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The rs3918188 and rs1799983 loci of eNOS gene are associated with susceptibility in patients with systemic lupus erythematosus in Northeast China

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To investigate the association between single nucleotide polymorphism (SNP) at the rs3918188, rs1799983 and rs1007311 loci of the endothelial nitric oxide synthase (*eNOS*) gene and genetic susceptibility to systemic lupus erythematosus (SLE) in northeastern China. The base distribution of *eNOS* gene rs3918188, rs1799983 and rs1007311 in 1712 human peripheral blood samples from Northeast China was detected by SNaPshot sequencing technology. The correlation between genotype, allele and gene model of these loci of the *eNOS* gene and the genetic susceptibility to SLE was investigated by logistic regression analysis. The results of the differences in the frequency distribution of their gene models were visualised using R 4.3.2 software. Finally, HaploView 4.2 software was used to analyse the relationship between the haplotypes of the three loci mentioned above and the genetic susceptibility to SLE. A multifactor dimensionality reduction (MDR) analysis was used to determine the best SNP-SNP interaction model. The CC genotype and C allele at the rs3918188 locus may be a risk factor for SLE (CC vs AA: OR = 1.827, $P < 0.05$; C vs A: OR = 1.558, $P < 0.001$), and this locus increased the risk of SLE in the dominant model and the recessive model (AC + CC vs AA: OR = 1.542, $P < 0.05$; CC vs AA + AC: OR = 1.707, $P < 0.001$), while the risk of SLE was reduced in the overdominant model (AC vs AA + CC: OR = 0.628, $P < 0.001$). The GT genotype and T allele at locus rs1799983 may be a protective factor for SLE (GT vs GG: OR = 0.328, $P < 0.001$; T vs G: OR = 0.438, $P < 0.001$) and this locus reduced the risk of SLE in the overdominant model (GT vs GG + TT: OR = 0.385, $P < 0.001$). There is a strong linkage disequilibrium between the rs1007311 and rs1799983 loci of the eNOS gene. Among them, the formed haplotype AG increased the risk of SLE compared to GG. AT and GT decreased the risk of SLE compared to GG. In this study, the *eNOS* gene rs3918188 and rs1799983 loci were found to be associated with susceptibility to SLE. This helps to deeply explore the mechanism of eNOS gene and genetic susceptibility to SLE. It provides a certain research basis for the subsequent exploration of the molecular mechanism of these loci and SLE, as well as the early diagnosis, treatment and prognosis of SLE.

Keywords Systemic lupus erythematosus, eNOS, Single nucleotide polymorphism, Northeast China

Systemic lupus erythematosus (SLE) is an autoimmune disease[1] characterised by devastating systemic organ involvement that can lead to decreased organ function and increased morbidity and mortality[2]. The global incidence of SLE is relatively high[3], among which the prevalence of systemic lupus erythematosus in China ranks second in the world[4]. SLE imposes a heavy burden on society and families[5]. Despite significant advances in the treatment of SLE, its mortality rate remains high[6]. The most common causes of death are kidney disease, cardiovascular and other systemic diseases, and infections[7]. The pathogenesis of SLE has not been elucidated.

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It is currently believed that SLE is mainly due to genetic and environmental influences[8]. With the development of molecular biology, many studies have confirmed that genetic susceptibility has an important impact on the pathogenesis of SLE[9–11]. Single nucleotide polymorphism (SNP) is a commonly used marker to understand disease susceptibility[12]. In GWAS studies, accurate estimation of SNP heritability can help us to better understand the extent to which the measured genetic variation affects the phenotype[13].

A literature review identified more than 100 SLE susceptibility loci in East Asian and European populations[14]. The endothelial nitric oxide synthase (*eNOS*) gene has been reported to be one of the candidate genes for SLE susceptibility[10,11,15]. *eNOS* catalyzes the production of nitric oxide, and the process of nitric oxide production is closely related to the pathogenesis of SLE[16]. The *eNOS* gene 7q35-36 is 21 kb in length and has 26 exons and 25 introns. There are about 10 polymorphic sites distributed in the promoter, exons and introns of the *eNOS*[17]. Among these loci, the common mutations that result in amino acid substitutions in the mature protein are the G894T or Glu298Asp (rs1799983) mutations, where a G base substitution to T would result in the substitution of glutamate (Glu) by aspartic acid (Asp) at position 298 of the corresponding amino acid in exon 7[18]. There are a number of common SNP loci on the *eNOS* gene such as rs1808593 has been found to have an important role in the development of pediatric SLE and central nervous system complications[19], but the exact mechanism is not clear.

