Gene polymorphisms and serum levels of sVEGFR-1 in patients with systemic lupus erythematosus

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Correlation between soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) concentration, VEGFR1 gene polymorphisms and systemic lupus erythematosus (SLE) risk remains unclear. The present case–control study comprised 254 SLE patients, 385 other rheumatic diseases patients and 390 healthy controls. Serum levels of sVEGFR-1 were detected by enzyme-linked immunosorbent assay. Seven VEGFR1 genetic variants (rs2296188, rs9943922, rs2296283, rs7324510, rs9554322, rs9582036, rs9554320) were genotyped by KASP. Serum levels of sVEGFR-1 were up-regulated in SLE and positively correlated with disease activity. Furthermore, serum sVEGFR-1 presented a distinctive elevation in SLE in comparison with other rheumatic diseases. Frequencies of allele T of rs2296283 and allele G of rs9554322 were significant lower in SLE patients (P = 0.003, P = 0.004). Frequencies of genotypes TT of rs2296188 and rs2296283 were declined in patients compared with healthy controls (P = 0.039, P = 0.033). CC genotype of rs7324510 and rs9582036 was negatively correlated with SLE risk (OR = 0.538, OR = 0.508). Distribution of GG, GC, GG + GC genotypes of rs9554322 were different between SLE patients and healthy controls (P = 0.027, P = 0.036, P = 0.010). Moreover, frequency of TC genotype of rs7324510 was higher in SLE patients with lupus headache (χ² = 9.924, P = 0.039) and frequency of TC genotype of rs9943922 was lower in patients with cylindruria (χ² = 7.589, P = 0.026). Frequencies of allele C of rs7324510 and allele T of rs9943922 were decreased in SLE patients with cylindruria and hypocomplementemia, respectively (χ² = 4.195, P = 0.041, χ² = 3.971, P = 0.046). However, frequency of allele C of rs9554322 was increased in SLE patients with pyuria (χ² = 11.702, P = 0.001). In addition, SLE patients carrying GG, GC, CC genotypes for rs9554322 had higher levels of serum sVEGFR-1. In conclusion, serum sVEGFR-1 was elevated in SLE patients and may be a disease marker. VEGFR1 gene polymorphisms related to risk of SLE in a Chinese Han population.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a heterogeneous organ involvement that hinges on multiple autoantibodies production. Considering the worldwide estimation of incidence of SLE, there was a regional variation of the disease. European countries had a lower incidence of SLE, whereas Asia, Australia and the Americas had higher incidence. Gender difference also exists in SLE. It is known that occurrence of the disorder in women is more frequent than men, with a ratio of approximately 6:1. The etiology of SLE has remained elusive, but genetic predisposition, environmental triggers and hormonal factors are demonstrated to involve in. A higher concordance rate in monozygotic twins than dizygotic twins and the high sibling recurrence risk ratio both support a strong heritability of SLE. In the past decade, a variety of genome-wide association studies (GWAS) have recognized over 40 SLE susceptibility loci existing in exons and introns.

Soluble vascular endothelial growth factor receptor-1 (sVEGFR-1, also named as sFlt-1) is a nature vascular endothelial growth factor receptor (VEGFR) competitor. The human VEGFR1 gene is located in chromosome 13q12. It was originally found to be expressed on vascular endothelial cells, and then discovered expressed on smooth muscle cells, monocytes, trophoblasts, mesangial cells and osteoblasts. sVEGFR-1 competes with signaling receptors of VEGF (VEGFR-1/VEGFR-2) by capturing their ligands. It has a negative role in angiogenesis after binding to the VEGF. However, sVEGFR-1 interacts with endothelial cells components, showing an angiogenesis effect. Therefore, sVEGFR-1 exists multiple effects on vessel growth progression and may be a new biomarker for SLE.

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therapeutic target for VEGF-mediated pathological signaling. In systemic sclerosis (SSc) patients, lower serum level of sVEGFR-1 was detected in comparison with healthy controls. In rheumatoid arthritis (RA) patients, expression of sVEGFR-1 was increased, correlating with VEGF concentration. Serum level of sVEGFR-1 in patients with osteoarthritis (OA) was higher compared with that in non-arthritic controls. VEGFR1 genetic variant associated with RA disease activity. Collectively, sVEGFR-1 plays important roles in inflammatory and autoimmune diseases. To date, relationship of sVEGFR-1 and lupus is limited. What is the expression profile of sVEGFR-1 in lupus, if VEGFR1 gene polymorphisms relate to SLE risk needs to be discussed.

