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Endothelin-1, a marker for systemic lupus erythematosus?

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Abstract

Objective: Systemic lupus erythematosus is a chronic rheumatic disorder. Endothelin-1, a vasoconstrictor, belongs to the endothelin family. To date, association between ET-1 and pathogenesis of SLE remains unclear.

Method: This case-control study was carried out by 314 SLE, 252 other inflammatory autoimmune diseases patients and 500 healthy controls. Serum ET-1, CCN3, IL-28B levels were detected by ELISA, and ET-1 gene polymorphisms (rs5369, rs5370, rs1476046, rs2070699, rs2071942, rs2071943, rs3087459, rs4145451, rs6458155, rs9369217) were genotyped with KASP.

Results: Raised ET-1 concentrations in SLE patients correlated with clinical characteristics. Serum CCN3, IL-28B expressions were higher in SLE patients, and ET-1 levels were positively correlated with the two cytokines. Rs5370, rs1476046, rs2070699, rs2071942, rs2071943, rs3087459, rs6458155 and rs2070699 were associated with SLE risk. Rs2070699 (T, TT) was related to alopecia. Rs5370 (T, TT, TG), rs1476046 (G,GA), rs2071942 (G,GA) and rs2071943 (G,GA) were associated with pericarditis, pyuria and fever manifestations. Rs3087459 (CC) and rs9369217 (TC) were relevant to anti-SSB indicator. Rs5369 (AA) was associated with IgG and CRP levels.

Conclusion: elevated serum ET-1 in SLE patients may be a potential disease marker, and its gene polymorphisms were relevant to SLE susceptibility.

1. Introduction

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease characterized by dysregulated production of autoantibodies and inflammatory cytokines [1]. The clinical manifestations caused by systemic inflammation and tissue damage in SLE are complex and varied, involving multiple organs, such as skin, kidney, joints, blood vessels [2]. The exact etiology of SLE remains obscure, but genetic susceptibility, environmental factors and sex hormones are demonstrated to play pivotal roles. To date, a large number of genome-wide association studies (GWASs) have identified roughly 180 genetic loci associated with SLE susceptibility [3].

Endothelin-1 (ET-1), the main component of the endothelin family, consists of 21 amino acids and two intramolecular disulphide bonds. ET-1 is mostly produced by endothelial cells and is expressed in a variety of cells, such as vascular endothelial cells, smooth muscle cells, cardiomyocytes, fibroblasts and macrophages [4, 5]. Being an endogenous long-acting vasoconstrictor, ET-1 can produce kinds of growth factors and stimulate the proliferation and contraction of vascular endothelial cells, vascular smooth muscle cells and vascular fibroblasts, which play key roles in regulating the dynamic balance of hemodynamics [6, 7]. Moreover, SLE is associated with different vascular processes resulting from endothelial dysfunction, such as vasculitis and Raynaud's phenomenon. Abnormal expression of ET-1 derived from endothelial cells may lead to endothelial dysfunction and further participate in the pathogenesis of SLE [8]. In rheumatoid arthritis (RA) patients, higher serum ET-1 levels were detected

when compared to controls, and were positively related to C-reactive protein (CRP) expression [9]. Expression of ET-1 was increased in both psoriasis vulgaris and systemic sclerosis (SSc) patients [10, 11]. These findings indicated that ET-1 may be abnormally expressed in inflammatory autoimmune diseases. To date, relationship of ET-1 and lupus was limited discussed and if ET-1 gene polymorphisms relate to SLE risk has not been elucidated.

Nephroblastoma overexpressed (NOV, CCN3), one of the first proteins identified in the CCN3 family, is highly expressed in many cells and may be secreted by regulatory T cells [12]. CCN3 is capable of inducing angiogenesis by binding ligands αvβ3 and α5β1, which will promote endothelial cell adhesion and migration [13]. In addition, abnormal expression of CCN3 has been reported to be associated with immune regulation and involved in various immune-related diseases such as RA, SSc, glomerulonephritis (GN) [14]. Therefore, CCN3 may associate with ET-1 and pathogenesis of SLE. Type I interferon is associated with development of SLE, and when type I interferon and its receptors are deficient, autoantibody production and autoimmune disease development are inhibited in animal models [15, 16]. Type III interferons, including IL-28A, IL-28B, IL-29 (also known as IFN-k2, IFN-k3 and IFN-k1, respectively), are functionally similar to type I interferon. Moreover, IL-28B has been proved to play a role in antiviral and antitumor immunity and involve in development of multiple immune diseases [17]. For example, IL-28B was highly expressed in lupus and was associated with disease activity [18]. However, the relationship between ET-1 and CCN3, IL-28B in lupus remains unclear.

In this study, serum ET-1 concentrations were assayed in SLE patients, evaluating potential of serum ET-1 as a SLE biomarker. In addition, we measured the serum levels of CCN3, IL-28B to explore the association of ET-1 and CCN3, IL-28B. Finally, we discussed 10 polymorphisms of ET-1 gene (rs5369, rs5370, rs1476046, rs2070699, rs2071942, rs2071943, rs3087459, rs4145451, rs6458155, rs9369217) and SLE susceptibility.

2. Methods

2.1 Subjects.

This case-control study recruited 314 SLE patients, 252 other inflammatory rheumatic diseases patients, and 500 healthy controls. All patients were from the Department of Rheumatology and Immunology of Affiliated Hospital of Southwest Medical University, and the healthy controls came from Medical Examination Center of the Center for Disease Control and Prevention in Jiangyang District, Luzhou. The study has three stages. Within the first part, we evaluated the ability of serum ET-1 to differentiate patients with SLE from healthy individuals, as well as other rheumatic diseases. A training cohort of 53 SLE patients and 80 healthy controls was used to investigate whether there was a difference in serum ET-1 between patients and healthy controls. SLE was diagnosed with 1997 American College of Rheumatology (ACR) revised criteria [19]. Then, a validation cohort was conducted to confirm the findings in training cohort, including 102 SLE patients, 90 patients with RA (according to 1987 ACR criteria for RA) [20], 95 with osteoarthritis (OA) (1986 ACR criteria for OA) [21], 55 with Sjogren’s syndrome (SS) (2016

ACR for SS) [22], 38 with ankylosing spondylitis (AS) (Modifed New York criteria for AS) [23]and 17 with systemic sclerosis (SSc) (2013 ACR/European League Against Rheumatism criteria for SSc) [24]. Based on the SLE disease activity index (SLEDAI), lupus patients were divided into less-active period (SLEDIA < 10) and active period (SLEDIA ≥ 10). In the second part, we discussed the correlation between ET-1 and CCN3, IL-28B, where we detected serum CCN3 and IL-28B concentrations in 53 SLE patients and 80 healthy Individuals. In the third stage, we genotyped 10 SNPs (rs5369, rs5370, rs1476046, rs2070699, rs2071942, rs2071943, rs3087459, rs4145451, rs6458155, rs9369217) of ET-1 gene in order to discuss the genetic susceptibility of SLE with ET-1 variations, including 314 SLE patients and 500 healthy controls in a Chinese Han population. This study was endorsed by the Ethics Committee of Affiliated Hospital of Southwest Medical University. Blood samples were taken from all subjects after they had signed the informed consent. Clinical and laboratory characteristics of all subjects are shown in Table 1.