In this study, we screened three common SNPs (rs3918188, rs1799983 and rs1007311) in the *eNOS* gene with minor allele frequencies greater than 0.05 using the NCBI (https://www.ncbi.nlm.nih.gov/snp/) database. We investigated the correlation between three SNPs in the *eNOS* gene and susceptibility to SLE in patients with SLE in northeastern China and in healthy individuals. Among these SNPs, the rs1799983 locus has been studied and reported to be strongly associated with hypertension[17], intracranial aneurysm[20], and obesity[21]. In addition, the rs1799983 locus was not associated with genetic susceptibility to SLE in Brazilian[22], southeastern Iranian[23], and Arabian[24] populations. Considering that genetic polymorphisms are associated with environmental geographic ethnicity, there is also a lack of studies on the association of this locus with SLE susceptibility in northeastern China. Therefore, it is of great interest to study the association between this locus and SLE susceptibility in northeastern China. In addition to the rs1799983 locus, the susceptibility of the other two loci to SLE patients has rarely been reported. rs3918188 is located in the promoter region[25], and it has been found to be susceptible to T2DM[26]. rs1007311 has been reported to have a potential association with chronic mountain sickness[27]. Since the polymorphisms at the rs3918188, rs1799983 and rs1007311 loci of the *eNOS* gene are not clearly associated with genetic susceptibility to SLE in northeastern China. Therefore, the present study used the above three loci of the *eNOS* gene in northeastern China as an entry point to investigate whether they might be associated with the genetic susceptibility of SLE patients.

Materials and methods

Subjects of the study

A total of 856 patients with SLE and 856 healthy individuals of Northeastern origin who attended the First Affiliated Hospital of Harbin Medical University from January 2020 to December 2022 were collected and divided into the SLE group and the control group. When recruiting SLE patients for this study, our team continuously recruited the control group until the number of controls matched the number of SLE patients. The study was ethically approved by the Ethics Committee of Hainan Medical College (approval number: HYLL-2017-001) and all study subjects signed an informed consent form. SLE group inclusion criteria: Age 18–70 years, both male and female; those who met the 2019 European League Against Rheumatism (EULAR/ACR) diagnostic criteria for SLE; those who signed the informed consent form. Inclusion criteria for the control group: those aged 18–70 years, both male and female; those with a normal health examination; those without substantial pathology of major organs such as heart, brain, liver, kidney and lung; those who signed the informed consent form. Exclusion criteria: Patients with overlapping other rheumatic diseases such as rheumatoid arthritis, scleroderma and polymyositis; patients with combined serious primary diseases of the circulatory, urinary and haematopoietic systems; patients with combined serious metabolic disorders, patients with tumours; professional athletes, pregnant and lactating women and women who are menstruating; those who do not wish to participate in this study.

Polymerase chain reaction (PCR) reactions

Primers were designed with reference to the literature[28,29] and the primer sequences for the target SNP fragments are shown in Table 1. Reaction system: DNA mold 1 μ, 10*buffer 1.5 μL, $MgCL_2$ (25 mmol/L) 1.5 μL, dNTP (10 mmol/L) 0.3 μL, primers (10 μmol/L) 0.15 μL/strip, Taqase (5 u/μL) 0.3 μL, ultrapure water made up to 15 μL.

Target loci	Primer orientation	Primer sequences (5'→3')	Expected product size (bp)
rs3918188	F	ATCGTTCTTGCTAACTCTGGC	472
	R	TACTTCACTGAGACTGAAGGG	
rs1007311	F	TGGAGATGAAGGCAGGAGACAG	255
	R	TCAATCCCTTTGGTGCTCACG	
rs1799983	F	TGGAGATGAAGGCAGGAGACAG	255
	R	CAGTCAATCCCTTTGGTGCT	

Table 1. Primers for PCR reactions.

