Association of leptin and leptin receptor gene polymorphisms with systemic lupus erythematosus in a Chinese population

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Abstract

To explore the association of LEPL and leptin receptor (LEPR) gene single-nucleotide polymorphisms (SNPs) with susceptibility to systemic lupus erythematosus (SLE) in a Chinese population. Four LEPL SNPs (rs11761556, rs12706832, rs2071045 and rs2167270) and nine LEPR SNPs (rs10749754, rs1137100, rs1137101, rs13306519, rs8179183, rs1805096, rs3790434, rs3806318 and rs7518632) were genotyped in a cohort of 633 patients with SLE and 559 healthy controls. Genotyping of SNPs was performed with improved multiple ligase detection reaction (iMLDR). No significant differences were detected for the distribution of allele and genotype frequencies of all 13 SNPs between patients with SLE and controls. The genotype effects of recessive, dominant and additive models were also analysed, but no significant evidence for association was detected. However, further analysis in patients with SLE showed that the TT genotype and T allele frequencies of the LEPL rs2071045 polymorphism were nominally significantly higher in patients with pericarditis (P = 0.012, P = 0.011, respectively). In LEPR, the GA/AA genotype and A allele frequencies of the rs1137100 polymorphism were both nominally associated with photosensitivity in patients with SLE (P = 0.043, P = 0.018, respectively). Moreover, the genotype and allele distribution of rs3806318 were also nominally associated with photosensitivity in patients with SLE (P = 0.013, P = 0.008, respectively). No significant differences in serum leptin levels were observed in patients with SLE with different genotypes. In summary, LEPL and LEPR SNPs are not associated with genetic susceptibility to SLE, but may contribute to some specific clinical phenotype of this disease; further studies are necessary to elucidate the exact role of LEPL and LEPR genes in the pathogenesis of SLE.

Keywords: leptin • single-nucleotide polymorphisms • systemic lupus erythematosus

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder involving multiple organs and tissues such as skin, kidneys, joints, lung and central nervous system. The aetiology and pathogenesis of SLE generally considers an involvement of environmental factors, which could trigger abnormal autoimmune responses in individuals who carry a predisposing genetic background [1]. Therefore, genetic variants within immune-modulating genes may confer the risk of SLE.

Leptin is an adipokine that plays a key role in the modulation of immune responses and the development and maintenance of inflammation [2]. It is a 16-kD non-glycosylated polypeptide hormone mainly produced by white adipose tissue (WAT), encoding by the obese (ob) gene of murine homolog of human LEPL gene [3]. Investigations have shown that leptin is increased during acute infection and inflammation, indicating that leptin acts as a pro-inflammatory cytokine. It enhances macrophage phagocytosis activity and stimulates them to produce several pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α [4]. Leptin exerts its biological actions through the activation of leptin receptor, which belongs to the class 1 cytokine receptor superfamily and are encoded by the diabetes (db) gene [5]. Numerous studies have shown abnormal increase in serum/plasma leptin levels in patients with SLE [6–9], but the data regarding association between leptin-related gene polymorphisms and SLE is still very limited. Afroz
et al. [10] showed that a LEP gene polymorphism (LEPR Q223R) was associated with SLE susceptibility in a Kashmiri population. A recent study assessed LEP and LEPR gene polymorphisms in four different ancestral groups with SLE, but the results did not support associations between leptin-related polymorphisms and increased SLE susceptibility [11]. In this study, we carried out a case–control study to explore whether LEP and LEPR gene polymorphisms are associated with SLE susceptibility in a Chinese population.

Materials and methods

Study participants

A total of 633 patients with SLE were recruited from the Department of Rheumatology and Immunology at the First Affiliated Hospital of Anhui Medical University, Anhui Provincial Hospital and Anqing Hospital Affiliated to Anhui Medical University. All patients met the 1997 American College of Rheumatology (ACR) revised criteria for the classification of SLE [12]. The disease severity was quantified according to the SLE disease activity index 2000 (SLEDAI-2K) [13]. More active SLE was defined as a SLEDAI-2K score >10, and those patients with SLEDAI-2K ≤10 were classed as relatively inactive [14, 15]. Normal controls were recruited from the general population and healthy blood donors and were geographically and ethnically matched with patients with SLE. All the normal controls did not have a history of SLE, other inflammatory/autoimmune diseases or cancer. The demographic and clinical features were collected from the medical records or by questionnaire and reviewed by experienced physicians. The study was approved by the Medical Ethics Committee of Anhui Medical University. All participants were enrolled after informed consent had been obtained.