Table 1
Characteristics of SLE patients and controls.

Characteristics	SLE	HC	P value
Age (years)	38.00 (27.00–49.00)	37.00 (34.00–40.00)	0.884
Female (%)/male (%)	89.17/10.83	92.60/7.40	0.092
Lupus headache, n (%)	21 (6.6)	–	–
Vasculitis, n (%)	28 (8.92)	–	–
Arthritis, n (%)	158 (50.32)	–	–
Myositis, n (%)	31 (9.87)	–	–
Rash, n (%)	132 (42.04)	–	–
Alopecia, n (%)	98 (31.21)	–	–
Oral ulcer, n (%)	45 (14.33)	–	–
Pleurisy, n (%)	25 (7.9)	–	–
Pericarditis, n (%)	25 (7.9)	–	–
Fever, n (%)	61 (19.43)	–	–
Hypocomplementemia, n (%)	157 (50.00)	–	–
ds-DNA+, n (%)	70 (22.29)	–	–
Thrombocytopenia, n (%)	47 (14.99)	–	–
Leukopenia, n (%), n (%)	36 (11.46)	–	–
Hematuria, n (%)	107 (34.08)	–	–
Proteinuria, n (%)	154 (49.04)	–	–
Pyuria, n (%)	27 (8.5)	–	–
C3 (g/L)	0.73 (0.46–0.94)	–	–
C4 (g/L)	0.14 (0.07–0.22)	–	–
ESR (mm/H)	25.00 (11.00–51.00)	–	–
RF (IU/ml)	9.70 (8.40–12.30)	–	–
IgA (mg/L)	2.55 (1.82–3.33)	–	–
IgM (mg/L)	1.01 (0.69–1.44)	–	–

SLE, systemic lupus erythematosus; HC, healthy controls; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; CRP, C-reactive protein.

Characteristics	SLE	HC	P value
IgG (g/L)	14.24 (10.58–19.75)	–	–
CRP (mg/L)	3.00 (0.5-14.59)	–	–

SLE, systemic lupus erythematosus; HC, healthy controls; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; CRP, C-reactive protein.

2.2 Serum, DNA preparation and genotyping

Peripheral blood was centrifuged to obtain serum, which was stored at -80°C until use. Genomic DNA was extracted by TIANamp Blood DNA kits (TIANGEN) on the basis of instructions. ET-1 polymorphisms (rs5369, rs5370, rs1476046, rs2070699, rs2071942, rs2071943, rs3087459, rs4145451, rs6458155, rs9369217) were genotyped using KASP™ method. Information of KASP primers for 10 single nucleotide polymorphisms (SNPs) was listed in Supplementary table 1.

2.3 Measurement of ET-1, CCN3, IL-28B by Enzyme-linked immune sorbent assay (ELISA)

The concentrations of ET-1, CCN3 and IL-28B in SLE patients and control group were determined by ELISA kits (Cusabio, Houston, USA). All steps were carried out in line with manufacturer’s instructions and each sample was tested repeatedly. The data were measured at 450nm and converted into concentration by linear standard curve.

2.4 Statistics

SPSS 23.0 and GraphPad Prism 5.01 were used for analysis of all data. For quantitative information, when the data obeyed normal distribution, means ± standard deviation (SD) and independent samples t-test were used to describe and analyze. When not obeyed, median (interquartile range) and Wilcoxon ranks sum test were used. For categorical data, we chose to describe them by frequency and percentage, and adopted chi-square test for comparison. Potential of serum ET-1 as the biomarker for lupus was evaluated by area of receiver operating characteristic (ROC) curve. Hardy-Weinberg equilibrium (HWE) assessed deviation of individual polymorphism in patients and healthy controls. Distribution of genotypes and alleles between patients and healthy controls was compared using chi-square test, then odds ratio (OR) and 95% confidence interval (CI) were analyzed by logistic regression model. The level of significance was set at $P < 0.05$ (two-sided).

3. Results

3.1 ET-1 serum levels in training cohort

ET-1 serum concentrations were significantly higher in 53 SLE patients compared to 80 healthy controls ($P < 0.001$, Fig. 1A). Patients with alopecia (N = 15) had increased serum levels of ET-1 when compared with that in patients without alopecia (N = 38) ($P = 0.031$, Fig. 1B). Patients with proteinuria (N = 31) had

increased serum concentrations of ET-1 when compared with that in patients without proteinuria (N = 22) (P = 0.007, Fig. 1C). Similarly, SLE patients with positive anti-Sm (N = 16) revealed elevated expression of ET-1 in serum than that in patients with negative anti-Sm antibody (N = 37) (P = 0.003, Fig. 1D). Moreover, SLE patients with active period (SLEDIA ≥ 10) had higher ET-1 levels compared to those with less active period (SLEDIA < 10) (P < 0.001, Fig. 1E). Correlation analysis revealed a positive correlation between serum ET-1 levels and SLEDAI score (r_s=0.463, P < 0.001, Fig. 1F), ESR (r_s=0.479, P = 0.005, Fig. 1G). Other clinical and laboratory features are not associated with serum ET-1 levels in lupus patients (Supplementary table 2 and 3). Then, to evaluate the ability of serum ET-1 to distinguish SLE patients from healthy individuals, we performed ROC curve analysis, which showed area under ROC curve (AUC) was 0.912 (95% CI = 0.866–0.959) (P < 0.001, Fig. 1H).

3.2 Elevated serum levels of ET-1 in validation cohort

In the validation cohort, 102 SLE patients were compared with 252 disease controls (including 90 RA, 95 OA, 55 SS, 38 AS, 17 SSc) to further confirm the potential of serum ET-1 as a biomarker for lupus. The results indicated that the serum ET-1 levels in SLE patients were significantly higher than that in other rheumatic diseases (all P < 0.001, Fig. 2A). The AUC was 0.803 by ROC curve analysis when comparing serum levels of ET-1 between SLE with RA patients (Fig. 2B). Similarly, serum ET-1 in SLE patients compared to that in OA, SS, AS, SSc, showed AUC 0.979, 0.856, 0.892 and 0.903, respectively (Fig. 2C-F). We compared serum ET-1 levels between SLE patients and non-SLE populations, showing that there was a significant difference (P < 0.001, Fig. 2G) and AUC was 0.900 (95% CI = 0.869–0.932) (Fig. 2H).