Reaction conditions: Predenaturation at 94 ℃ for 3 min, denaturation at 94 ℃ for 15 s, annealing at 55 ℃ for 15 s, extension at 72 ℃ for 30 s, cycling for 35 cycles, terminal extension at 72 ℃ for 3 min, and ultra-pure water was supplemented to 15 μL. At the end of the reaction, 3 μL of PCR product was taken from each spot, mixed, placed on ice, purified by adding 0.2 μL of nucleic acid exonuclease I and 0.5 μL of alkaline phosphatase, and reacted for 15 min at 37 ℃, followed by 15 min at 80 ℃. The remaining primers in the reaction products were removed primarily with nucleic acid exonuclease I and the remaining dNTP in the reaction was removed with alkaline phosphatase. The above products are stored in the refrigerator at − 20 ℃.

Detection of SNPs at target loci using SNaPshot

SNaPshot sequencing reaction primers were designed according to the sequences of the target loci, as shown in Table 2. SNaPshot sequencing reaction system: PCR amplification purification product 2 μL, Snapshot Mix reagent 1 μL, extension primer (10 μmol/L) 0.2 μL/strip, ddH_2O make up to 6 μL. Reaction conditions: Predenaturation at 96 ℃ for 1 min, predenaturation at 96 ℃ for 10 s, annealing at 52 ℃ for 5 s, extension at 60 ℃ for 30 s, and cycling for 30 cycles. After the reaction, the reaction products were placed on ice, mixed with 0.2 μL alkaline phosphatase and purified, reacted at 37 ℃ for 60 min, and then reacted at 75 ℃ for 15 min, mainly using alkaline phosphatase to remove the remaining dNTP in the reaction. After purification, 1 μL of each SNP loci was taken, 9 μL of HIDI was added and mixed well, and the reaction was allowed to stand at 95 ℃ for 3 min, followed by an ice-water bath and electrophoretic sequencing. The remaining products were stored in a refrigerator at 4 ℃.

Statistical analyses

In this study, two researchers (Huitao Wu and Qi Zhang) independently entered the data separately, and the data were verified and organised. The *t*-test and χ^2 test were used to compare the general information between the two groups. The Hardy–Weinberg law of genetic equilibrium was used to test the goodness of genetic stability of the samples selected for this study, and the differences in the frequency distributions of genotypes, alleles, and gene models of the *eNOS* gene at the rs3918188, rs1799983, and rs1007311 loci were analysed by logistic regression, and the results were expressed as odds ratio (OR) and 95% Confidence Interval (CI), and results with $P < 0.05$ were considered statistically significant. Finally, HaploView 4.2 software was used for chain imbalance analysis as well as haplotype analysis. In addition, we used multifactor dimensionality reduction (MDR) analysis to determine the best SNP-SNP interaction model. we did not match for age and gender when conducting the study.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Hainan Medical College (HYLL-2017–001).

Informed consent

Informed consent was obtained from all subjects involved in the study.

Results

General clinical information

This study included 856 patients with SLE and 856 healthy controls in northeastern China. The mean age of the control group was 34.81 ± 10.8 years, with 89 males (10.4%) and 767 females (89.6%). The mean age of the SLE group was 35.28 ± 14.2 years, with 78 (9.1%) males and 778 (90.9%) females. There was no statistical difference between the two groups in terms of age and gender ($P > 0.05$), as shown in Table 3.

Hardy–Weinberg law of genetic equilibrium test

The distribution of genotypes at the rs3918188, rs1799983, and rs1007311 loci of the *eNOS* gene in northeastern China among the controls all conformed to the Hardy–Weinberg law of genetic equilibrium ($P > 0.05$).

Distribution of genotypes and allele frequencies in Northeast China

In SLE patients from northeastern China, the CC genotype and C allele frequency at the rs3918188 locus were significantly higher compared with controls (CC vs AA: OR = 1.827, 95% CI 1.225–2.725, $P < 0.05$; C vs A: OR = 1.558, 95% CI 1.321–1.836, $P < 0.001$), which may be a risk factor for SLE. The GT genotype and T allele frequency at the rs1799983 locus were significantly lower compared to controls (GT vs GG: OR = 0.328, 95% CI 0.292–0.501, $P < 0.001$; T vs G: OR = 0.438, 95% CI 0.334–0.558, $P < 0.001$), which may be a protective factor for SLE. The polymorphism of rs1007311 was not associated with genetic susceptibility to SLE in either genotype or allele ($P > 0.05$). As shown in Table 4. We performed multiple corrections for comparisons of genotypes using

Target loci	Chromosome location	Polymorphism	Extension product	Primer orientation	Primer length (bp)	Primer sequences
rs3918188	151005693	[A/C]	AC	F	42	TTTTTTTTTTTTTTTTTTTTGAGCAAGGCA CACGTACAAGGG
rs1007311	150998920	[A/G]	AG	F	22	AGGGCATGAGGCTCAGCCCCAG
rs1799983	150999023	[G/T]	GT	F	42	TTTTTTTTTTTTTTTTTTTTGCTGCTGCA GGCCCCAGATGA

Table 2. Primers for SNaPshot sequencing reactions.

