Heterozygous	AC vs. CC	1.60 (0.95–2.69)	.073
rs3761548	Homozygous	AA vs. CC	3.65 (1.12–11.87)	**.023**
	Dominant	AC + AA vs. CC	1.78 (1.09–2.91)	**.020**
	Recessive	AA vs. AC + CC	3.22 (1.00–10.39)	**.039**
	Allele	A vs. C	1.80 (1.18–2.74)	**.006**
−924 (A > G)	Heterozygous	AG vs. AA	1.57 (0.97–2.55)	.067
rs2232365	Homozygous	GG vs. AA	1.20 (0.56–2.58)	.645
	Dominant	AG + GG vs. AA	1.49 (0.94–2.38)	.091
	Recessive	GG vs. AG + AA	0.94 (0.46–1.92)	.856
	Allele	G vs. A	1.22 (0.87–1.69)	.249
−6054 (del > ATT)	Heterozygous	del/ATT vs. del/del	1.53 (0.93–2.52)	.093
rs5902434	Homozygous	ATT/ATT vs. del/del	1.24 (0.63–2.40)	.535
	Dominant	del/ATT + ATT/ATT vs. del/del	1.44 (0.91–2.30)	.122
	Recessive	ATT/ATT vs. del/ATT + del/del	0.99 (0.54–1.82)	.969
	Allele	ATT vs. del	1.20 (0.87–1.66)	.275

p values < 0.05 were considered as statistically significant.

3.6. Analyses stratified by immunologic marker

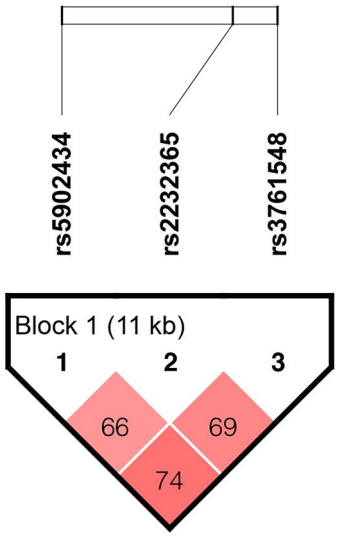
When the genetically defined subgroups with special immunologic characteristics (including AnA, anti-dsDNA, ESR, hs-CRP and C3 and C4 levels) were analysed, lower serum complement C4 levels occurred more frequently (80.0%) in patients carrying -3279 AC + AA genotype (p = .005) than the patients with CC genotype (51.9%) (Table 7). As for FOXP3 -924 (A > G) and -6054 (del > ATT), those SNPs were not associated with immunological indices. Although -6054 (del > ATT) has been associated with other autoimmune diseases [30], the genetic mutations alone do not have much effect [33]. Since a combined analysis can better understand the role of FOXP3 variants in SLE, we studied the association of FOXP3 haplotype with immunological indices. However, no significant association was found between FOXP3 haplotypes and special immunologic markers.

3.7. SNP–SNP interaction analysis

Table 8 presents the details of the SNP–SNP interactions in SLE patients. The generalized MDR analysis of SNP–SNP interactions indicated that the two-locus model (-924 A/G, -6054 del/ATT) and three-locus model (-3279 C/A, -924 A/G and -6054 del/ATT) had 10/10 cross-valid consistency and high-test accuracy when compared with the one-locus model. The statistical test set in the two-locus model (p < .0001) and three-locus model were highly significant, followed by the one-locus model of SNP–SNP interaction (p < .05). This indicates a strong SNP–SNP interaction in the two-locus and three-locus models for SLE risk among the three SNPs of FOXP3.

3.8. Functional annotation

The results revealed that the variants -3279 C/A, -924 A/G and -6054 del/ATT were identified as enhancer



L1	L2	D'	r^2
rs5902434	rs2232365	0.663	0.396
rs5902434	rs3761548	0.696	0.158
rs2232365	rs3761548	0.743	0.161

Haplotype	Frequency (%)
del/A/A	54.1
ATT/G/C	16.5
ATT/G/A	10.8
ATT/A/C	8.2
del/G/C	6.5
ATT/A/A	1.6
del/A/A	1.5

Figure 2. Linkage disequilibrium analysis between the three SNPs of *FOXP3*. The value in the LD block represents *D'* as a percentage. The redder the colour, the stronger the linkage. Seven common haplotypes for these polymorphisms were constructed.