SNP selection, genotyping and enzyme-linked immunosorbent assay (ELISA)

We conducted a search for the LEP and LEPR gene single-nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) ≥0.05 within the Han Chinese population (CHB) of Beijing, China, as listed in the international HapMap Project database (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36; HapMap Data Rel 24/phasell Nov08, on NCBI B36 assembly, dbSNP b126). Then, linkage disequilibrium (LD) analysis with an \( r^2 \) threshold of 0.8 was performed with Haploview 4.2 software (Broad Institute, Cambridge, MA, USA) for tagging SNP selection. Under these criteria, six tag SNPs in LEP and 26 tag SNPs in LEPR were selected for further evaluation. We used the bioinformatics tools F-SNP (http://compbio.cs.queensu.ca/F-SNP/) and SNP function prediction (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm) to assess the predicted functional effects of each tag SNP [16, 17]. The test for functional SNP aimed to evaluate the potentially deleterious functional impact at the splicing, transcriptional, translational and post-translational level. The basic information of these tag SNPs is shown in Table S1. In addition, the existing literature studies about the LEP and LEPR gene polymorphisms were reviewed. Finally, a total of four tag SNPs (rs11761556, rs12706832, rs2071045 and rs2167270) in LEP and nine tag SNPs (rs10749754, rs1137100, rs1137101, rs13306519, rs8179183 (rs1805094), rs1805096, rs3790434, rs3806318 and rs7518632) in LEPR were included for genotyping in our study cohort. The selected SNPs were genotyped in both case and control groups performed with improved multiple ligase detection reaction (iMLDR) genotyping assays, with technical support from the Center for Genetic & Genomic Analysis, Genesky Biotechnologies Inc., Shanghai.

Serum leptin level was determined by ELISA kits according to the manufacturer’s instruction (R&D Systems, Inc. Minneapolis, MN, USA), and the results were expressed as nanogram per millilitre.

Statistical analysis

Differences in genotype and allele frequencies between the two groups were analysed using chi-square or Fisher’s exact test. Comparisons of serum leptin level between different groups of clinical features and genotypes were conducted using nonparametric test. Odds ratios (ORs) and 95% confidence interval (CIs) were estimated by non-conditional logistic regression analyses. All these statistical analyses were performed using SPSS 10.01 software (SPSS Inc., Chicago, IL, USA).

Hardy–Weinberg equilibrium (HWE) was evaluated in normal controls, and haplotype tests were performed by the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [18]. A two-sided \( P \) value of <0.05 was considered as statistically significant. The Bonferroni correction was used for multiple testing.

Results

In this study, we included a total of 633 SLE cases and 559 healthy controls. In patients, there were 60 males and 573 females with a mean age of 39.4 ± 12.74 years. The demographic characteristics and clinical features of 633 patients with SLE are shown in Table 1. The most commonly reported clinical manifestations were arthritis (287 (46.52)%), followed by photosensitivity (57 (9.24)%), oral ulcers (74 (11.99)%), discoid rash (65 (10.53)%), malar rash (225 (36.47)%), and pleurisy (22 (3.57)%). The numbers of female and male patients were 573 (90.52)% and 63 (9.48)% respectively. The characteristics of patients with SLE are presented in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients with SLE (n = 633)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Age, year</td>
<td>39.4 ± 12.74</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>60 (9.48)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>573 (90.52)</td>
</tr>
<tr>
<td><strong>Clinical manifestations</strong></td>
<td></td>
</tr>
<tr>
<td>Malar rash, n (%)</td>
<td>225 (36.47)</td>
</tr>
<tr>
<td>Discoid rash, n (%)</td>
<td>65 (10.53)</td>
</tr>
<tr>
<td>Photosensitivity, n (%)</td>
<td>57 (9.24)</td>
</tr>
<tr>
<td>Oral ulcers, n (%)</td>
<td>74 (11.99)</td>
</tr>
<tr>
<td>Arthritis, n (%)</td>
<td>287 (46.52)</td>
</tr>
<tr>
<td>Pleurisy, n (%)</td>
<td>22 (3.57)</td>
</tr>
<tr>
<td>Pericarditis, n (%)</td>
<td>16 (2.59)</td>
</tr>
<tr>
<td>Renal disorder, n (%)</td>
<td>78 (12.64)</td>
</tr>
<tr>
<td>Neurological disorder, n (%)</td>
<td>22 (3.57)</td>
</tr>
</tbody>
</table>