Item		Control	SLE	P. value
Age, years (mean ± SD)		34.81 ± 10.8	35.28 ± 14.2	0.442^{ns}
Sex [n (%)]	Male	89 (10.4%)	78 (9.1%)	0.370^{ns}
	Female	767 (89.6%)	778 (90.9%)	
Skin erythema [n (%)]		–	324 (37.9%)	–
Arthritis [n (%)]		–	278 (32.5%)	–
Alopecia [n (%)]		–	252 (29.4%)	–
Thrombocytopenia [n (%)]		–	592 (69.2%)	–
ANA (+) [n (%)]		–	242 (28.3%)	–
Anti-dsDNA antibody (+) [n (%)]		–	186 (21.7%)	–
Anti-Sm antibody (+) [n (%)]		–	128 (15.0%)	–
Anti-SSA antibody (+) [n (%)]		–	292 (34.1%)	–
Low levels of C3 [n (%)]		–	126 (14.7%)	–
Low levels of C4 [n (%)]		–	140 (16.4)	–

Table 3. Demographics and clinical characteristics of participants. *ns* no statistical difference, *SD* standard deviation, *ANA* anti nuclear antibody, *Anti dsDNA antibody* anti double stranded DNA antibody, *Anti Sm antibody* anti Smith antibody, *Anti-SSA antibody* anti-Sj gren syndrome A antibody.

Target loci	Genotype	Control (%)	SLE (%)	P. value	OR (95% CI)
rs3918188	AA	66 (7.7)	44 (5.1)	1	1
	AC	304 (35.5)	220 (25.7)	0.701	1.086 (0.714–1.651)
	CC	486 (56.8)	592 (69.2)	0.003**	1.827 (1.225–2.725)
	A	436 (25.5)	308 (18.0)	1	1
	C	127 (74.5)	1404 (82.0)	< 0.001***	1.558 (1.321–1.836)
rs1007311	AA	230 (26.9)	248 (29.0)	1	1
	AG	441 (51.5)	438 (51.2)	0.47	0.921 (0.737–1.151)
	GG	185 (21.6)	170 (19.9)	0.254	0.254 (0.852–0.647)
	A	901 (52.6)	934 (54.6)	1	1
	G	811 (47.4)	778 (45.4)	0.258	0.925 (0.809–1.058)
rs1799983	GG	644 (75.2)	758 (88.6)	1	1
	GT	200 (23.4)	90 (10.5)	< 0.001***	0.328 (0.292–0.501)
	TT	12 (1.4)	8 (0.9)	0.216	0.556 (0.23–1.394)
	G	1488 (86.6)	1606 (93.8)	1	1
	T	224 (13.4)	106 (6.2)	< 0.001***	0.438 (0.334–0.558)

Table 4. Distribution of genotypes and allele frequencies of control and SLE groups in Northeast China. "***" means P < 0.001, "**" means P < 0.01.

the Bonferroni method. The results showed that the genotype frequency distribution of rs3918188 CC (vs AA) and rs1799983 GT (vs GG) remained different between the healthy control and SLE groups.

Comparison of gene models in Northeast China

The rs3918188 locus had an elevated risk of SLE development in AC + CC gene carriers compared to AA gene carriers in the dominant model (AC + CC vs AA: OR = 1.542, 95% CI 1.04–2.286, $P < 0.05$); in the recessive model, CC gene carriers had an elevated risk of SLE occurrence compared to AA + AC: OR = 1.707, 95% CI 1.40–2.082, $P < 0.001$); and in the overdominant model, AC gene carriers had a reduced risk of SLE compared to AA + CC gene carriers (AC vs AA + CC: OR = 0.628, 95% CI 0.51–0.773, $P < 0.001$). As shown in Fig. 1.