3.3 Connections between ET-1 and CCN3, IL-28B concentrations in SLE

There were increased serum CCN3 levels in SLE patients compared with that in healthy subjects (P = 0.006, Fig. 3A). Lupus patients with thrombocytopenia are more likely to express high levels of CCN3 (P = 0.020, Fig. 3B) (Supplementary table 4). SLEDAI score and other indicators of clinical characteristics were not found to be significantly correlated with CCN3 levels (Supplementary table 5). For IL-28B, its expression was elevated in the serum of SLE patients, compared to the healthy group (P < 0.001, Fig. 4A). Lupus patients with hematuria, proteinuria, cylindruria had higher levels of IL-28B (P = 0.014, Fig. 4B; P = 0.012, Fig. 4C; P = 0.032, Fig. 4D) (Supplementary table 6). Higher serum IL-28B levels in SLE patients with active period (SLEDIA ≥ 10) were obtained when compared to patients with less-active period (SLEDIA < 10) (P = 0.047, Fig. 4E). Correlation analysis observed that SLEDAI score, ESR levels were positively correlated with IL-28B concentrations, respectively (r_s=0.406, P = 0.003, Fig. 4F; r_s 0.461, P = 0.007, Fig. 4G) (Supplementary table 7). Subsequently, we explored the correlation between ET-1 and CCN3, IL-28B levels. By calculating the correlation, it is found that ET-1 levels were positively correlated with CCN3 levels and IL-28B levels in SLE patients (r_s=0.338, P = 0.007, Fig. 3C; r_s=0.441, P = 0.001, Fig. 4H).

3.4 Association of ET-1 SNPs with SLE

To discuss whether polymorphisms of ET-1 gene correlate with SLE risk, a total of 314 lupus patients and 500 age, sex-matched healthy volunteers were selected. The HWE test showed no deviation in patients and controls for all 10 polymorphisms ($P > 0.05$, Table 2). The alleles and genotypes were shown in Table 3. Frequency of rs5370 genotype TG was higher in SLE patients compared to healthy controls (TG vs GG: OR = 1.443, 95% CI: 1.072–1.943, P = 0.016). In the dominant model (TT + TG vs GG), frequency of genotypes TT + TG of rs5370 was increased in SLE patients (OR = 1.334, 95% CI: 1.005–1.770, P = 0.046). For rs1476046, frequency of genotype GG in SLE patients was markedly lower than that in controls [GG vs GA + AA (recessive model): OR = 0.693, 95% CI: 0.521–0.920, P = 0.011]. Compared with healthy controls, frequency of rs2070699 allele T was declined in SLE patients (T vs G: OR = 0.811, 95% CI: 0.664–0.990, P = 0.039). Similarly, decreased frequencies of TT, TG, TT + TG genotypes of rs2070699 were found in SLE patients [TT vs GG: OR = 0.669, 95% CI: 0.452–0.986, P = 0.042; TG vs GG: OR = 0.674, 95% CI: 0.475–0.957, P = 0.027; TT + TG vs GG (dominant model) : OR = 0.672, 95% CI : 0.485–0.931, P = 0.017]. Frequency of GG genotype for rs2071942 was increased in SLE patients when compared with that in healthy subjects (GG vs GA: OR = 1.705, 95% CI: 1.033–2.812, P = 0.037). With respect to rs2071943, there was decreased frequency of GG in SLE patients than that in control group [GG vs GA + AA (recessive model): OR = 0.707, 95% CI: 0.532–0.938, P = 0.016]. In the dominant model, higher frequencies of CA + CC genotypes of rs3087459 were more likely to be found in patients with SLE (CA + CC vs AA: OR = 1.352, 95% CI: 1.013–1.805, P = 0.040). In addition, SLE patients with rs6458155 had an enhanced frequency of TC genotype (TC vs CC: OR = 1.392, 95% CI: 1.021–1.899, P = 0.0037). For rs5369, rs4145451, rs9369217 polymorphism, we compared the allele and genotypes distribution between SLE cases and healthy controls, and there were no significant differences.

Table 2
The Hardy-Weinberg's expectation test in SLE patients and healthy controls for SNPs.

SNPs	SLE		HC	
rs5369	$\chi^2 = 4.594$	$P = 0.101$	$\chi^2 = 4.735$	$P = 0.094$
rs5370	$\chi^2 = 0.918$	$P = 0.632$	$\chi^2 = 2.062$	$P = 0.357$
rs1476046	$\chi^2 = 0.516$	$P = 0.772$	$\chi^2 = 1.568$	$P = 0.457$
rs2070699	$\chi^2 = 1.847$	$P = 0.397$	$\chi^2 = 0.036$	$P = 0.982$
rs2071942	$\chi^2 = 0.190$	$P = 0.909$	$\chi^2 = 5.628$	$P = 0.060$
rs2071943	$\chi^2 = 0.308$	$P = 0.857$	$\chi^2 = 2.067$	$P = 0.356$
rs3087459	$\chi^2 = 3.917$	$P = 0.141$	$\chi^2 = 3.179$	$P = 0.204$
rs4145451	$\chi^2 = 0.657$	$P = 0.720$	$\chi^2 = 2.351$	$P = 0.309$
rs6458155	$\chi^2 = 0.540$	$P = 0.763$	$\chi^2 = 1.582$	$P = 0.453$
rs9369217	$\chi^2 = 1.141$	$P = 0.565$	$\chi^2 = 1.095$	$P = 0.578$

SNP, single nucleotide polymorphisms. SLE, systemic lupus erythematosustis; HC, healthy controls.

Table 3
Frequencies of alleles and genotypes for ET-1 gene polymorphisms in SLE patients and healthy controls.

Polymorphism			SLE, n (%)	Controls, n (%)	OR (95% CI)	*P* value
rs5369	Genotype	GG	292 (93.0)	455 (91)	0.770 (0.233–2.546)	0.668
		GA	17 (5.4)	39 (7.8)	0.523 (0.140–1.951)	0.335
		AA	5 (1.6)	6 (1.2)	Reference	
	Allele	G	601 (95.7)	949 (94.9)	1.196 (0.742–1.928)	0.462
		A	27 (4.3)	51 (5.1)	Reference	
	Recessive model	GG	292 (93.0)	455 (91.0)	1.313 (0.772–2.232)	0.315
		GA + AA	22 (7.0)	45 (9.0)	Reference	
	Dominant model	GA + GG	309 (98.4)	494 (98.8)	0.751 (0.227–2.480)	0.638
		AA	5 (1.6)	6 (1.2)	Reference	
rs5370	Genotype	TT	24 (7.7)	48 (9.6)	0.918 (0.541–1.560)	0.753
		TG	143 (45.5)	182 (36.4)	1.443 (1.072–1.943)	0.016
		GG	147 (46.8)	270 (54.0)	Reference	
	Allele	T	191 (30.4)	278 (27.8)	1.135 (0.912–1.413)	0.257
		G	437 (69.6)	722 (72.2)	Reference	
	Recessive model	TT	24 (7.7)	48 (9.6)	0.779 (0.497-1.300)	0.340
		TG + GG	290 (92.3)	452 (90.4)	Reference	
	Dominant model	TT + TG	167 (53.2)	230 (46.0)	1.334 (1.005–1.770)	0.046
		GG	147 (46.8)	270 (54.0)	Reference	

SLE, systemic lupus erythematosus; OR, odd ratio; 95% CI, 95% confidence interval