\( n \): number; SLE: systemic lupus erythematosus.
mean age of 39.40 ± 12.74 years, while six males and 553 females in controls with a mean age of 42.92 ± 16.57 years. The clinical features of the patients are shown in Table 1. The main clinical manifestations were arthritis (46.52%), malar rash (36.47%), renal disorder (12.64%) and oral ulcers (11.99%) (Table 1). The observed genotype frequencies of all detected SNPs were distributed in compliance with the HWE in control groups (all \( P > 0.05 \)).

**Association of LEP and LEPR gene polymorphisms with risk of SLE**

The result of allele frequency and genotype frequency of 13 SNPs in patients with SLE and controls are shown in Tables 2 & 3. There were no significant differences in allele and genotype distribution between patients with SLE and controls in all of these SNPs (all \( P > 0.05 \)). We also evaluated the association of LEP and LEPR gene polymorphisms with SLE under dominant, recessive and additive model (Tables 2 & 3). Consistently, none of these polymorphisms achieved a significant difference between cases and controls (all \( P > 0.05 \)).

**Association of LEP and LEPR gene polymorphisms with clinical features in patients with SLE**

To examine the potential genetic association between leptin-related gene polymorphisms and specific clinical features in SLE, we conducted a case-only analysis and summarized the results in Tables 4 & 5. In LEP, the TT genotype and T allele frequencies of the rs2071045 polymorphism were significantly higher in patients with pericarditis compared with patients without this feature (\( P = 0.012, P = 0.011 \), respectively). The A allele of the rs11761556 was significantly higher in patients with malar rash (\( P = 0.044 \)). However,

### Table 2 Genotype frequencies of LEP SNPs in patients with SLE and healthy controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Analysed model</th>
<th>SLE (( N = 633 ))</th>
<th>Control (( N = 559 ))</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11761556</td>
<td>Genotypes</td>
<td>AA 337</td>
<td>305</td>
<td>0.962</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 249</td>
<td>211</td>
<td>0.740</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 47</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>AA 337</td>
<td>305</td>
<td>0.962</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 47</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>rs12706832</td>
<td>Genotypes</td>
<td>GG 347</td>
<td>317</td>
<td>0.924</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 249</td>
<td>209</td>
<td>0.813</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 37</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>GG 347</td>
<td>317</td>
<td>0.924</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 37</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>rs2071045</td>
<td>Genotypes</td>
<td>CC 191</td>
<td>187</td>
<td>0.774</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT 336</td>
<td>263</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT 106</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>CC 191</td>
<td>187</td>
<td>0.774</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT 106</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>rs2167270</td>
<td>Genotypes</td>
<td>GG 391</td>
<td>369</td>
<td>0.727</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 215</td>
<td>167</td>
<td>0.760</td>
</tr>
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<td></td>
<td></td>
<td>AA 27</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>GG 391</td>
<td>369</td>
<td>0.726</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 27</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

*\( N \): number; SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

*The \( P \) values are not corrected for multiple testings, Bonferroni corrected \( P = 0.0167 \).
no significant associations between other SNPs in *LEP* and clinical features of SLE were observed. In *LEPR*, the GA/GG genotype and G allele frequencies of the rs3806318 polymorphism achieved significant difference between patients with and without photosensitivity ($P = 0.013$, $P = 0.008$, respectively). In addition, the GA/AA genotype and A allele of rs1137100 also demonstrated an increased