The rs1799983 locus was associated with a reduced risk of SLE development in GT gene carriers compared to gene carriers of GG + TT in the overdominant model (GT vs GG + TT: OR = 0.385, 95% CI 0.294–0.505, $P < 0.001$); and was not associated with genetic susceptibility to SLE in the recessive model and the dominant model were not associated with genetic susceptibility to SLE ($P > 0.05$). The polymorphism of rs1007311 was not associated with susceptibility to SLE in any of the three models ($P > 0.05$). As illustrated in Fig. 1.

Haplotype analysis

After using Haplo View 4.2 software for haplotype construction and linkage disequilibrium analysis of the three SNP loci on the *eNOS* gene, an LD map consisting of rs1007311 and rs1799983 was constructed (shown in Fig. 2). Strong linkage disequilibrium was regarded as strong with $r^2 > 80\%$[30]. The difference in the frequency

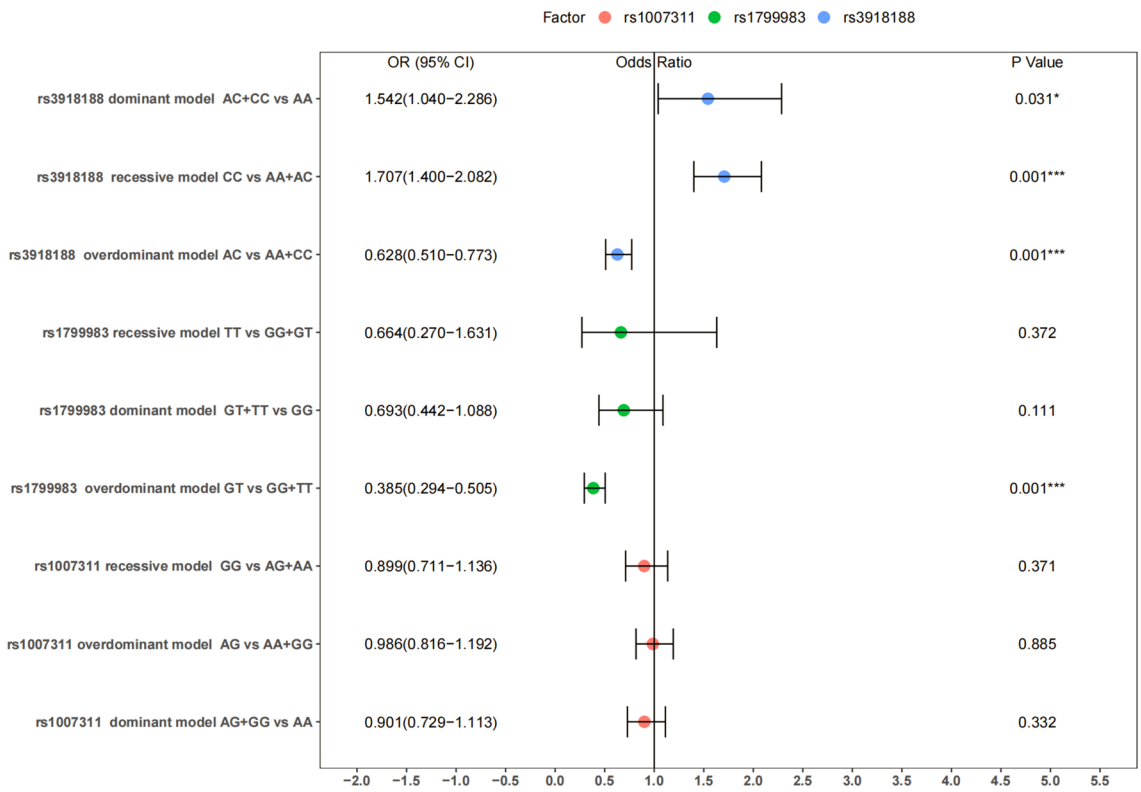

Fig. 1. Comparison of genetic models of SLE patients in Northeast China.

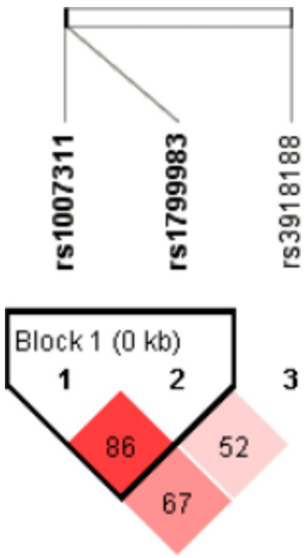

Fig. 2. Gene single nucleotide polymorphisms were analysed for linkage disequilibrium (unnumbered red squares show complete LD. Larger numbers in the squares indicate a higher degree of LD. *LD* linkage disequilibrium).