Polymorphism			SLE, n (%)	Controls, n (%)	OR (95% CI)	*P* value
rs1476046	Genotype	GG	134 (42.7)	259 (51.8)	0.825 (0.506–1.344)	0.439
		GA	148 (47.1)	190 (38.0)	1.241 (0.759–2.029)	0.388
		AA	32 (10.2)	51 (10.2)	Reference	
	Allele	G	416 (66.2)	708 (70.8)	0.823 (0.665–1.019)	0.074
		A	212 (33.8)	292 (29.2)	Reference	
	Recessive model	GG	134 (42.7)	259 (51.8)	0.693 (0.521–0.920)	0.011
		GA + AA	180 (57.3)	241 (48.2)	Reference	
	Dominant model	GA + GG	282 (89.8)	449 (89.8)	1.001 (0.628–1.596)	0.997
		AA	32 (10.2)	51 (10.2)	Reference	
rs2070699	Genotype	TT	85 (27.1)	150 (30.0)	0.669 (0.454–0.986)	0.042
		TG	140 (44.6)	245 (49.0)	0.674 (0.475–0.957)	0.027
		GG	89 (28.3)	105 (21.0)	Reference	
	Allele	T	310 (49.4)	546 (54.6)	0.811 (0.664–0.990)	0.039
		G	318 (50.6)	454 (45.4)	Reference	
	Recessive model	TT	85 (27.1)	150 (30.0)	0.866 (0.633–1.185)	0.369
		TG + GG	229 (72.9)	350 (70.0)	Reference	
	Dominant model	TT + TG	225 (71.7)	395 (79.0)	0.672 (0.485–0.931)	0.017
		GG	89 (28.3)	105 (21.0)	Reference	

SLE, systemic lupus erythematosus; OR, odd ratio; 95% CI, 95% confidence interval

Polymorphism			SLE, n (%)	Controls, n (%)	OR (95% CI)	*P* value
rs2071942	Genotype	GG	146 (46.5)	264 (52.8)	1.185 (0.725–1.938)	0.499
		GA	140 (44.6)	176 (35.2)	1.705 (1.033–2.812)	0.037
		AA	28 (8.9)	60 (12.0)	Reference	
	Allele	G	432 (68.8)	704 (70.4)	0.927 (0.746–1.151)	0.491
		A	196 (31.2)	296 (29.6)	Reference	
	Recessive model	GG	146 (46.5)	264 (52.8)	0.777 (0.585–1.031)	0.080
		GA + AA	168 (53.5)	236 (47.2)	Reference	
	Dominant model	GA + GG	286 (91.1)	440 (88.0)	1.393 (0.868–2.234)	0.169
		AA	28 (8.9)	60 (12.0)	Reference	
rs2071943	Genotype	GG	143 (45.6)	271 (54.2)	0.873 (0.528–1.445)	0.598
		GA	142 (45.2)	181 (36.2)	1.299 (0.799–2.164)	0.316
		AA	29 (9.2)	48 (9.6)	Reference	
	Allele	G	428 (68.2)	723 (72.3)	0.820 (0.660–1.019)	0.074
		A	200 (31.8)	277 (27.7)	Reference	
	Recessive model	GG	143 (45.6)	271 (54.2)	0.707 (0.532–0.938)	0.016
		GA + AA	171 (54.4)	229 (45.8)	Reference	
	Dominant model	GA + GG	285 (90.8)	452 (90.4)	1.044 (0.643–1.694)	0.863
		AA	29 (9.2)	48 (9.6)	Reference	
rs3087459	Genotype	CC	8 (2.6)	10 (2.0)	1.435 (0.556-3.700)	0.455

SLE, systemic lupus erythematosus; OR, odd ratio; 95% CI, 95% confidence interval

Polymorphism			SLE, n (%)	Controls, n (%)	OR (95% CI)	*P* value
		CA	127 (40.4)	169 (33.8)	1.348 (1.004–1.808)	0.047
		AA	179 (57.0)	321 (64.2)	Reference	
	Allele	C	143 (22.8)	189 (18.9)	1.265 (0.991–1.616)	0.059
		A	485 (77.2)	811 (81.1)	Reference	
	Recessive model	CC	8 (2.6)	10 (2)	1.281 (0.500-3.281)	0.606
		CA + AA	306 (97.4)	490 (98.0)	Reference	
	Dominant model	CA + CC	135 (43.0)	179 (35.8)	1.352 (1.013–1.805)	0.040
		AA	179 (57.0)	321 (64.2)	Reference	
rs4145451	Genotype	CC	93 (29.6)	163 (32.6)	1.161 (0.772–1.748)	0.473
		CA	165 (52.6)	223 (44.6)	1.506 (1.032–2.198)	0.034
		AA	56 (17.8)	114 (22.8)	Reference	
	Allele	C	351 (55.9)	549 (54.9)	1.041 (0.852–1.272)	0.695
		A	277 (44.1)	451 (45.1)	Reference	
	Recessive model	CC	93 (29.6)	163 (32.6)	0.870 (0.641–1.182)	0.373
		CA + AA	221 (70.4)	337 (67.4)	Reference	
	Dominant model	CA + CC	258 (82.2)	386 (77.2)	1.361 (0.953–1.944)	0.090
		AA	56 (17.8)	114 (22.8)	Reference	
rs6458155	Genotype	TT	46 (14.6)	81 (16.2)	1.067 (0.694–1.642)	0.766

SLE, systemic lupus erythematosus; OR, odd ratio; 95% CI, 95% confidence interval

Polymorphism			SLE, n (%)	Controls, n (%)	OR (95% CI)	*P* value
		TC	160 (51.0)	216 (43.2)	1.392 (1.021–1.899)	0.037
		CC	108 (34.4)	203 (40.6)	Reference	
	Allele	T	252 (40.1)	378 (37.8)	1.103 (0.899–1.353)	0.348
		C	376 (59.9)	622 (62.2)	Reference	
	Recessive model	TT	46 (14.6)	81 (16.2)	0.888 (0.599–1.315)	0.553
		TC + CC	268 (85.4)	419 (83.8)	Reference	
	Dominant model	TC + TT	206 (65.6)	297 (59.4)	1.304 (0.972–1.748)	0.076
		CC	108 (34.4)	203 (40.6)	Reference	
rs9369217	Genotype	TT	4 (1.3)	7 (1.6)	0.939 (0.272–3.243)	0.920
		TC	89 (28.3)	130 (26)	1.125 (0.819–1.544)	0.469
		CC	221 (70.4)	363 (72.6)	Reference	
	Allele	T	97 (15.4)	144 (14.4)	1.806 (0.821–1.436)	0.563
		C	531 (84.6)	856 (85.6)	Reference	
	Recessive model	TT	4 (1.3)	7 (1.6)	0.909 (0.264–3.130)	0.879
		TC + CC	310 (98.7)	493 (98.4)	Reference	
	Dominant model	TC + TT	93 (29.6)	137 (27.2)	1.115 (0.816–1.523)	0.494
		CC	221 (70.4)	363 (72.6)	Reference	

SLE, systemic lupus erythematosus; OR, odd ratio; 95% CI, 95% confidence interval