<table>
<thead>
<tr>
<th>SNP</th>
<th>Analysed model</th>
<th>SLE ($N = 633$)</th>
<th>Control ($N = 559$)</th>
<th>$P$ value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10749754</td>
<td>Genotypes</td>
<td>AA 468</td>
<td>415</td>
<td>0.801</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 156</td>
<td>135</td>
<td>0.766</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>AA 468</td>
<td>415</td>
<td>0.801</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>rs1137100</td>
<td>Genotypes</td>
<td>GG 440</td>
<td>385</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 165</td>
<td>160</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 28</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>GG 440</td>
<td>385</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 28</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>rs1137101</td>
<td>Genotypes</td>
<td>GG 478</td>
<td>427</td>
<td>0.987</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 145</td>
<td>123</td>
<td>0.901</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>GG 478</td>
<td>427</td>
<td>0.987</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA 10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>rs13306519</td>
<td>Genotypes</td>
<td>CC 426</td>
<td>393</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG 171</td>
<td>145</td>
<td>0.208</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 36</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>CC 426</td>
<td>393</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 36</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>rs8179183</td>
<td>Genotypes</td>
<td>CC 579</td>
<td>502</td>
<td>0.283</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC 53</td>
<td>54</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>CC 579</td>
<td>502</td>
<td>0.343†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>rs1805096</td>
<td>Genotypes</td>
<td>AA 480</td>
<td>437</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 145</td>
<td>112</td>
<td>0.327</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>AA 480</td>
<td>437</td>
<td>0.506</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 8</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Genotype frequencies of *LEPR* SNPs in patients with SLE and healthy control
risk of photosensitivity ($P = 0.043$, $P = 0.018$, respectively) in patients with SLE. No significant associations of other SNPs in LEPR with clinical features were found. Furthermore, there was no significant difference in the genotype distribution between patients with SLEDAI >10 and patients with SLEDAI ≤10 (Tables 4 & 5).

**Table 3. Continued**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Analysed model</th>
<th>SLE ($N = 633$)</th>
<th>Control ($N = 559$)</th>
<th>$P$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3790434</td>
<td>Genotypes</td>
<td>CC 446</td>
<td>415</td>
<td>0.235</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT 178</td>
<td>130</td>
<td>0.088</td>
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<td></td>
<td></td>
<td>TT 9</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>CC 446</td>
<td>415</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT 9</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>rs3806318</td>
<td>Genotypes</td>
<td>AA 512</td>
<td>448</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA 112</td>
<td>107</td>
<td>0.214</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Additive model</td>
<td>AA 512</td>
<td>448</td>
<td>0.279†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG 9</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>rs7518632</td>
<td>Genotypes</td>
<td>AA 403</td>
<td>352</td>
<td>0.324</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 198</td>
<td>186</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
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<td>CC 32</td>
<td>21</td>
<td></td>
</tr>
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<td></td>
<td>Additive model</td>
<td>AA 403</td>
<td>352</td>
<td>0.323</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC 32</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

*The $P$ values are not corrected for multiple testings, Bonferroni corrected $P = 0.0167$. †Calculated by Fisher’s exact test (exact $P$ value).

**Table 4** The positive findings of association between genotype frequencies in LEP and clinical characteristics

<table>
<thead>
<tr>
<th>Gene (SNP)</th>
<th>Allele (M/m)</th>
<th>Clinical features</th>
<th>Group</th>
<th>Genotypes $n$ (%)</th>
<th>$P$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2071045</td>
<td>C/T</td>
<td>Pericarditis</td>
<td>Positive</td>
<td>MM 2, Mm 7, mm 7</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>181, 322, 98</td>
<td></td>
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</tbody>
</table>

*The $P$ values are not corrected for multiple testings, Bonferroni corrected $P = 0.0167$. Bold value means $P < 0.05$.

Association of serum leptin levels with LEP and LEPR genotypes in patients with SLE

We examined the associations between serum leptin levels with different LEP and LEPR genotypes (Table 6). However, no significant differences in serum leptin levels were observed between patients with different LEP or LEPR genotypes.