of haplotypes between the SLE group and the control group was analysed. The results showed that there was a strong linkage disequilibrium between rs1007311 and rs1799983, and the two SNPs formed haplotypes GG, AG, AT and GT. The haplotype AG individuals formed had an increased risk of SLE (AG vs GG: OR = 1.230, 95% CI 1.068–1.416, $P < 0.01$), and the haplotype AT individuals formed had a decreased risk of SLE (AT vs GG: OR = 0.505, 95% CI 0.391–0.654, $P < 0.001$), and the haplotype GT formed had a decreased risk of SLE (GT vs GG: OR = 0.241, 95% CI 0.081–0.721, $P < 0.001$), as shown in Table 5.

Haplotype	SLE (%)	Control (%)	P. value	OR (95% CI)
GG	774 (45.2)	794 (46.4)	1	–
AG	832 (48.6)	694 (40.5)	< 0.01**	1.230 (1.068–1.416)
AT	102 (6.0)	207 (12.1)	< 0.001***	0.505 (0.391–0.654)
GT	4 (0.2)	17 (1.0)	< 0.05*	0.241 (0.081–0.721)

Table 5. Haplotype analysis of the population in Northeast China. *OR* odds ratio, *CI* confidence interval. "*" means $P < 0.05$, "**" means $P < 0.01$ "***" means $P < 0.001$.

MDR analysis for eNOS variants

The MDR method was used to analyze the interactions between *eNOS* SNPs and SLE risk. The results of the MDR model analysis of SNP-SNP interactions are shown in Table 6 and Fig. 3 below. Strongly interacting loci are very close to each other in branching, while weakly interacting loci are far away from each other. The best single-digit point model is rs1799983 (test accuracy: 0.5584, $P < 0.001$, cross-validation consistency (CVC): 9/10); the best two-digit point models are rs1799983 and rs3918188 (test accuracy: 0.6086, $P < 0.001$, CVC: 10/10); the best of the three-locus model was rs1007311, rs1799983 and rs3918188 (test accuracy: 0.6209, $P < 0.001$, CVC: 10/10), which was the most effective SNP-SNP interaction model.

Discussion

SLE is a systemic autoimmune disease[31], which is characterised by immune dysregulation and often leads to multi-system and multi-organ involvement[32]. The etiology of SLE has both genetic and environmental components and is a multifactorial disease[33]. At present, the important influence of genetic susceptibility on the development of SLE has been demonstrated[9,34,35]. For example, IFN-α was found to inhibit NO production in insulin-stimulated human umbilical vein endothelial cells, which in turn caused endothelial dysfunction in patients with systemic lupus erythematosus[36]. *eNOS* gene have several SNP sites on them, some of which have been reported to be associated with SLE[28]. For example, Shiva et al[37]. included 504 subjects in a case–control study and found that the 27-bp VNTR (4b/a) gene polymorphism in the *eNOS* gene may be a significant risk factor for the development of SLE in South Indian subjects. To further explore the relationship between the rs3918188, rs1799983 and rs1007311 loci of the *eNOS* gene and genetic susceptibility to SLE, we collected a cumulative total of 1712 population samples from northeastern China for SNP analysis.

Firstly, we performed the Hardy–Weinberg law of genetic equilibrium test. After confirming that all three loci were in balance, logistic regression analysis of genotype, allele, and gene model frequency was performed. In this study, we found that rs1799983 was associated with susceptibility to SLE in a population in northeast China, and the frequency of GT genotype and T allele carried by SLE patients was significantly reduced compared with the control group. In addition, in the overdominant model, GT gene carriers had a reduced risk of SLE compared with GG + TT gene carriers. We found that the GT genotype and T allele of the rs1799983 locus may be protective factors for systemic lupus erythematosus. In a overdominant model, this locus reduced the

Model	Training bal. acc. (%)	Testing bal. acc. (%)	CVC	OR (95% CI)	P. value
rs1799983	0.5670	0.5584	9/10	2.5462 (1.9604, 3.3070)	< 0.001
rs1799983, rs3918188	0.6123	0.6086	10/10	2.4911 (2.0509, 3.0257)	< 0.001
rs1007311, rs1799983, rs3918188	0.6294	0.6209	10/10	3.9855 (3.1532, 5.0377)	< 0.001

Table 6. Impact of SNP-SNP interaction on SLE risk.