## Results

### Demographical and clinical characterization of study subjects.

The demographical characteristics of the patients with SLE and controls are shown in Table 1. For SLE patients, age was 38 (27.1–48.4) years. The age was 36 (29.0–40.0) years for RA patients, 42 (39.0–44.0) years for OA patients, 38 (26.50–46.75) years for gout patients, 44 (44.0–46.7) years for Sjögren’s syndrome (SS) patients, 37 (32.0–45.5) years for ankylosing spondylitis (AS) patients (Table 1). The Age of SLE group was matched among all control groups. For gender information about SLE group and controls, there was no gender difference between SLE patients and other study group excepting gout (P < 0.001) and AS (P < 0.001). In addition, the hypocomplementemia, proteinuria, arthritis and rash were the four dominant clinical characteristics in SLE patients (the proportion of 51.57%, 45.28%, 41.73% and 40.15%, respectively). Other clinical information was contained in Table 1.

### Correlation between serum levels of sVEGFR-1 and SLE.

Concentration of sVEGFR-1 in patients with 61 SLE was higher in comparison with that in 94 healthy subjects (7.738 (7.604–26.286) vs 12.115 (8.655–12.115) ng/ml, P = 0.015, Fig. 1A). Concentration of sVEGFR-1 levels and SLE disease activity index (SLE-DAI) was calculated, showing a statistically significant correlation (r = 0.557, P < 0.001, Fig. 1B). Higher levels of serum sVEGFR-1 were discovered in active SLE patients than in less-active patients (7.968 (5.325–20.421) vs 22.435 (13.491–36.642) ng/ml, P < 0.001, Fig. 1C). When discussing the association of serum levels of sVEGFR-1 and SLE clinical and laboratory characteristics, differences were existed in the patients with arthritis, alopecia, ds-DNA, hematuria (P = 0.001, P = 0.021, P = 0.012, P = 0.017, respectively, Fig. 1D–G). The other information was contained in Table 1.
about the relationships between sVEGFR-1 and SLE clinical characteristics were exhibited in Table 2. Moreover, ROC curve showed that the AUC was 0.615 (95% CI 0.511–0.720) (Fig. 1H). These results suggested that serum sVEGFR-1 was up-regulated in SLE and positively correlated with disease activity. In validation set, 100 SLE patients and 385 disease controls (including RA, OA, gout, SS and AS) were compared to evaluate difference of serum levels of sVEGFR-1. Analysis indicated that serum concentration of sVEGFR-1 was significantly higher in SLE than that in RA, OA, gout, SS and AS groups (20.987 (13.080–40.885) vs 6.151 (3.614–12.134), 6.208 (4.129–10.293), 3.862 (2.790–5.817), 10.975 (5.513–15.963), 7.851 (5.268–7.851) ng/ml, all \( P < 0.001\), Fig. 2A–E).

Serum sVEGFR-1 in SLE patients compared with that in RA, OA, gout, SS and AS patients showed AUC of 0.843, 0.878, 0.960, 0.776, 0.850, respectively (Fig. 2F–J). Thus, these results revealed that serum sVEGFR-1 presented a distinctive elevation in SLE.

Polymorphisms of VEGFR1 and risk of SLE. To investigate whether polymorphisms of VEGFR1 gene affect SLE, seven SNPs were analyzed between SLE patients and healthy controls. No deviation from the HWE test was observed in patients and controls for each polymorphism (\( P > 0.05\), Table 3). Results found that SLE associated with genotypes or alleles of following SNPs: rs2296188, rs9943922, rs2296283, rs7324510, rs9554322, rs9582036. The genotypes and alleles frequencies were summarized in Table 4. Compared with healthy controls, frequency of rs2296283 allele T was strongly declined in SLE patients (OR = 0.710, 95% CI 0.567–0.891, \( P = 0.003\)). Similarly, frequency of rs9554322 allele G was significantly associated with SLE (OR = 0.667, 95% CI 0.468–0.901, \( P = 0.004\)). As for frequencies of VEGFR1 genotypes, the most significant difference was observed in rs2296283, by which the genotype TT was dramatically decreased in SLE patients compared with healthy controls (OR = 0.513, 95% CI 0.327–0.804, \( P = 0.004\)). There were decreased frequencies of TT + TC in patients with

### Table 2. Analysis of serum sVEGFR-1 levels in SLE by clinical features. sVEGFR-1 soluble vascular endothelial growth factor receptor-1, SLE systemic lupus erythematosus, SLEDAI SLE disease activity index.