**Haplotype analyses**

Five main haplotypes (AATC, GATC, GGCA, GGCC and GGTA) for LEP gene and five main haplotypes (ACACGAGC, ACGCAGCA, ACGGAGCA, ATGCAGCAA and GGCAGCAGA) for LEPR gene were identified using SHEsis software (Tables 7 & 8). The results revealed that the haplotypes ACGCAGCA and ATGCAGCAA were significantly associated with SLE, and the ACGCAGCAGA appeared to be a significant
and osteoarthritis (OA) [26]. Various autoimmune diseases such as rheumatoid arthritis (RA) can be triggered by genetic factors and environmental factors [25]. Similar studies have also been conducted among different populations to evaluate the role of plasma/serum leptin level between patients with SLE and normal controls [23, 24]. Our recent study demonstrated lower or unchanged circulating leptin levels in patients with SLE compared to healthy controls [23, 24].

Systemic lupus erythematosus is a severe autoimmune disease with multiple serological alterations and affecting various organs. Although the aetiology is still unclear, it is generally acknowledged that this disease has complex genetic and environmental backgrounds. Recent studies have shown that leptin levels are elevated in patients with SLE and involved in the pathogenesis of this disease [19–22]. However, some other groups have demonstrated lower or unchanged circulating leptin levels in patients with SLE compared to healthy controls [23, 24]. Our recent work, a meta-analysis, also found no significant difference in plasma/serum leptin level between patients with SLE and normal controls [25]. Similar studies have also been conducted among various autoimmune diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) [26–28]. A recent study showed that serum leptin level and serum leptin/leptin receptor ratio imbalance were positively correlated with anticyclic citrullinated peptide antibodies in RA [29] and might act as a predictor for disease activity [30]. Therefore, LEP and LEPR gene polymorphisms might affect its expression and activity, and thereby involved in SLE pathogenesis.

In the current study, we investigated the association of LEP and LEPR single-nucleotide polymorphisms with susceptibility to SLE in a Chinese population. However, we failed to detect any significant association between these SNPs and SLE risk. This result is inconsistent with a previous study by Afroze et al. They firstly reported an association between LEPR Q223R polymorphisms and risk of SLE in 100 Kashmiri individuals [10]. They found that the carriers of variant genotype (A/G + G/G) or G allele were at increased risk of SLE and the different genotypes of LEPR Q223R might be involved in the development of different clinical manifestations associated with SLE. However, the sample size of this study is relatively small to get a powerful conclusion. Another study by Zhao et al. [11] also analysed the association of leptin pathway-related gene polymorphisms with SLE risk in four different ancestral groups. Then, they conducted a trans-ancestry meta-analysis across four ancestral groups to elevate the overall effect among these SNPs. Their results suggested that although several SNPs showed weak associations, these associations did not remain significant after correction for multiple testing. This result was similar with our findings. We also examined the potential associations of LEP and LEPR gene polymorphisms with specific clinical characteristics in patients with SLE. In LEP, none of the SNPs were associated with any clinical characteristics except the rs2071045 polymorphism. The TT genotype and T allele frequencies of the rs2071045 were significantly increased in patients with pericarditis, suggesting that the T allele of rs2071045 in SLE might elevate the risk of pericarditis. In LEPR, we found positive association between the GA/GG genotype and G allele frequencies of the rs3806318 polymorphism and the risk of photosensitivity in patients with SLE. Our results also demonstrated that rs1137100 might increase the risk of skin photosensitivity in SLE; however, the differences did not reach the statistical significance after correction for multiple testing.

**Table 5** The positive findings of association between genotype frequencies in LEPR and clinical characteristics

<table>
<thead>
<tr>
<th>Gene (SNP)</th>
<th>Allele (M/m)</th>
<th>Clinical features</th>
<th>Group</th>
<th>Genotypes n (%)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1137100</td>
<td>G/A</td>
<td>Photosensitivity</td>
<td>Positive</td>
<td>34 17 6</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>396 142 22</td>
<td></td>
</tr>
<tr>
<td>rs3806318</td>
<td>A/G</td>
<td>Photosensitivity</td>
<td>Positive</td>
<td>40 14 3</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>458 96 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arthritis</td>
<td>Positive</td>
<td>237 43 7</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>261 67 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLEDAI</td>
<td>More active (SLEDAI &gt;10)</td>
<td>33 3 2</td>
<td>0.020†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Less active (SLEDAI≤10)</td>
<td>37 16 1</td>
<td></td>
</tr>
</tbody>
</table>

n: number; SNP: single-nucleotide polymorphism; M: major alleles; m: minor alleles; SLEDAI: systemic lupus erythematosus disease activity index.