3.5 Relationship of ET-1 polymorphisms with clinical, laboratory features in SLE patients

Qualitative and quantitative indicators of different clinical features associated with ET-1 gene polymorphisms in SLE patients are shown in Supplementary table 8, 9, 10 and 11, respectively. Compared to patients without these clinical features, frequency of rs5070 genotypes TT + TG was elevated in patients with pericarditis and positive ANA (P = 0.034, P = 0.045), and was decreased in patients with fever (P = 0.005). Moreover, there was a higher frequency of allele T of rs5370 in patients with pericarditis, and a lower frequency of allele T of rs5370 in patients with pyuria compared with those in patients without the features (P = 0.013, P = 0.047). For rs1476046, decreased G allele frequency in patients with pericarditis and increased G allele frequency in patients with pyuria was observed when compared to patients without these specific features. A lower frequency of genotype GA of rs1476046 in SLE patients with fever and pyuria was noted (P = 0.013, P = 0.028). Significant differences for genotypes and allele frequencies of rs2070699 polymorphism was showed in patients with alopecia compared with patients without this clinical feature (P = 0.013, P = 0.035). Distribution of GG, GA, AA genotypes of rs2071942 and rs2071943 was different between SLE patients with and without fever (P = 0.025, P = 0.010). Patients with pericarditis had a declined frequency of the G allele of rs2071942 and rs2071943 compared to patients without pericarditis (P = 0.019, P = 0.025). The frequency of G allele of rs2071942 and rs2071943 in patients with pyuria was higher (P = 0.035, P = 0.028). About rs3087459, decreased frequencies of C allele and CC genotype in patients with positive ANA and increased frequency of CA genotype in patients with positive anti-SSB were observed when compared to patients without these specific features (P = 0.045, P = 0.029, P = 0.027). In addition, the distribution of TT, TC and CC genotypes of rs9369217 was disparate in SLE patients with and without anti-SSB (P = 0.029) (Supplementary table 8 and 9). Other SNPs were not related to the clinical and laboratory manifestations of SLE (Supplementary table 10).

When discussing quantitative indicators of clinical features, levels of IgG between patients with GG + GA genotype and AA genotype for rs5369 was different (P = 0.025). SLE patients carrying rs5369 AA genotype had higher expression of CRP as compared to the patients carrying GG + GA genotype (P = 0.012) (Supplementary table 11).

3.6 ET-1 haplotypes and SLE risk

Considering the genetic linkage disequilibrium, we analyzed the correlation between ET-1 haplotypes and SLE risk. We constructed two blocks, one consisting of rs6458155, rs4145451 and the other including rs2071942, rs2071943, rs5370 (Fig. 3). Results revealed that frequency of haplotype CA was lower in SLE patients compared to healthy controls (P = 0.012). Reduced frequency of haplotype AGG was found in patients with SLE (P = 0.041). No significant differences were showed in the other haplotypes (Table 4).

Table 4
Haplotype analysis of ET−1 gene polymorphisms between SLE and healthy controls.

Block*	Haplotype	Frequency	SLE ratio	Control ratio	χ^2	P values
1	CC	0.551	0.556	0.548	0.093	0.761
	TA	0.385	0.398	0.377	0.723	0.395
	CA	0.062	0.043	0.074	6.351	0.012
2	GGG	0.691	0.674	0.701	1.399	0.237
	AAT	0.286	0.304	0.275	1.652	0.199
	AGG	0.016	0.008	0.021	4.179	0.041

*Block 1 consists of rs6458155 and rs4145451. Block 2 consists of rs2071942, rs2071943 and rs5370. SLE, systemic lupus erythematosus. SLE patient versus controls by 2×2 chi-square test.

4. Discussion

SLE is a rheumatic immune disease with heterogeneous clinical symptoms that results from endothelial cell activation and immune disorders [25]. Endothelial cells can cause proliferative vasculopathy by regulating vascular tension, immune and coagulation systems, thereby contributing to autoimmune disease pathogenesis. When endothelial cells are activated under inflammatory stimulation, expression of surface adhesion molecules was increased, which promotes migration and accumulation of leukocytes to endothelial cells. Then, this induces vascular obstruction and tissue hypoxia, leading to apoptosis and tissue fibrosis [26]. Hence, measuring biomarkers related to endothelial cell activation is important for diagnosis of SLE. Endothelium-derived ET-1 plays a pathogenic role in connective tissue disease, pulmonary hypertension, and cancer by affecting angiogenesis, inflammation, and fibrosis [27]. In osteoarthritis, ET-1 induces an increase of IL-18 through the ET-1/ETAR axis and PI3K-dependent manner, promoting osteoblast proliferation and exacerbating the disease [28]. Previous study showed that compared with control group, increased ET-1 levels were observed in RA patients, particularly with kidney and cardiovascular system damage [29]. Moreover, ET-1 expression is elevated in other autoimmune diseases such as SSc, psoriasis and type 1 diabetes [30, 31]. According to our study, serum ET-1 levels were significantly higher in SLE patients in training cohort when compared with healthy controls, which was similar to previous findings [8, 32, 33]. Moreover, we found that serum ET-1 levels were associated with clinical symptoms of cylindruria, alopecia and laboratory indice (anti-Sm antibody). Similarly, Yoshio et al. proposed that serum ET-1 in SLE patients correlated with IgM antibody expression [33]. A study with small sample size suggested that serum ET-1 was higher in active SLE patients than in inactive SLE patients and controls, and ET-1 concentrations were higher in patients with visceral manifestation [32]. Consistently, we used a two-stage case-control study with a large sample size to confirm a positive correlation between serum ET-1 concentrations and SLEDAI score or disease activity, and that patients with active SLE had higher ET-1 levels. Urinary ET-1 may be a useful measurement of

renal inflammatory activity and may serve as a marker of lupus nephritis disease activity [34]. In the present study, we also explored the potential of serum ET-1 as a biomarker for SLE. In training cohort, AUC of ET-1 was 0.912 (95% CI: 0.866–0.959), indicating that serum ET-1 could distinguish SLE patients from healthy subjects. In the validation cohort, ET-1 concentrations were higher in SLE patients than in other rheumatic diseases, including RA, OA, SS, AS, SSc. Compared with non-SLE diseases, the AUC of serum ET-1 in SLE was higher than or close to 0.900, and the AUC of SLE and OA patients was 0.979. Therefore, serum ET-1 has good discriminatory ability for SLE patients and is a promising marker for SLE disease.