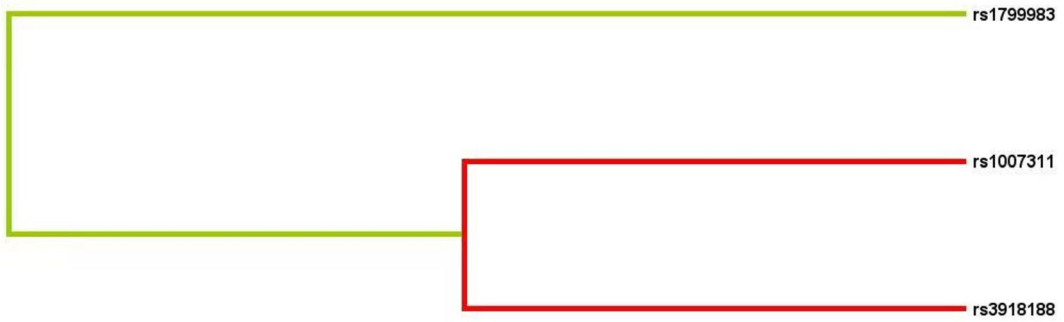

Fig. 3. Dendrogram of the effect of *eNOS* SNP-SNP interactions on SLE risk.

risk of SLE. Previously, many scholars have studied the association between rs1799983 polymorphism and susceptibility to SLE. Some studies have found that this loci is not associated with genetic susceptibility to SLE in Brazil[22], Southeast Iran[23], and Arabia[24]. Interestingly, our study found that this loci was associated with SLE genetic susceptibility, suggesting that the genetic susceptibility of this loci and SLE may be affected by different regions and populations[38]. Lee YH et al.[24] found by a Meta-analysis that the TT + TG genotype at the rs1799983 locus reduced the risk of SLE compared to GG in an Asian population. However, this effect was not observed for the TT + TG genotype compared to GG in the dominant model we studied. Interestingly, in the overdominant model, we observed that the GT genotype at the rs1799983 locus reduced the risk of developing SLE compared to GG + TT. This may be due to differences in sample size, region, and race[39]. Meanwhile, some studies have been conducted to correlate the rs1799983 locus with specific clinical subtypes of SLE. Li et al.[15] found that variation at this locus was significantly associated with the development of SLE nephropathy in northeastern China. Others[40] found that the rs1799983 locus polymorphism was significantly associated with genetic susceptibility to SLE combined with femoral head necrosis, but not with SLE. This suggests that there may be a genetic susceptibility between the rs1799983 locus and certain clinical subtypes resulting from the progression of SLE. In the future, the clinical subtypes of SLE could be considered as an entry point for a more in-depth study of the potential mechanisms behind the genetic susceptibility of this locus to SLE.

Currently, the rs1007311 locus of the *eNOS* gene has been less studied for susceptibility to disease, and in the present study, no association was found between this locus and susceptibility to SLE. However, rs3918188 has a wide range of research fields. When a scholar[26] first evaluated the potential role of this loci on type 2 diabetes (T2DM) susceptibility in Chinese Han population, it was found that the genetic polymorphism of this loci had an impact on T2DM susceptibility. Kang JH et al.[41] observed that in women with age at menarche < 13 years, the risk of POAG was significantly lower in wild-type AA purebreds compared to CC purebreds with rs3918188; however, for women with age at menarche ≥ 13 years, this SNP was not associated with POAG. Al-Forkan M et al.[42] concluded that the AC genotype at this locus significantly increased the odds of cardiac tissue damage in CVD patients in arsenic-affected regions. However, studies on the association of rs3918188 with SLE have not been reported in the literature. In this study, we found that the CC genotype and C allele frequency at the rs3918188 locus were significantly higher in SLE patients in the Northeast compared with controls, which may be a risk factor for SLE. And all three gene models at this locus are associated with genetic susceptibility to SLE. In the dominant model, AC + CC gene carriers had an increased risk of SLE compared to AA gene carriers, in the recessive model, CC gene carriers had an increased risk of SLE compared to AA + AC gene carriers, and in the overdominant model, AC gene carriers had a decreased risk of SLE compared to AA + CC gene carriers.