*The P values are not corrected for multiple testings, Bonferroni corrected P = 0.0167. Bold value means P <0.05.

†Calculated by Fisher’s exact test (exact P value).

**Discussion**

Systemic lupus erythematosus is a severe autoimmune disease with multiple serological alterations and affecting various organs. Although the aetiology is still unclear, it is generally acknowledged that this disease has complex genetic and environmental backgrounds. Recent studies have shown that leptin levels are elevated in patients with SLE and involved in the pathogenesis of this disease [19–22]. However, some other groups have demonstrated lower or unchanged circulating leptin levels in patients with SLE compared to healthy controls [23, 24]. Our recent work, a meta-analysis, also found no significant difference in plasma/serum leptin level between patients with SLE and normal controls [25]. Similar studies have also been conducted among various autoimmune diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) [26–28]. A recent study showed that serum leptin level and serum leptin/leptin receptor ratio imbalance were positively correlated with anticyclic citrullinated peptide antibodies in RA [29] and might act as a predictor for disease activity [30]. Therefore, LEP and LEPR gene polymorphisms might affect its expression and activity, and thereby involved in SLE pathogenesis.

In the current study, we investigated the association of LEP and LEPR single-nucleotide polymorphisms with susceptibility to SLE in a Chinese population. However, we failed to detect any significant association between these SNPs and SLE risk. This result is inconsistent with a previous study by Afroze et al. They firstly reported an association between LEPR Q223R polymorphisms and risk of SLE in 100 Kashmiri individuals [10]. They found that the carriers of variant genotype (A/G + G/G) or G allele were at increased risk of SLE and the different genotypes of LEPR Q223R might be involved in the development of different clinical manifestations associated with SLE. However, the sample size of this study is relatively small to get a powerful conclusion. Another study by Zhao et al. [11] also analysed the association of leptin pathway-related gene polymorphisms with SLE risk in four different ancestral groups. Then, they conducted a trans-ancestry meta-analysis across four ancestral groups to elevate the overall effect among these SNPs. Their results suggested that although several SNPs showed weak associations, these associations did not remain significant after correction for multiple testing. This result was similar with our findings. We also examined the potential associations of LEP and LEPR gene polymorphisms with specific clinical characteristics in patients with SLE. In LEP, none of the SNPs were associated with any clinical characteristics except the rs2071045 polymorphism. The TT genotype and T allele frequencies of the rs2071045 were significantly increased in patients with pericarditis, suggesting that the T allele of rs2071045 in SLE might elevate the risk of pericarditis. In LEPR, we found positive association between the GA/GG genotype and G allele frequencies of the rs3806318 polymorphism and the risk of photosensitivity in patients with SLE. Our results also demonstrated that rs1137100 might increase the risk of skin photosensitivity in SLE; however, the differences did not reach the statistical significance after correction for multiple testing.