CCN3, as a pro-angiogenic factor and fibrosis inhibitor, involves in numerous autoimmune diseases [14]. Serum CCN3 concentrations were elevated in RA patients and positively correlated with expression of IL-6 [35]. CCN3 expression was increased in SSc, multiple sclerosis patients [36, 37]. For osteoarthritis, CCN3 could inhibit PI3K/AKT/mTOR pathway by reducing HMGB1 levels and decrease extracellular matrix catabolism [38]. In our study, we found that serum CCN3 levels were significantly higher in SLE patients compared to normal subjects and were associated with thrombocytopenia. In addition, we observed a positive correlation between ET-1 levels and CCN3 levels in SLE patients. CCN3 inhibits expression of vascular adhesion molecules and reduces monocytes adhesion. CCN3 negatively regulates activation of NF-κB pathway, affecting endothelial cell inflammation and cardiovascular homeostasis [39]. Type III interferons (IFNs) and type I IFNs may promote THP-1 cell differentiation, which contributes to follicular B cell activation and participates in the pathogenesis of autoimmune diseases [40]. IL-28B, known as IFN-λ3, belongs to a subtype of type III IFNs. IL-28B regulates innate and adaptive immune responses. SSc patients with pulmonary fibrosis have higher IL-28B serum levels and IL-28B gene polymorphism (rs12979860) is associated with risk of pulmonary fibrosis in a Caucasian population with SSc [41]. With respect to SLE, IL-28B SNPs (rs8099917, rs12979860) are risk factors for lupus nephritis in Taiwanese. Moreover, serum concentrations of IL-28B were elevated in SLE patients compared to healthy controls, which were related to complement expression and SLE disease activity [42]. IL-28B expression is associated with lupus disease activity, such as skin involvement [18]. According to our study, IL-28B levels in SLE patients were positively associated with disease activity and ESR levels. Lupus patients with hematuria, proteinuria, cylindruria showed higher levels of IL-28B, which are typical clinical manifestations of lupus. We analyzed correlation between ET-1 and IL-28B levels and observed that expression of ET-1 was positively correlated with IL-28B expression. The above data suggest that ET-1 is associated with both CCN3 and IL-28B. High expression of ET-1 may regulate the expression of IL-28B, CCN3 and then contribute to the pathogenesis of lupus. However, more functional studies are needed to reveal the mechanism of ET-1 regulation of CCN3 and IL-28B.

It is now accepted that SNP as a new genetic marker can be used for discovery of high-risk patients. ET-1 gene polymorphisms are widely discussed in vascular-related diseases and cancer, such as hypertension, coronary atherosclerosis, and papillary thyroid cancer [43, 44]. However, ET-1 gene polymorphisms have been less studied in autoimmune diseases. In this study, we explored the relationship between ET-1 gene polymorphisms and SLE risk through a case-control study in a Chinese Han population. We found that genotypes of rs5370 (TG,TT + TG), rs1476046 (GG), rs2070699 (TT,TG), rs2071942 (GA), rs2071943 (GG), rs3087459 (CA + AA), rs6458155 (TC) and allele of rs2070699 (T) were associated with SLE

susceptibility. For rs5370, TG, TT + TG genotype frequencies were higher in SLE patients, suggesting that rs5370 polymorphism may increase the risk of SLE in Chinese Han population. The GG genotype frequency of rs2071942 was increased in SLE patients compared to healthy subjects and was positively associated with SLE risk. Mantaka et al. investigated the association of ET-1 rs2071942 and rs5370 polymorphisms with primary biliary cirrhosis (PBC). The genotypes and alleles distribution of both loci were not significantly different from controls and PBC patients, but rs2071942 allele A and rs5370 allele T were associated with stage of disease progression [45]. This inconsistency may be due to differences in sample size, ethnicity, disease type, and duration. Similarly, in RA patients, frequencies of the genotypes and alleles of rs5370 and rs18000541 were not significantly different from those and healthy controls, but TT genotype of rs5370, rs18000541 was related to RA patients complicated with hypertension, indicating that rs5370 and rs18000541 loci were associated with cardiovascular risk in RA [46]. In our findings, rs5370, rs1476046, rs2070699, rs2071942, rs2071943 rs5369, rs3087459, and rs9369217 polymorphisms correlated with some clinical features and laboratory manifestations in SLE patients. Patients carrying rs2070699 T allele and TT genotype were more likely to develop symptoms of alopecia. Regarding rs2071942 and rs2071943, allele G was associated with pericarditis and pyuria symptoms, and genotype GA was related to fever. Moreover, significant correlation between rs5370 T allele, rs1476046 G allele and pericarditis, pyuria in SLE cases was observed. Rs3087459 (CC) and rs9369217 (TC) were associated with anti-SSB laboratory indicator. Rs5369 AA genotype correlated with IgG and CRP levels, suggesting that mutations at the rs5369 locus may affect the expression of these disease markers in SLE patients. Indeed, CRP is an acute phase protein produced by hepatocytes in response to inflammation and has important pathogenic significance in active lupus nephritis. IgG immune complexes are deposited in the spleen, causing damage to the immune barrier of the spleen, and then a large number of antibodies are produced [47]. Notably, one study proposed that in graves' disease (GD), ET-1 gene polymorphisms (rs5370 and rs1800541) were not associated with disease susceptibility, but were associated with autoantibody production in GD patients [48]. To the best of our knowledge, this study is the first to discuss the relationship between ET-1 gene polymorphisms and lupus, which may offer new insights and basis for further discussion of ET-1 genetic mutation and SLE in the future.

Nevertheless, our study has several limitations. First, we measured cross-sectional serum ET-1 levels in a relatively limited number of samples. Therefore, a large number of multicenter clinical samples and longitudinal data are warranted to confirm serum ET-1 as a disease marker of SLE. Second, the related mechanism of ET-1 regulating CCN3 and IL-28B and then affecting the pathogenesis of SLE remains to be discussed.

In conclusion, high expression of ET-1 is associated with pathogenesis of SLE and may be a potential disease biomarker. ET-1 gene polymorphisms were related to SLE susceptibility in Chinese Han population.

Declarations

AUTHOR CONTRIBUTION

Study conception and design: RL, WDX. Acquisition of data, analysis and interpretation of data: YYT, DCW, CY. Drafting the article: RL, WDX. Final approval of the version of the article to be published: all authors, and that all authors agree to be accountable for all aspects of the work.

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DATA AVAILABILITY STATEMENT

Datasets are available from the corresponding author on reasonable request.

CONFLICT OF INTERESTS

None.