| Clinical features | Positive n (%) | Negative n (%) | Z    | P   |
|-------------------|----------------|----------------|------|-----|
| Vasculitis        | 10 (16.39)     | 51 (83.61)     | 1.013 | 0.311 |
| Arthritis         | 21 (34.43)     | 40 (65.57)     | 3.931 | 0.001 |
| Rash              | 23 (37.70)     | 38 (62.30)     | 0.551 | 0.582 |
| Alopecia          | 15 (24.59)     | 46 (75.41)     | 2.311 | 0.021 |
| Fever             | 11 (18.03)     | 50 (81.97)     | 1.163 | 0.245 |
| Hypocomplementemia| 21 (34.43)     | 40 (65.57)     | 1.199 | 0.230 |
| ds-DNA            | 23 (37.70)     | 38 (62.30)     | 2.188 | 0.029 |
| Thrombocytopenia  | 17 (27.87)     | 44 (72.13)     | 0.434 | 0.664 |
| Hematuria         | 19 (31.15)     | 42 (68.85)     | 2.383 | 0.017 |
| Proteinuria       | 31 (50.82)     | 30 (49.18)     | 1.774 | 0.076 |
| SLEDAI            | 38 (62.30)     | 23 (37.70)     | 3.199 | 0.001 |

Figure 1. Serum levels of sVEGFR-1 in the discovery set. (A) Enzyme-linked immunosorbent assay was used to detect serum sVEGFR-1 levels in 61 systemic lupus erythematosus (SLE) patients and 94 healthy controls. Every symbol expresses an independent SLE patient and healthy control. (B) Correlation between SLEDAI and serum levels of sVEGFR-1. (C) Difference of serum levels of sVEGFR-1 in SLE patients with less-active period and active period. (D–G) Difference of serum levels of sVEGFR-1 in several characteristics of SLE patients. (H) Potential of sVEGFR-1 as a disease marker for SLE, analyzed by receiver operating characteristic curve analysis.
SLE as compared with healthy controls (OR = 0.588, 95% CI 0.394–0.876, \( P = 0.009 \)). Frequencies of all rs9554322 genotypes (GG, GC, GG + GC) were lower in SLE group (OR = 0.417, 95% CI 0.192–0.907, \( P = 0.027 \); OR = 0.694, 95% CI 0.493–0.977, \( P = 0.036 \); OR = 0.649, 95% CI 0.468–0.901, \( P = 0.010 \)). For rs2296188 and rs9943922, frequencies of TT were both lower in SLE patients when compared with controls (OR = 0.578, 95% CI 0.344–0.972, \( P = 0.039 \); OR = 0.619, 95% CI 0.398–0.962, \( P = 0.033 \)). Moreover, we found marginal differences of genotype CC in rs7324510 and rs9582036 in SLE patients compared with healthy controls (OR = 0.538, 95% CI 0.296–0.976, \( P = 0.041 \); OR = 0.508, 95% CI 0.262–0.907, \( P = 0.046 \)). Frequencies of rs9554320 genotypes, although not statistically significant, were lower in SLE patients as compared with healthy subjects (data not show).

**VEGFR1 haplotypes and SLE risk.** In the present study, we exerted the haplotype analysis through constructing a block which comprised rs2296283, rs9943922 and rs7324510 (\( D' = 0.915, r^2 = 0.521 \); \( D' = 0.716, r^2 = 0.221 \); \( D' = 0.700, r^2 = 0.131 \)) (Fig. 3). Results revealed that the frequencies of CTA haplotype were higher in SLE patients in comparison with healthy controls (OR = 1.435, 95% CI 1.137–1.810, \( P = 0.002 \)). Proportions of CTC and TTA haplotypes were fewer in SLE patients (OR = 0.234, 95% CI 0.081–0.674, \( P = 0.003 \); OR = 0.303, 95% CI 0.197–0.465, \( P = 0.001 \)). The other haplotypes were not figured out statistical significance (Table 5).