To further explore the relationship between these loci, we performed haplotype analyses and MDR analysis. The results showed a strong linkage disequilibrium between the rs1007311 and rs1799983 loci, implying that these two loci are non-independent factors that may be able to influence each other. We then performed haplotype correlation analysis. Compared with GG haplotypes, individuals with AG haplotypes had an increased risk of SLE ($P < 0.01$), and individuals with AT and GT haplotypes had a decreased risk of SLE ($P < 0.05$). MDR analysis results suggested strong synergy between the rs1007311 and rs3918188 loci, and weak synergy between these two and the rs1799983 locus.

In conclusion, this study found that the CC genotype and C allele of the rs3918188 locus may be a risk factor for SLE. In the dominant model, the AC + CC genotype (vs AA) at rs3918188 increases the risk of systemic lupus erythematosus. In recessive models, CC genotype (vs AA + AC) at rs3918188 increases the risk of systemic lupus erythematosus. In an overdominant model, the AC genotype (vs AA + CC) at rs3918188 reduces the risk of systemic lupus erythematosus. The GT genotype and T allele of the rs1799983 locus may be protective factors for SLE. In an overdominant model, GT (vs GG + TT) at rs1799983 reduced the risk of systemic lupus erythematosus. This locus reduces the risk of SLE in the overdominant model. There is a strong linkage disequilibrium between the rs1007311 and rs1799983 loci of the *eNOS* gene. Among them, the formed haplotype AG increased the risk of SLE compared to GG. AT and GT decreased the risk of SLE compared to GG. However, the deeper molecular mechanism of these locus and genetic susceptibility to SLE is still unclear and needs to be explored in the future.

There is still a deficiency in this study; We didn't stratify racial groups. In addition to Han, there are Manchu, Korean and other ethnic minorities in Northeast China. The lack of ethnic stratification means that our results can only reflect the overall effect of SNP polymorphisms in SLE in the Northeast China population. The results may not be suitable for some peoples. In fact, the incidence of SLE has distinct geographical and ethnic characteristics[3]. A study identified 38 novel SLE loci, which helped explain the genetic basis of SLE differences between East Asian and European populations[43]. This suggests that SNP polymorphisms may provide an explanation for the genetic basis of SLE incidence in different populations. If the sample size can be expanded and the stratification of different ethnic groups can be improved in the future, the association between the SNP locus of *eNOS* gene and the incidence of SLE of a certain ethnic group can be further revealed. Second, this study focused on the association of genetic markers with disease susceptibility and did not explore the functional impact of these genetic variants. Therefore, additional experiments could be conducted in the future to explore the functional impact of these genetic variants in depth. Moreover, We did not perform associations between genotypes and SLE phenotypes such as lupus nephritis, nor did we perform subgroup analyses based on *eNOS* phenotypes and NO (nitrite and nitrate) levels. The lack of phenotypic stratification may affect the generalization of our findings. As the results of our study are global effects, we can only confirm whether SNP sites are associated with SLE, and it is difficult to assess their association with different SLE phenotypes. These analyses need to be improved and supplemented in the future. Finally, this study mainly used one-way and multifactorial logistic regression for statistical analysis. In fact, there was some overfitting of the multiple regression model. Although this study complements the MDR analysis and the multiple test correction, there is still room for improvement in statistical methods, and other appropriate tests should be supplemented in the future to ensure the validity of the results.

Conclusions

In this study, based on DNA sequencing analysis of the *eNOS* gene in SLE patients and healthy individuals in Northeast China, we found that the rs3918188 and rs1799983 loci of the *eNOS* gene were associated with susceptibility to SLE. In addition, in haplotype analysis, we also found a strong linkage imbalance between the *eNOS* gene rs1007311 and rs1799983, and haplotype AG, AT and GT were associated with genetic susceptibility to SLE. In the MDR analysis, we identified a possible synergy between rs1007311 and rs3918188. This helps to deeply explore the mechanism of *eNOS* gene and genetic susceptibility to SLE. It provides a certain research basis for the subsequent exploration of the molecular mechanism of these loci and SLE, as well as the early diagnosis, treatment and prognosis of SLE.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conceptualization, X.Z., G.L. and Q.Z.; methodology and software, H.W.; validation, Z.W.; formal analysis, W.X.; investigation,Z.H.; data curation, L.S.; writing—original draft preparation, X.Z., G.L. and Q.Z.; writing—review and editing, Y.Z. and A.G.; visualization, W.X.; supervision, Y.Z. and A.G.

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Competing interests
The authors declare no competing interests.

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