Table 6. Frequency distribution of FOXP3 haplotype in SLE patients and controls.

Haplotype	SLE (%)	Control (%)	*p* Value	OR (95% CI)
−3279A/-924G/-6054ATT	18.7	10.8	**.002**	1.91 (1.25–2.92)
−3279C/-924A/-6054del	54.5	54.1	.870	1.03 (0.75–1.40)
−3279C/-924A/-6054ATT	1.1	8.2	**<.001**	0.13 (0.04–0.44)
−3279C/-924G/-6054del	0.4	6.5	**<.001**	0.06 (0.01–0.44)
−3279C/-924G/-6054ATT	21.0	16.5	.123	1.35 (0.92–1.99)

Controls and cases with a frequency of <0.03 were discarded and ignored in the analysis. *p* values < 0.05 were considered as statistically significant.

histone marks, and the variants -3279 C/A and -6054 del/ATT were identified as promoter histone marks. In addition, -3279 C/A was related to the motif change for some genes (Table 9).

4. Discussion

SLE is distinguished by autoantibodies, produced due to various immunological changes, such as the breakdown of immune self-tolerance. $CD4^+CD25^+$ T cells, the subset of T lymphocytes exhibiting negative regulatory functions, are instrumental in maintaining self-tolerance and preventing autoimmune reactions and undesirable responses from auto-reactive T cells [9,10]. The transcription factor FOXP3 is pivotal in governing the development and activation of Tregs. Variation in the promoter region of *FOXP3* could downregulate the FOXP3 protein expression and eventually impair the suppressive function of Treg cells [34,35]. Therefore, SNPs within the promoter region of the FOXP3 gene may contribute to the susceptibility to SLE. Numerous studies have indicated that variations in the FOXP3 gene, specifically those found in the regulatory regions, have been linked to various autoimmune conditions, including asthma [36], type I diabetes (TID) [37], MS [38] and rheumatoid arthritis [19]. It has been observed that several autoimmune disorders may have overlapping genetic factors. Consequently, our study aimed to examine the potential correlation between three specific FOXP3 promoter region SNPs (-3279 C/A, -924 A/G and -6054 del/ATT) and the susceptibility to SLE.

In this study, -3279 (C > A) was significantly associated with SLE susceptibility. The A allele and AA genotype of the *FOXP3* promoter -3279 increased the risk of SLE. Considering these SNPs located in the promoter region, the -3279 AA genotype can accelerate the severity of SLE [39]. Previous studies found that the mutation from C to A at the position of -3279 reduced the relative luciferase activity of the Foxp3 promoter, decreased FOXP3 mRNA expression and eventually reduced FOXP3 protein expression [40]. Another study showed that SNPs located in the promoter regions of the FOXP3 gene could modulate the relative gene expression, and the allelic variants are associated with a reduced expression of the relative genes in alopecia areata patients [41]. In addition, one study found that the immune-suppressive activities of T cells with attenuated Foxp3 expression were nearly abolished *in vitro* and *in vivo* [15]. The frequency of the -924 AG + GG genotype was significantly higher than that of healthy controls, indicating that the AG + GG genotype was susceptible to SLE, while -6054 (del > ATT) was not associated with SLE susceptibility. Similarly, a team from Iran [42] investigated the relationship between *FOXP3* promoter SNPs (−3279 C/A) and SLE and found that the -924 (A > G) AA

Table 7. Association analysis of the FOXP3 polymorphisms with special immunologic characteristics in SLE patients.