**Assocation of VEGFR1 gene polymorphisms with clinical and laboratory features in SLE.** Given that SLE patients had diverse phenotypes and genetic predisposition, we explored association between VEGFR1 polymorphisms and clinical, laboratory features. The genotype and allele frequencies of VEGFR1 polymorphisms in SLE patients with different clinical manifestations were listed in Table 6. As the results shown, there was an increased frequency of TC genotype in patients with lupus headache in contrast to patients without this feature for rs7324510 (\( \chi^2 = 9.924, P = 0.039 \)). For rs9943922, TC genotype was different in patients with cylindruria as compared with patients without this feature (\( \chi^2 = 7.589, P = 0.026 \)). Distribution of CC, CA, AA genotypes of rs9582036 and rs9554320 was different between SLE patients with and without pyuria (\( \chi^2 = 14.437, P = 0.003 \); \( \chi^2 = 15.074, P = 0.001 \)). In addition, C allele frequency of rs7324510 was lower in SLE patients with hypocomplementemia comparing to those without (\( \chi^2 = 4.195, P = 0.041 \)). T allele frequency of rs9943922 polymorphism was different between SLE patients with cylindruria and those without (\( \chi^2 = 3.971, P = 0.046 \)). It was found that the C allele frequency of rs9554320 was increased in SLE patients with pyuria.
(χ² = 11.702, P = 0.001). No significant association was found between the other SNPs and clinical, laboratory manifestations of SLE (Supplementary Table 1).

**Variation of VEGFR1 and concentration of serum sVEGFR-1.** To discuss possible significance of VEGFR1 SNPs on serum sVEGFR-1 concentration, serum sVEGFR-1 levels were compared according to genotypes of individual SNP. Analysis indicated that serum levels of sVEGFR-1 were significantly different among SLE patients carrying GG, GC, CC genotypes for rs9554322 (P = 0.027, Fig. 4A). The other SNPs were not related to serum levels of sVEGFR-1 (Fig. 4B–G).

| SNP          | Genotype | SLE (N = 254) n (%) | Controls (N = 390) n (%) | OR (95% CI) | P value |
|--------------|----------|---------------------|--------------------------|-------------|---------|
| rs2296188    | TT       | 25 (9.8)            | 62 (15.9)                | 0.578 (0.344–0.972) | 0.039   |
|              | TC       | 109 (43.0)          | 156 (40.0)               | 1.001 (0.714–1.404) | 0.993   |
|              | TT + TC  | 134 (52.8)          | 218 (55.9)               | 0.881 (0.641–1.121) | 0.434   |
|              | CC       | 120 (47.2)          | 172 (44.1)               | Reference    |         |
|              | T        | 159 (31.3)          | 280 (35.9)               | 0.814 (0.614–1.032) | 0.089   |
|              | C        | 349 (68.7)          | 500 (64.1)               | Reference    |         |
| rs9443922    | TT       | 62 (24.4)           | 127 (32.6)               | 0.619 (0.398–0.962) | 0.033   |
|              | TC       | 125 (49.2)          | 178 (45.6)               | 0.891 (0.601–1.321) | 0.565   |
|              | TT + TC  | 187 (73.6)          | 305 (78.2)               | 0.778 (0.538–1.124) | 0.181   |
|              | CC       | 67 (26.4)           | 85 (21.8)                | Reference    |         |
|              | T        | 249 (49.0)          | 432 (55.4)               | 0.775 (0.619–0.969) | 0.025   |
|              | C        | 259 (51.0)          | 348 (44.6)               | Reference    |         |
| rs2296283    | TT       | 79 (31.1)           | 154 (39.5)               | 0.513 (0.327–0.804) | 0.004   |
|              | TC       | 115 (45.3)          | 176 (45.1)               | 0.654 (0.427–1.003) | 0.051   |
|              | TT + TC  | 194 (76.4)          | 330 (84.6)               | 0.588 (0.394–0.876) | 0.009   |
|              | CC       | 60 (23.6)           | 60 (15.4)                | Reference    |         |
|              | T        | 273 (53.8)          | 484 (62.1)               | 0.710 (0.567–0.891) | 0.003   |
|              | C        | 235 (46.2)          | 296 (37.9)               | Reference    |         |
| rs7324510    | CC       | 17 (6.7)            | 46 (11.8)                | 0.538 (0.296–0.976) | 0.041   |
|              | CA       | 94 (37.0)           | 136 (34.9)               | 1.005 (0.717–1.410) | 0.975   |
|              | CC + CA  | 111 (43.7)          | 182 (46.7)               | 0.887 (0.646–1.220) | 0.460   |
|              | AA       | 143 (56.3)          | 208 (53.3)               | Reference    |         |
|              | C        | 128 (25.2)          | 229 (29.3)               | 0.810 (0.629–1.044) | 0.103   |
|              | A        | 380 (74.8)          | 551 (70.7)               | Reference    |         |
| rs9554322    | GG       | 9 (3.5)             | 28 (7.2)                 | 0.417 (0.192–0.907) | 0.027   |
|              | GC       | 77 (30.3)           | 144 (36.9)               | 0.694 (0.493–0.977) | 0.036   |
|              | GG + GC  | 86 (33.8)           | 172 (44.1)               | 0.649 (0.468–0.901) | 0.010   |
|              | CC       | 168 (66.2)          | 218 (55.9)               | Reference    |         |
|              | G        | 95 (18.7)           | 200 (25.7)               | 0.667 (0.507–0.878) | 0.004   |
|              | C        | 413 (81.3)          | 580 (74.3)               | Reference    |         |
| rs9582036    | CC       | 13 (5.1)            | 37 (9.4)                 | 0.508 (0.262–0.907) | 0.046   |
|              | CA       | 80 (31.5)           | 120 (30.8)               | 0.965 (0.682–1.364) | 0.840   |
|              | CC + CA  | 93 (36.6)           | 157 (40.2)               | 0.857 (0.619–1.188) | 0.354   |
|              | AA       | 161 (63.4)          | 233 (59.8)               | Reference    |         |
|              | C        | 106 (20.8)          | 183 (24.8)               | 0.802 (0.613–1.049) | 0.108   |
|              | A        | 402 (79.2)          | 587 (75.2)               | Reference    |         |
| rs9554320    | CC       | 141 (55.5)          | 234 (60.0)               | 1.019 (0.498–2.088) | 0.957   |
|              | CA       | 100 (39.4)          | 134 (34.3)               | 1.263 (0.607–2.632) | 0.532   |
|              | CC + CA  | 241 (94.9)          | 368 (94.3)               | 1.109 (0.548–2.242) | 0.775   |
|              | AA       | 13 (5.1)            | 22 (5.6)                 | Reference    |         |
|              | C        | 382 (75.2)          | 602 (77.2)               | 0.931 (0.397–1.869) | 0.841   |
|              | A        | 126 (24.8)          | 178 (22.8)               | Reference    |         |

