Impact of IL-10 gene polymorphisms and its interaction with environment on susceptibility to systemic lupus erythematosus

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Abstract
This study aims to explore the impact of interleukin (IL)-10 single nucleotide polymorphisms (SNPs) and its interaction with environment on the risk of systemic lupus erythematosus (SLE). Chi-square testing method was used to investigate whether the distributions for genotype of four SNPs were differed from Hardy-Weinberg equilibrium (HWE). Logistic regression was used to test the association between IL-10 SNPs and SLE risk. The best interaction combinations between IL-10 SNPs and environmental factors were assessed by generalized multifactor dimensionality reduction (GMDR). Both rs1800896-G and rs1800871-T alleles were associated with increased risk of SLE, the odds ratios (ORs) (95% confidence interval (CI)) for the two SNPs were 1.68 (1.25–2.09) and 1.47 (1.12–1.94), respectively. Then, we used the GMDR method to analyze the high-order interactions of four SNPs within IL-10 gene and environmental factors on SLE risk. We found a significant interaction combination (two-locus model with P = 0.001) between rs1800896 and smoking, after adjusting for gender, age, body mass index (BMI), and alcohol drinking. We also used two-variable stratified analysis by logistic regression to analyze the synergistic effect between two variables (rs1800896 and smoking), which had significant significance in GMDR model. We found that current smokers with rs1800896-AG or GG genotype have the highest SLE risk, compared with never smokers with the rs1800896-AA genotype, OR (95% CI) = 2.24 (1.52–3.58). The rs1800896-G and rs1800871-T alleles and interaction between rs1800896 and current smoking were all associated with increased risk of SLE.

Keywords
interaction, interleukin (IL)-10, single nucleotide polymorphisms, smoking, systemic lupus erythematosus

Date received: 29 September 2019; accepted: 19 June 2020

Introduction
Systemic lupus erythematosus (SLE) was a common and incurable autoimmune disease. It occurs alternately in slow and rapid courses and can cause damage to the kidney and central nervous system; the incidence was higher in young or middle-aged females.1,2 The incidence of SLE in Asian population is higher than that in European population, and there are differences between males and females. The incidence of SLE in male population is about 9 times higher than that in female population.3,4 The etiology and pathogenesis of SLE were complex and not yet fully understood. At present, the main view is that SLE is an autoimmune disease caused by environmental factors acting on people...
with certain genetic background. So more and more studies have suggested that genetic factors could influence the susceptibility to SLE.

Interleukin-10 (IL-10) is an important inflammatory factor and plays important role in various pathophysiological processes. Previous study has shown that IL-10 level may be associated with SLE risk and may influence the activity of disease in SLE. IL-10 gene is located at the chromosome 1, and several IL-10 single nucleotide polymorphism (SNPs) have been reported with susceptibility to SLE in previous studies; however, these studies concluded conflicted results. Some studies suggested minor alleles of IL-10 gene were related with increased SLE risk, some studies suggested that IL-10 SNPs play a protective role in SLE, and some others studies also concluded negative results. So the relationship between IL-10 SNPs and susceptibility to SLE remains non-deterministic, and the impact of interactions on SLE risk was also need to be investigated. Our study thus aims to examine the impact of IL-10 SNPs and the interaction with the environmental factors on the susceptibility to SLE risk in Chinese population.

Materials and methods

Subjects

The current study was a case–control study, and a total of 1176 participants hospitalized in the Anqing Hospital Affiliated to Anhui Medical University were selected, including 391 SLE patients and 785 control participants. All SLE patients were diagnosed according to the criteria of the American College of Rheumatology (ACR) in 1997 by dermatologist. Those participants who suffered from autoimmune diseases and critical diseases, such as cancer, and participants with family history of autoimmune diseases were excluded from the control group. The participants selected in this study were all Han Chinese, and no genetic correlation was found among these subjects. The study was approved by the local ethical committee of the Anqing Hospital Affiliated to Anhui Medical University (20180013). Written informed consents were signed from each participant. The sample size was calculated according the following formula

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n = \frac{2pq(z_α + z_β)^2}{(p_1 - p_0)^2}\]

In this study, \(p_0 = 0.1\), odds ratio (OR) = 2, \(α = 0.05\), \(β = 0.1\), at last, the sample size = 380 pairs, so the sample size of this study meets the requirements of research.

Genomic DNA extraction and genotyping

Blood samples were obtained from all participants and were acquired and treated with EDTA. The genotyping for four SNPs were tested by using polymerase chain reaction (PCR) along with restriction fragment length polymorphism (RFLP). The descriptions for all selected four SNPs were shown in Table 1. The PCR reaction mixtures and conditions were referred to a previous study.