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Figures

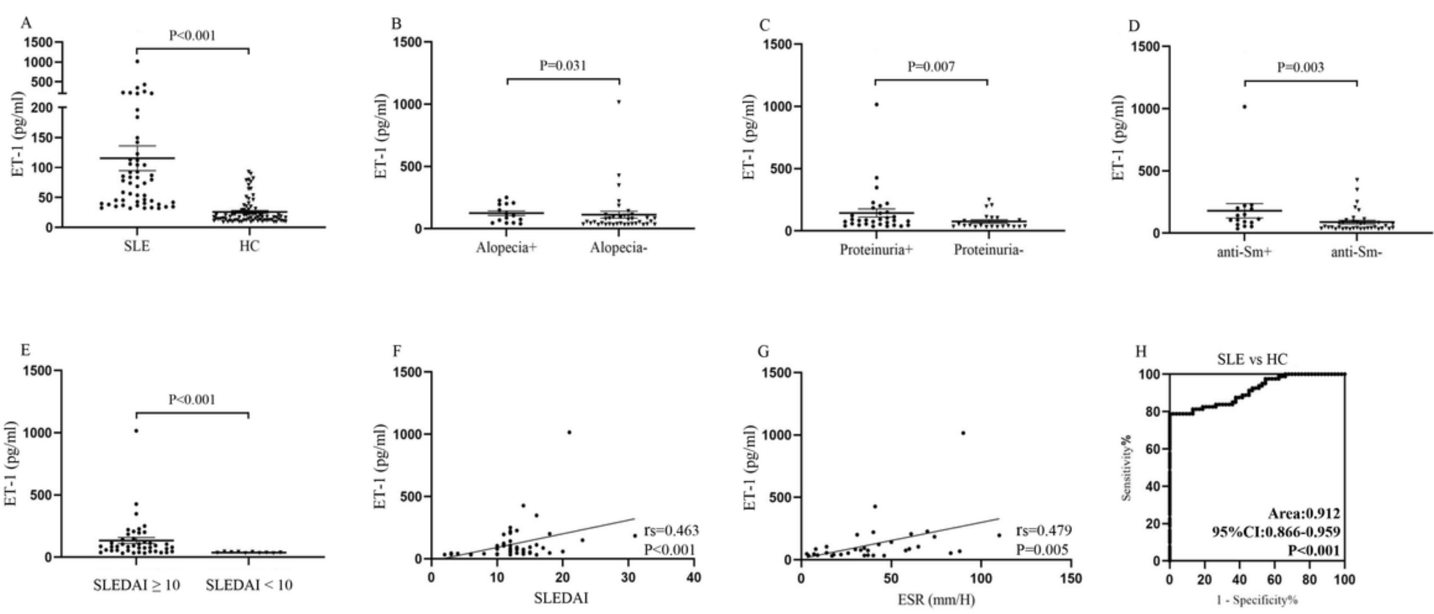

Figure 1

Comparison of ET-1 levels between SLE patients and healthy controls in the training cohort. (A) Serum ET-1 concentrations in 53 SLE patients and 80 healthy individuals were examined by ELISA. Each symbol stands for an independent sample. (B-D) ET-1 expression in SLE patients distributed according to alopecia, proteinuria and anti-Sm. (E) Difference of serum levels of ET-1 in SLE patients with less-active period and active period. (F-G) Relationship between ET-1 levels and SLEDAI, ESR. (H) Receiver-operating characteristic (ROC) curve analysis of serum ET-1 for the biomarker of SLE.

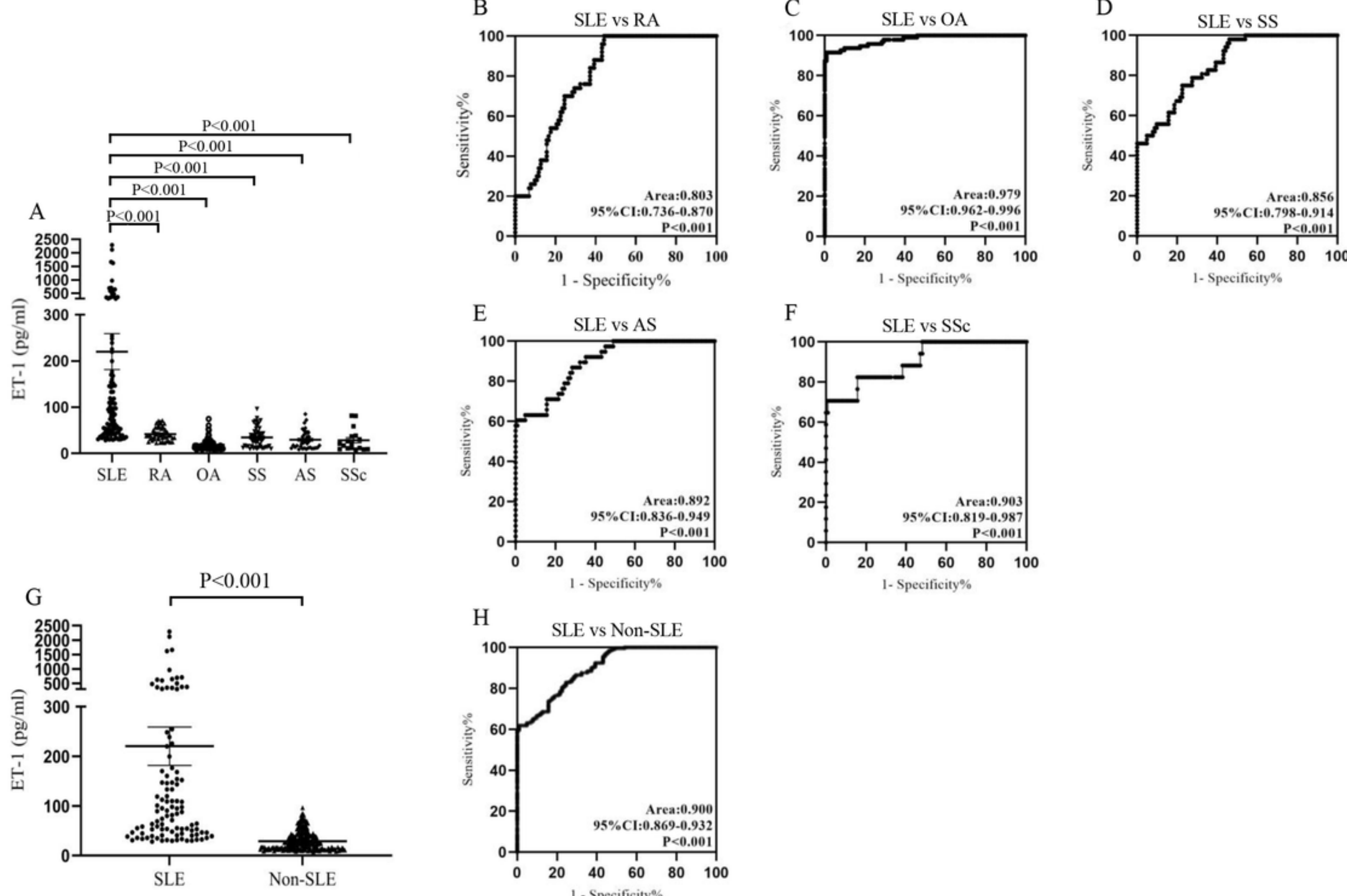

Figure 2

ET-1 concentrations in SLE patients from validation cohort. (A) Comparison of serum ET-1 levels in SLE patients (N=102) and disease controls (N=252, including 90 RA, 95 OA, 55 SS, 38 AS, 17 SSc) by ELISA. (B-F) Receiver operating characteristic (ROC) analysis was used to assess the potential of ET-1 in differentiating SLE from RA, OA, SS, AS, and SSc.(G) Analysis of the difference in serum ET-1 between SLE and non-SLE patients. (H) Receiver-operating characteristic (ROC) curve analysis of serum ET-1 between SLE and non-SLE patients.