Table 4. Allele and genotype frequencies of seven SNPs in the VEGFR1 gene in SLE patients and healthy controls. SNP single nucleotide polymorphism, SLE systemic lupus erythematosus, OR odds ratio, CI confidence interval.
Statistical power. According to \( \alpha = 0.05 \), \( OR = 1.8 \), and the MAF of each SNP, we calculated the statistical power. In the present study, the following powers were listed: 0.953 for rs2296188, 0.953 for rs9943922, 0.953 for rs2296283, 0.948 for rs7324510, 0.951 for rs9554322, 0.941 for rs9582036, respectively.

Discussion
In the present study, we revealed that sVEGFR-1 serum levels associated with severity of SLE as well as \( VEGFR1 \) genetic variants existed in Chinese Han population. These findings added evidence that serum sVEGFR-1 and \( VEGFR1 \) gene were implicated in SLE pathogenesis. To our knowledge, this is the first study describing the network of serum sVEGFR-1, \( VEGFR1 \) gene and SLE risk. According to the findings, we observed that SLE patients showed higher serum levels of sVEGFR-1 which positively correlated with disease activity. Furthermore, genotype TT of rs2296188, TT, TT + TC of rs2296283, CC of rs7324510, GG, GC, GG + GC of rs9554322 and CC of rs9582036 related to genetic susceptibility of SLE. SLE patients carrying rs2296283 G allele, rs9554322 T allele had decreased risk of SLE. Moreover, we found that \( VEGFR1 \) rs9554322 polymorphism may be a genetic factor for regulating sVEGFR-1 expression.

SLE is an autoimmune disease with a panel of clinical manifestations such as lupus nephritis that results from a variety of immunological and vascular abnormalities. Relationship between sVEGFR-1 and SLE is not clearly elucidated to date. VEGF is a crucial factor in circulating angiogenesis. It is able to modulate proliferation of endothelial cells, regulate chemotaxis, and capillary hyperpermeability in angiogenesis. Several studies found that serum concentration of VEGF was elevated in SLE patients. Renal glomerular microvasculature

Figure 3. Linkage disequilibrium of seven SNPs. Seven SNPs (rs2296188, rs9943922, rs2296283, rs7324510, rs9554322, rs9582036, rs9554320) were selected in the study. The intensity of LD is reflected in the color and numeric value (\( D' \)) of each box. Red and pink represent significant linkage, light blue and white indicate no linkage. \( D' \) value varies from 0 to 1, and the 1 value represents the maximum linkage.