	−3279 (C > A), *n* (%)			−924 (A > G), *n* (%)			−6054 (del > ATT), *n* (%)		
	CC	AC + AA	*p* Value	AA	AG + GG	*p* Value	del/del	del/ATT + ATT/ATT	*p* Value
Characteristics	77 (63.1%)	45 (36.9%)		46 (37.7%)	76 (62.3%)		47 (38.5%)	75 (61.5%)	
Anti-dsDNA Ab	32 (41.6%)	22 (48.9%)	.432	23 (50.0%)	31 (40.8%)	.321	23 (48.9%)	31 (41.3%)	.411
ANA	71 (92.2%)	41 (91.9%)	>.999	42 (91.3%)	70 (92.1%)	>.999	43 (91.5%)	69 (92.0%)	>.999
Decreased C3	27 (35.1%)	18 (40.0%)	.743	21 (45.7%)	24 (31.6%)	.098	20 (42.6%)	25 (33.3%)	.268
Decreased C4	40 (51.9%)	36 (80.0%)	**.005**	27 (58.7%)	49 (64.5%)	.594	26 (55.3%)	50 (66.7%)	.238
Increased hs-CRP	12 (15.6%)	8 (17.8%)	.752	11 (23.9%)	9 (11.8%)	.081	10 (21.3%)	10 (13.3%)	.249
Increased ESR	34 (44.2%)	23 (51.1%)	.458	18 (39.1%)	39 (51.3%)	.191	17 (36.2%)	40 (53.3%)	.064

p values < 0.05 were considered as statistically significant.

Table 8. Determination of SLE risk by SNP–SNP interaction analysis.

Model no.	Best combination of genes	Training accuracy	Testing accuracy	CVC	*p* Value	Total sensitivity	Total specificity	χ^2	OR (95% CI)	*F*-measure	Kappa
1	S1	0.5614	0.4967	6/10	.0424	0.6721	0.4366	4.1186	1.59 (1.01–2.49)	0.4620	0.0871
2	S1, S2	0.6254	0.6085	10/10	<.0001	0.6148	0.6343	21.0736	2.77 (1.78–4.30)	0.5085	0.2236
3	S1, S2, S3	0.6603	0.6301	10/10	<.0001	0.6557	0.6642	34.8949	3.77 (2.40–5.92)	0.5479	0.2890

S1: -924 (A > G); S2: -6054 (del > ATT); S3: -3279 (C > A); CVC: CV consistency; OR: odds ratio.

Table 9. Functional annotation for genetic variants that have an association with SLE risk.

Variant	Gene	Promoter histone marks	Enhancer histone marks	DNase	Proteins bound	Motifs changed	NHGRI/EBI GWAS hits	GRASP QTL hits	Selected eQTL hits
−3279 (C > A)	FOXP3	BLD, THYM	BLD	–	–	DEC, Egr1, Myb	–	–	–
−924 (A > G)	FOXP3	–	BLD, THYM	–	–	–	–	–	–
−6054 (del > ATT)	FOXP3	BLD	BLD, THYM	–	–	–	–	–	–

DNase: deoxyribonuclease; GWAS: genome-wide association study; QTL: quantitative trait locus; eQTL: expression quantitative trait locus.

genotype is associated with disease susceptibility in male patients with SLE. However, another research team [6] found no significant differences between SLE patients and controls in rs2232365 genotype and allele frequencies. Meanwhile, the FOXP3 haplotype (-3279 C/A and -924 A/G) is associated with SLE susceptibility. A subgroup analysis was performed based on gender in our study, and we found that only -3279 (C > A) was significantly associated with the SLE risk in females. Nevertheless, the study only included 12 male patients and 26 male controls, and as a result, subgroup analysis based on gender was not performed for the male participants. Differences in allele/genotype frequency between studies can be attributed to differences in the heterogeneity of the diseases studied and the ethnicity.