Previous studies have also evaluated the role of LEP and LEPR genes in multiple autoimmune diseases; however, the results are controversial [31–34]. A recent study demonstrated that the genotype and allele frequencies of the LEP rs2167270 gene polymorphism had no association with the risk of RA [31]. However, Farrokhi et al. revealed a significant role of LEP G-
<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes</th>
<th>Number</th>
<th>Serum leptin level M (P25, P75)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11761556</td>
<td>AA</td>
<td>46</td>
<td>6.43 (3.75, 19.60)</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>37</td>
<td>6.34 (3.66, 18.54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>8</td>
<td>8.87 (3.03, 13.14)</td>
<td></td>
</tr>
<tr>
<td>rs12706832</td>
<td>GG</td>
<td>49</td>
<td>7.12 (3.94, 19.29)</td>
<td>0.334</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>36</td>
<td>5.75 (3.35, 16.53)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>6</td>
<td>12.53 (5.60, 21.25)</td>
<td></td>
</tr>
<tr>
<td>rs2071045</td>
<td>CC</td>
<td>30</td>
<td>7.14 (3.59, 19.56)</td>
<td>0.928</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>47</td>
<td>5.86 (4.07, 20.36)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>14</td>
<td>8.87 (3.41, 16.45)</td>
<td></td>
</tr>
<tr>
<td>rs2167270</td>
<td>GG</td>
<td>57</td>
<td>7.12 (3.73, 20.02)</td>
<td>0.428</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>31</td>
<td>5.65 (3.38, 14.66)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>3</td>
<td>13.49 (6.17, 18.98)</td>
<td></td>
</tr>
<tr>
<td>rs10749754</td>
<td>AA</td>
<td>69</td>
<td>6.34 (3.57, 18.39)</td>
<td>0.832</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>18</td>
<td>5.53 (4.32, 19.51)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>4</td>
<td>14.27 (4.20, 20.89)</td>
<td></td>
</tr>
<tr>
<td>rs1137100</td>
<td>GG</td>
<td>67</td>
<td>6.34 (3.61, 19.17)</td>
<td>0.454</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>18</td>
<td>11.26 (4.89, 19.51)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>6</td>
<td>5.13 (2.38, 15.95)</td>
<td></td>
</tr>
<tr>
<td>rs1137101</td>
<td>GG</td>
<td>72</td>
<td>6.41 (3.63, 18.69)</td>
<td>0.955</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>16</td>
<td>5.47 (4.15, 20.08)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>3</td>
<td>12.52 (1.43, 16.02)</td>
<td></td>
</tr>
<tr>
<td>rs13306519</td>
<td>CC</td>
<td>63</td>
<td>8.21 (4.11, 19.17)</td>
<td>0.186</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>21</td>
<td>5.05 (3.44, 12.46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>7</td>
<td>16.70 (5.29, 22.51)</td>
<td></td>
</tr>
<tr>
<td>rs8179183</td>
<td>CC</td>
<td>81</td>
<td>7.12 (3.70, 19.29)</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>10</td>
<td>4.40 (3.85, 7.29)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>rs1805096</td>
<td>AA</td>
<td>73</td>
<td>6.26 (3.74, 18.39)</td>
<td>0.272</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>17</td>
<td>12.52 (4.05, 19.80)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>1</td>
<td>1.43</td>
<td></td>
</tr>
</tbody>
</table>
and LEPR Q223R polymorphisms in the risk of multiple sclerosis and its severity [32]. Furthermore, two recent studies showed that genetic polymorphisms of the leptin gene might influence lung function via Notch and JAK/STAT3 signalling pathway [34, 35]. Thus, LEP and LEPR gene polymorphisms might influence the production and activity of inflammatory cytokines and thereby play a role in the development of various autoimmune diseases.

### Table 6. Continued

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes</th>
<th>Number</th>
<th>Serum leptin level M (P25, P75)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3790434</td>
<td>CC</td>
<td>61</td>
<td>6.34 (3.57,19.08)</td>
<td>0.647</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>28</td>
<td>8.21 (4.12,18.96)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>2</td>
<td>4.68 (4.40,4.95)</td>
<td></td>
</tr>
<tr>
<td>rs3806318</td>
<td>AA</td>
<td>69</td>
<td>6.48 (3.92,19.85)</td>
<td>0.396</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>19</td>
<td>10.81 (3.52,15.92)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3</td>
<td>3.69 (3.61,5.21)</td>
<td></td>
</tr>
<tr>
<td>rs7518632</td>
<td>AA</td>
<td>65</td>
<td>6.48 (3.66,19.08)</td>
<td>0.632</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>17</td>
<td>10.96 (4.18,19.80)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>9</td>
<td>5.54 (2.95,14.27)</td>
<td></td>
</tr>
</tbody>
</table>

SNP: single-nucleotide polymorphism; M: median.