| Haplotype | SLE n (%) | Controls n (%) | \( \chi^2 \) | \( P \) values | OR (95% CI) |
|-----------|-----------|----------------|------------|---------------|-------------|
| CTA       | 217.23 (42.8) | 266.00 (41.1) | 9.320 | 0.002 | 1.435 (1.137–1.810) |
| CTC       | 4.03 (0.8) | 25.54 (3.5) | 8.557 | 0.003 | 0.234 (0.081–0.674) |
| TCA       | 125.43 (24.7) | 164.34 (21.1) | 2.101 | 0.147 | 1.218 (0.933–1.589) |
| TCC       | 119.83 (23.6) | 173.21 (22.2) | 0.252 | 0.615 | 1.071 (0.820–1.398) |
| TTA       | 27.74 (5.5) | 123.62 (15.8) | 32.701 | 0.001 | 0.303 (0.197–0.465) |
| TTC       | 0.00 (0.0) | 20.84 (2.7) | – | – | – |
| CCA       | 9.61 (1.9) | 0.04 (0.0) | – | – | – |
| CCC       | 4.13 (0.8) | 6.41 (0.8) | – | – | – |

Table 5. Haplotype analysis between SLE patients and Healthy controls. Haplotype comprised rs2296283, rs9943922 and rs7324510. SLE systemic lupus erythematosus, \( OR \) odds ratio, \( CI \) confidence interval.
is susceptible to local VEGF-A\textsuperscript{52}, VEGF mediates the glomerular endothelial cell proliferation and survival in damaged glomerular capillaries\textsuperscript{23}. The dysregulation of VEGF is involved in initiation of glomerular injury\textsuperscript{24}. Therefore, regulation of VEGF is important in lupus nephritis development. sVEGFR-1 was encoded by a specifically spliced form of VEGFR-1 mRNA. It is comprised of 656 N-terminal residues of the receptor, with a specific 30 amino acid tail at C-terminus. In SLE patients, plasma levels of sVEGFR-1 were found higher in patients with active lupus nephritis\textsuperscript{25}. Similarly, in our study, we found that serum sVEGFR-1 was up-regulated in SLE and positively correlated with disease activity. In the inflammatory circumstance, sVEGFR-1 was secreted by activated monocytes\textsuperscript{25}. High concentration of sVEGFR-1 antagonized the protective effect of VEGF, aggravating endothelial cell damage\textsuperscript{26}. Therefore, it is hypothesized that sVEGFR-1 was up-regulated in the inflammatory circumstance of SLE, which bound to VEGF, leading to endothelial cell damage, further promoting inflammation in SLE.

Concerning VEGFR1 gene polymorphisms, our findings found that six SNPs (rs2296188, rs9943922, rs2296283, rs7324510, rs9554322, rs9582036) correlated with risk of SLE. It is worthy that rs9554322 polymorphisms may negatively correlate with the risk of SLE in Chinese Han population (OR = 0.417, OR = 0.694, OR = 0.649). Genotype CC of rs9582036 was related to SLE risk (OR = 0.508). It is possible that regulatory, structural or quantitative polymorphisms at the VEGFR1 locus may affect VEGF signaling pathway and enhance susceptibility to some

| Clinical features | rs9943922 | Allele frequency, n (%) | P value | rs7324510 | Allele frequency, n (%) | P value |
|------------------|-----------|-------------------------|---------|-----------|-------------------------|---------|
|                  | TT       | TC                      | CC      | P         | T          | C       | P         | C          | A         | P         |
| Lupus headache   | Positive | 2 (12.50)               | 6 (37.50) | 8 (50.00) | 0.099      | 11 (34.38) | 21 (65.62) | 0.087      | 4 (25.00) | 6 (37.50) | 0.039      |
|                  | Negative | 60 (25.21)              | 119 (50.00) | 59 (24.79) | 238 (50.00) | 238 (50.00) | 13 (5.46) | 88 (36.98) | 137 (57.56) | 116 (24.37) | 360 (75.63) |

| Hypocomplementemia | Positive | 31 (23.66) | 65 (49.62) | 35 (27.18) | 0.960 | 128 (48.86) | 134 (51.14) | 0.940 | 5 (3.82) | 46 (38.12) | 80 (61.06) | 0.095 | 56 (21.37) | 206 (78.63) | 0.041 |
|                    | Negative | 31 (25.20) | 60 (48.78) | 32 (26.02) | 121 (49.19) | 125 (50.81) | 12 (9.76) | 48 (39.02) | 63 (51.22) | 72 (29.27) | 174 (70.73) |