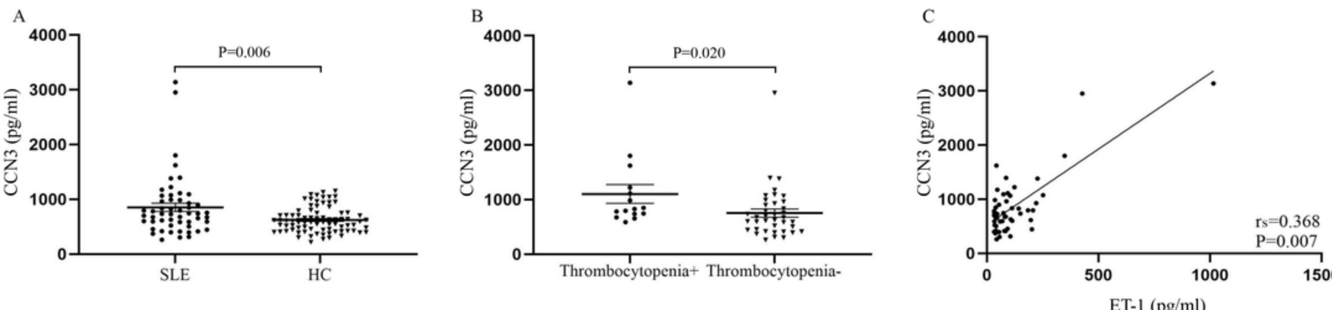

Figure 3

CCN3 expression in SLE patients and healthy controls. (A) Serum CCN3 expression was tested by ELISA in 53 SLE patients and 80 healthy controls. (B) serum ET-1 in SLE patients distributed in accordance with thrombocytopenia. (C) Correlation between ET-1 and CCN3 levels in SLE patients.

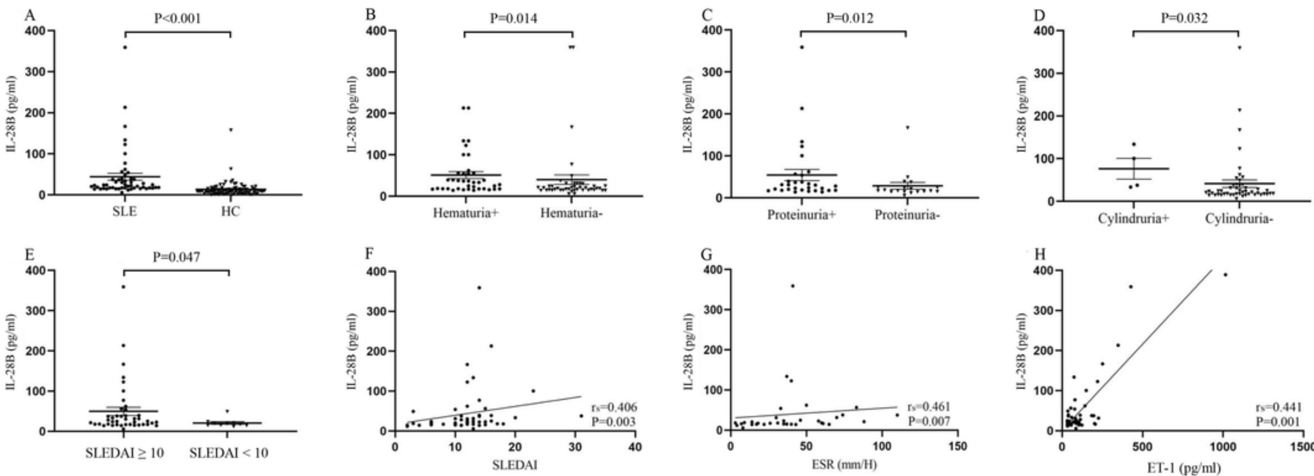

Figure 4

Comparison of IL-28B levels between SLE patients and healthy controls. (A) Serum IL-28B concentrations were detected by ELISA in 53 SLE patients and 80 healthy controls. (B-D) Distribution of serum IL-28B in SLE patients with hematuria, proteinuria and cylindruria. (E) The difference of serum IL-28B in SLE patients with active disease and less active disease. (F-G) The relationship between IL-28B concentrations and SLEDAI score, ESR levels. (H) Correlation between ET-1 and IL-28B expression in SLE patients.

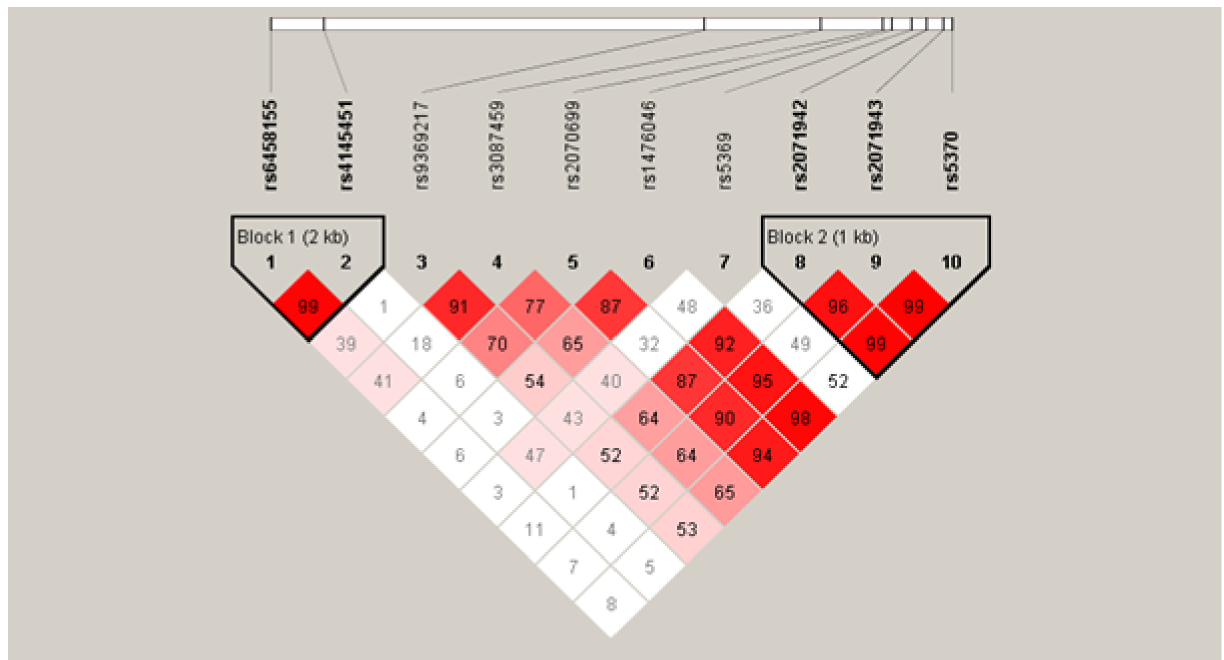

Figure 5

Linkage disequilibrium (LD) analysis for ten SNPs.

The color and numerical value (D') of each box represent the Te intensity of LD. Red and pink indicate significant linkage, while light blue and white indicate no linkage. The value of D' varies from 0 to 1, and the value of 1 represents the maximum link. Block 1 consists of rs6458155 and rs4145451. Block 2 consists of rs2071942, rs2071943 and rs5370.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable1.docx
- Supplementarytable2.docx
- Supplementarytable3.docx
- Supplementarytable4.docx
- Supplementarytable5.docx
- Supplementarytable6.docx
- Supplementarytable7.docx
- Supplementarytable8.docx
- Supplementarytable9.docx
- Supplementarytable10.docx
- Supplementarytable11.docx