### Table 7 Haplotype analysis of four SNPs in LEP gene in patients with SLE and controls

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Case [n (%)]</th>
<th>Control [n (%)]</th>
<th>( \chi^2 )</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2167270-</td>
<td>223.98 (17.7)</td>
<td>178.29 (15.9)</td>
<td>1.308</td>
<td>0.253</td>
<td>0.881 (0.710, 1.094)</td>
</tr>
<tr>
<td>rs12706832-</td>
<td>51.11 (4.0)</td>
<td>55.50 (5.0)</td>
<td>1.193</td>
<td>0.275</td>
<td>1.242 (0.841, 1.833)</td>
</tr>
<tr>
<td>rs2071045-</td>
<td>646.32 (51.1)</td>
<td>577.65 (51.7)</td>
<td>0.089</td>
<td>0.765</td>
<td>1.025 (0.870, 1.209)</td>
</tr>
<tr>
<td>rs11761556-</td>
<td>64.28 (5.1)</td>
<td>57.97 (5.2)</td>
<td>0.014</td>
<td>0.906</td>
<td>1.022 (0.710, 1.472)</td>
</tr>
<tr>
<td></td>
<td>226.09 (17.9)</td>
<td>201.06 (18.0)</td>
<td>0.006</td>
<td>0.939</td>
<td>1.008 (0.817, 1.245)</td>
</tr>
</tbody>
</table>

Total \( \chi^2 = 2.274, P = 0.069. \)
All the haplotypes with a frequency <0.03 were ignored in the analysis.

### Table 8 Haplotype analysis of four SNPs in LEPR gene in patients with SLE and controls

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Case [n (%)]</th>
<th>Control [n (%)]</th>
<th>( \chi^2 )</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3806318-</td>
<td>65.57 (5.2)</td>
<td>44.72 (4.0)</td>
<td>1.914</td>
<td>0.167</td>
<td>1.318 (0.891, 1.949)</td>
</tr>
<tr>
<td>rs3790434-</td>
<td>587.70 (46.4)</td>
<td>577.78 (51.7)</td>
<td>9.038</td>
<td>0.003</td>
<td>0.745 (0.615, 0.903)</td>
</tr>
<tr>
<td>rs1137100-</td>
<td>158.79 (12.5)</td>
<td>121.20 (10.8)</td>
<td>1.754</td>
<td>0.185</td>
<td>1.189 (0.920, 1.537)</td>
</tr>
<tr>
<td>rs13306519-</td>
<td>110.74 (8.7)</td>
<td>72.77 (6.5)</td>
<td>4.327</td>
<td>0.038</td>
<td>1.390 (1.018, 1.897)</td>
</tr>
<tr>
<td>rs10749754-</td>
<td>49.31 (3.9)</td>
<td>42.96 (3.8)</td>
<td>0.005</td>
<td>0.942</td>
<td>1.016 (0.668, 1.545)</td>
</tr>
</tbody>
</table>

Total \( \chi^2 = 10.469, P = 0.033. \) Bold value means \( P<0.05. \)
All the haplotype with a frequency <0.03 were ignored in the analysis.

---

"2548-A and LEPR Q223R polymorphisms in the risk of multiple sclerosis and its severity [32]. Furthermore, two recent studies showed that genetic polymorphisms of the leptin gene might influence lung function via Notch and JAK/STAT3 signalling pathway [34, 35]. Thus, LEP and LEPR gene polymorphisms might influence the production and activity of inflammatory cytokines and thereby play a role in the development of various autoimmune diseases."
However, one limitation of our study should be acknowledged. The sample size for analysing the serum leptin level in patients with SLE may not be sufficient to get a solid conclusion. Therefore, the findings should be interpreted with caution; further studies with larger sample size are needed to confirm these results.

In conclusion, LEP and LEPR gene polymorphisms are not associated with genetic susceptibility to SLE in the Chinese population. However, some SNPs are associated with specific clinical phenotype of SLE, such as skin rash, pericarditis and arthritis. Compared with previous similar studies, some of these contradictions may be due to studies with different ethnic background, sample size, pathogenesis and patients with different disease activity, duration and treatment. Therefore, further studies based on larger sample size in different populations are required to confirm this result, and the gene–gene or genes–environment interaction should be taken into consideration.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (81573222, 81373073).

Conflict of interest

The authors confirm that there are no conflict of interest.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Characteristics of the 32 Tag SNPs
Table S2 Genotype and allele frequencies of LEP SNPs in SLE patients and health controls
Table S3 Genotype and allele frequencies of LEPR SNPs in SLE patients and health controls
Table S4 Association of clinical characteristics with genotype and allele frequencies in LEP
Table S5 Association of clinical characteristics with genotype and allele frequencies in LEPR

References