| Cylindruria | Positive | 3 (25.00) | 2 (16.67) | 7 (58.33) | 0.026 | 7 (21.17) | 17 (70.83) | 0.046 | 1 (8.33) | 3 (25.00) | 8 (66.67) | 0.676 | 5 (20.83) | 19 (79.17) | 0.614 |
|            | Negative | 59 (24.38) | 123 (50.83) | 60 (24.79) | 12 (9.76) | 48 (39.02) | 63 (51.22) | 72 (29.27) | 174 (70.73) | 361 (74.57) |

| Pyuria | Positive | 2 (9.09) | 13 (59.09) | 7 (31.82) | 0.216 | 17 (0.386) | 27 (61.36) | 0.150 | 1 (4.54) | 7 (31.82) | 14 (63.64) | 0.744 | 9 (20.45) | 35 (79.55) | 0.448 |
|        | Negative | 60 (25.86) | 112 (48.28) | 60 (25.86) | 123 (50.00) | 233 (50.00) | 16 (6.90) | 87 (37.50) | 129 (55.60) | 119 (25.65) | 345 (74.35) |

**Table 6.** Analysis of VEGFR1 gene polymorphisms in SLE by clinical, laboratory features. SLE systemic lupus erythematosus.
angiogenic conditions. In our study, rs2296283 located in the functional 3′-UTR region and other three SNPs (rs2296188, rs9943922 and rs7324510) were within the introns. In a Polish study, there was no statistic difference in allele or genotypes frequencies of rs2296283 between RA patients and healthy controls. On the contrary, we obtained a statistical difference in the frequencies of genotype TT and TT + TC for rs2296283 in SLE patients (OR = 0.513, OR = 0.588). Compared with the Polish study, we further observed statistic differences in genotype TT of rs2296188, TT of rs9943922 and CC of rs7324510 (OR = 0.578, OR = 0.619, OR = 0.538), which were encoded in the introns. Therefore, rs2296283 located in the functional region of VEGFR1 gene may influence the mRNA translation and stability through regulating polyadenylation, miRNA-mRNA and protein-mRNA interactions. However, further study needs to verify the effect of rs2296283 within the functional region. The significance of SNPs located in non-coding region of VEGFR1 were not clear. Several studies showed that polymorphisms in non-coding region of VEGFR1 possibly silenced or enhanced the transcriptional activity of sVEGFR-1. Thus, it is postulated that polymorphisms in the non-coding region may implicate in selective splicing of RNA and promote the transcriptional activity of target protein. It is well known that SNP is the predominant pattern in the genomic DNA sequence variation. The majority of SNPs affected the gene expression instead of the protein composition. In agreement with previous studies, our results exhibited that six of seven SNPs (including rs2296188, rs9943922, rs2296283, rs7324510, rs9554322, rs9582036, rs9554320) were not related with sVEGFR-1 levels. Several GWAS studies indicated that although most of SNPs did not possess the direct ability to change the gene expression, these SNPs sometimes served for the functional counterparts which regulated gene expression and protein assembly. Thus, we hypothesized that the six SNPs selected in this study may not directly influence sVEGFR-1 concentration in SLE patients. On the other side, serum levels of sVEGFR-1 were different among rs9554322 genotypes (P = 0.029). SLE patients with rs9554322 GG genotype had higher sVEGFR-1 protein levels, suggesting that VEGFR1 rs9554322 G/C genetic variant may contribute to abnormal sVEGFR-1 serum levels. However, what is the exact role of polymorphisms in VEGFR1 gene, sVEGFR-1 protein expression needs to be discussed and whether the selected SNPs in the current study can affect VEGFR1 gene, sVEGFR-1 protein expression needs to be conducted by functional study in the future.

There are several limitations in this study. First, the sample size in our study is relatively limited, where cases were recruited from two hospitals. Considering the large number of Chinese Han population, larger scale and multi-center studies are needed in the future. Second, the clear mechanism of sVEGFR-1 involves in SLE pathogenesis needs to discuss.

In summary, the present study showed that serum levels of sVEGFR-1 were elevated in SLE patients, may be a disease marker, and VEGFR1 gene polymorphisms related to risk of SLE in a Chinese Han population.