Haplotype analysis was performed on the three SNPs to study better the role of the FOXP3 gene polymorphism in SLE. The -3279 A/-924G/-6054ATT haplotype was associated with SLE susceptibility, while the -3279 C/-924A/-6054ATT haplotype was protective against the disease. However, no significant association was found between various FOXP3 haplotypes and immunological markers in SLE patients. Furthermore, the immunological index analysis suggests that lower serum complement C4 levels occurred more frequently in patients carrying -3279 AC + AA genotype than the patients with CC genotype, while FOXP3 -924 A/G and -6054 (del > ATT) were not associated with immunological indices. In contrast to our findings, Lin et al. [17] found that lower levels of anti-dsDNA antibodies in women carrying the -3279 A allele and the -6054 ATT allele were associated with developing lupus nephritis in women with SLE. Similarly, Qiu et al. evaluated the association of 17 SNP polymorphisms with SLE, including -3279 (C > A). They found that different alleles can affect promoter activity and corresponding serum factor levels in patients with SLE, revealing that SLE-related SNPs play an important role in the pathogenesis of SLE [11].

The clinical presentation of SLE is variable. In human studies, Treg production or maintenance is impaired in SLE patients, suggesting that this feature is correlated with SLE disease activity and autoantibody titres [9]. FOXP3 gene mutation results in reduced production or dysfunction of Treg cells, increased expansion of autoreactive T cells, and accelerated production of autoantibodies and inflammatory cytokines [43]. Complement activation can cause tissue inflammation and damage, which plays a key role in the pathogenesis of SLE [44]. Early complement components are involved in clearing

immune complexes and apoptotic debris, and defects in complement components (such as C4 and C1q) often lead to the development of SLE [45]. Studies have found that the -3279 (C > A) CC genotype is associated with decreased complement C4 and increased ESR. The -924 (A > G) variation was significantly associated with increased hs-CRP levels, and patients with the -924 AA genotype had higher hs-CRP levels. Studies have shown that high levels of hs-CRP and ESR are associated with disease activities and major damage to organs or systems [46]. However, the role of hs-CRP in systemic SLE remains controversial. Barnes et al. [47] found no association between hs-CRP and SLE disease activity. These data suggest a relationship between *FOXP3* SNPs and disease presentation and pathology. In addition, most SLE patients have changes in immunological markers when the disease is active, inactive or mild, and anti-dsDNA antibodies, cytokines and complement levels fluctuate significantly during SLE. Zhang et al. [48] found that the proportion of Tregs in patients with active SLE was significantly lower than in non-active SLE patients, suggesting that Treg cell depletion accelerates disease progression.

Limitations of our study include examining only three SNP loci within the FOXP3 gene, which may not fully elucidate the genetic complexity of the gene and its involvement in the immune response in SLE. Additionally, variations in the expression levels of immune markers and FOXP3 mRNA may occur in different disease activity states, suggesting the need for future investigations involving diverse disease activity levels. Furthermore, the small sample size utilized in this study underscores the necessity for larger sample sizes in future research, which should also consider the influence of environmental factors, diverse ethnicities and gender to validate the current findings. In addition, further replicative association studies in different populations and functional characterization of these SNPs would be essential to draw unequivocal conclusions on the roles of the FOXP3 gene in SLE pathogenesis and progression.

5. Conclusions

In conclusion, the results suggest that the *FOXP3* -3279 (C > A) and -924 (A > G) gene polymorphism are associated with an increased risk of SLE. The study indicates that the Foxp3 SNPs may significantly impact the development of SLE and could serve as a potential target for immunotherapy in SLE. Consequently, it is imperative to undertake functional investigations to explore the influence of genetic variations/SNPs on the expression or functions of relevant genes associated with SLE and advance our understanding of its pathogenesis.

Author contributions

Conceptualization, S.D., X.S. and R.L.; methodology, R.L. and S.D.; software, S.D. and L.Z.; validation, S.D. and J.W.; formal analysis, S.D.; resources, R.L.; data curation, S.D. and L.Z.; writing – original draft preparation, S.D.; writing – review and editing, X.S. and R.L.; funding acquisition, X.S. and R.L. The final version of the manuscript to be published is approved by all authors, and all authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical statement

This study was approved by the Ethics Committee of The First Affiliated Hospital of Henan University of Science and Technology (2023-03-K0049), and written consent was obtained from all participants.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

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