Materials and methods

Study subjects. Case–control studies were conducted for sVEGFR-1 serum levels and gene polymorphisms. A total of 254 SLE patients, 385 other rheumatic diseases patients and 390 healthy controls were recruited in the present study. Test for serum levels of sVEGFR-1 was performed in two stages. The discovery set included 61 SLE and 94 healthy controls, and the validation set comprised another independent 100 SLE and 385 SLE-free disease controls [100 RA, 100 OA, 100 gout, 44 Sjögren’s syndrome (SS) and 41 ankylosing spondylitis (AS)]. The study of gene polymorphisms consisted of 254 SLE and 390 healthy controls. All patients were recruited...
from the Department of Rheumatology and Immunology, Affiliated Hospital of Southwest Medical University and Affiliated Minda Hospital of Hubei Minzu University, classified by 1997 American College of Rheumatology (ACR) revised criteria for SLE31, 1987 ACR revised criteria for RA32, Osteoarthritis Criteria Subcommittee of the American Rheumatism Association (ARA) criteria for OA33, 1977 ARA criteria for gout34, American-European classification criteria for SS35 and Modified New York criteria for AS36. The SLE disease activity index (SLEDAI) was calculated to evaluate the disease activity of SLE patients37. Based on the SLEDAI score, the disease activity of SLE patients were divided into less-active period (SLEDIA < 10) and active period (SLEDIA ≥ 10). Age and sex matched healthy controls were selected from Jiangyang district center for disease control and prevention in Luzhou, having no history of SLE and other inflammatory autoimmune diseases. All the participants were Chinese Han origin. The Medical Ethics Committee of Southwest Medical University approved our study protocol. Written informed consent was obtained from each subject. Blood samples were collected from patients and healthy controls.

SNP selection. A systemic search for previous literature about VEGFR1 gene was performed. Based on the 1,000-genome project (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/), all candidate SNPs complied with following screening criteria: pairwise tagging of HapMap population with r² ≥ 0.8; a minor allele frequency (MAF) ≥ 5%; Chinese Han Beijing (CHB) ethnicity. At last, seven SNPs including rs2296188, rs9943922, rs2296283, rs7324510, rs9554322, rs9582036, rs9554320 were selected.

DNA extraction and genotyping analysis. Peripheral blood was collected from ulnar veins in the fasting and clearheaded state. Samples were centrifuged and serum was stored at −80 °C until analysis. Genomic DNA was extracted utilizing TIANamp Blood DNA kits (TIANGEN, Beijing, China) in line with manufacturer’s instructions. VEGFR1 genotyping reactions were completed by Gene company using KASP (Gene Company, Shanghai, China). Information of KASP primers (Primer_AleleFAM, Primer_AleleHEX and Primer_Common) was listed in Supplementary Table 2. To prove the reliability of genotyping results, five percent of the whole samples were repeatedly genotyped. Concordance rate of the repeated cases performed 100%, demonstrating that the results were reliable in this study.

sVEGFR-1 protein measurement. sVEGFR-1 protein levels of SLE patients and control groups were assessed by enzyme-linked immunosorbent assay (ELISA) kits (Cusbio, Houston, USA) in accordance with the manufacturer’s protocol. All samples were measured in duplicates and plates were read automatically at an absorbance of 450 nm using LT-4000MS reader (Labtech International Ltd, East Sussex, UK). Concentration was calculated on the basis of a linear standard curve. The detection limit was 0.039 ng/ml.

Statistical analysis. Data was performed by Statistical Package for the Social Sciences (SPSS Inc., Chicago, version 17.0). Categorical data were expressed as frequency and percentage. According to Shapiro–Wilks test, measurement data were expressed by mean ± standard deviation (SD) when it was normally distributed or median (inter-quartile range) when it was not normally distributed. For comparison of genotype and allele distribution between cases and controls, chi-squared or Fisher’s exact test were used as appropriate. Odds ratio (OR) and 95% confidence interval (CI) were analyzed by logistic regression model. Relationship between two variables was evaluated using Spearman’s rank test. Area of receiver operating characteristic (ROC) curve evaluated the specificity and sensitivity of predictive power of sVEGFR-1 in SLE. Hardy–Weinberg equilibrium (HWE) of genotypes in patients and healthy controls was estimated by chi-squared test. HaploView 4.1 software was used to analyze linkage disequilibrium (LD). VEGFR1 haplotypes were assessed by using online software SHEsis (https://analysis.bio-x.cn). Statistical power was assessed (https://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). P value lower than 0.05 was significant.

Data availability All data supporting the results of this study are available in the article and supplementary information files or are available from the authors upon request.

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Author contributions
Z.-C.Y. contributed to the data analysis and manuscript editing. J.-M.W. was in charge of sample collection and DNA extraction. Q.W. and J.Z. performed DNA extraction. W.-D.X. and A.-F.H. contributed to ELISA detection and reviewing of the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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