Statistical analysis

SPSS 22.0 software was used for statistical analysis. Chi-square testing method was used to investigate whether the distributions for genotype of four SNPs were differed from Hardy-Weinberg equilibrium (HWE). Means ± standard deviations were calculated for continuous variables, to test whether

| SNPs | Chromosome | Major/ | Primers | Restriction enzymes |
|------|------------|--------|---------|--------------------|
| rs1800896 -1082A > G | 1:20673552 | A/G | Forward: 5'-AACACTAAGGCTCCTTTGGA-3' | MnlI |
| rs1800872 -592C > A | 1:20673062 | C/A | Forward: 5'-GGTGAGCATCCTGACTGC-3' | RsaI |
| rs1800871 -819C/T | 1:20673289 | C/T | Forward: 5'-CTCAGGCCAAACCTGGGC-3' | RseI |
| rs3024498 +4529(A/G) | 1:206768184 | A/G | Forward: 5'-CTGAAAATTTGGTTCTAGCAGCGC-3' | PvuII |

SNPs: single nucleotide polymorphisms.
these variables were different between two groups using the t-test. Percentages were calculated for categorical variables, to test whether these categorical variables were different between two groups using chi-square test. Logistic regression was used to test the association between IL-10 SNPs and SLE risk. The best interaction combinations were assessed by generalized multifactor dimensionality reduction (GMDR). Some parameters were also calculated to verified which combination was the best one, such as the testing balanced accuracy, the sign test, and cross-validation consistency. We estimating the sample size by genetic power calculator at 5% significance level and 80% significance level, respectively, and the sample size remained the same. All significance is considered by two-tailed $P$-value, and those significances with $P < 0.05$ were considered as statistical.

**Results**

Table 2 shows a comparison of general demographic characteristics and related indicators between SLE patients and control group. All studied subjects are Han Chinese people consisting of 391 SLE patients (136 males and 255 females, mean age was $50.7 \pm 10.9$ years) and 785 control subjects (288 males and 497 females, mean age was $51.3 \pm 11.5$ years). Some parameters including age, gender, and body mass index (BMI) are not statistically significant different between the two groups ($P > 0.05$). But smoking and alcohol drinking rates were statistically higher for cases than controls ($P < 0.05$).

In this study, the frequency for the rs1800896-G allele was 30.4% in SLE patients and was 19.7% in controls ($P < 0.05$). The frequency for the rs1800871-T allele was 24.3% in SLE cases and was 18.2% in controls ($P < 0.05$). Results obtained from logistic model indicated that both rs1800896-G and rs1800871-T alleles were correlated with increased risk of SLE, the ORs (95% confidence interval (CI)) of which were $1.68 (1.25–2.09)$ and $1.47 (1.12–1.94)$, respectively (Table 3).

We used the GMDR method to test the effect for the interaction among four SNPs and environment on the risk of SLE (Table 4). We found a significant interaction combination (two-locus model with $P=0.001$) between rs1800896 and smoking. After adjustment for covariates including gender, age, BMI, and alcohol drinking, the cross-validation consistency was 10/10 and the prediction error was 0.632. We also used two-variable stratified analysis to analyze the synergistic effect between two variables (rs1800896 and smoking), which has statistical significance in GMDR model by using logistic regression. We found that smokers with rs1800896-AG or GG genotype have the highest SLE risk, compared with never smokers with rs1800896-AA genotype, OR (95% CI) = 2.24 (1.52–3.58), after covariates adjusting (Figure 1).

**Discussion**

In our study, we found that both rs1800896-G and rs1800871-T alleles were correlated with the increased susceptibility to SLE. However, there was no correlation of rs1800872 and rs3024498

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**Table 2.** General characteristics of study participants in case and control group.

| Variables                      | SLE cases (n = 391) | Controls (n = 785) | $P$-values |
|-------------------------------|---------------------|--------------------|------------|
| Age (years) (means ± SD)      | 50.7 ± 10.9         | 51.3 ± 11.5        | 0.391      |
| BMI (kg/m²) (means ± SD)      | 23.4 ± 7.9          | 23.8 ± 8.1         | 0.421      |
| Duration of disease (years)   | 6.4 (3.7–9.1)       |                    |            |
| SLEDAI                        | 14 (4–28)           |                    |            |
| Gender, n (%)                 |                     |                    |            |
| Males                         | 136 (34.8)          | 288 (36.7)         | 0.521      |
| Females                       | 255 (65.2)          | 497 (63.3)         |            |
| Smoking status, n (%)         |                     |                    |            |
| Current                       | 102 (26.1)          | 141 (18.0)         | 0.001      |
| Never                         | 289 (73.9)          | 644 (82.0)         |            |
| Alcohol drinking, n (%)       |                     |                    |            |
| Current                       | 143 (36.6)          | 205 (26.1)         | 0.0002     |
| Never                         | 248 (63.4)          | 580 (73.9)         |            |

SLE: systemic lupus erythematosus; BMI: body mass index; SD: standard deviation; SLEDAI: systemic lupus erythematosus disease activity index.
Table 3. Association analysis for four target SNPs within IL-10 gene and SLE risk.

| SNPs          | Genotypes or alleles | Frequencies, n (%) | OR (95% CI)a | P-values for HWE test |
|---------------|----------------------|--------------------|--------------|----------------------|
|               |                      | Controls (n = 785) | SLE cases (n = 391) |                      |
| rs1800896 -1082A > G | Codominant           |                    |              |                      |
|                | AA genotype          | 511 (65.1)         | 193 (49.4)   | 1.00 (ref)           | 0.063                |
|                | AG genotype          | 239 (30.4)         | 158 (40.4)   | 1.57 (1.19–1.96)     |                      |
|                | GG genotype          | 35 (4.5)           | 40 (10.2)    | 2.02 (1.32–2.71)     |                      |
|                | A allele             | 1261 (80.3)        | 544 (69.6)   | 1.00 (ref)           |                      |
|                | G allele             | 309 (19.7)         | 238 (30.4)   | 1.68 (1.25–2.09)     |                      |
| rs1800872 -592C > A | Codominant           |                    |              |                      |
|                | CC genotype          | 481 (61.3)         | 226 (57.8)   | 1.00 (ref)           | 0.467                |
|                | CA genotype          | 264 (33.6)         | 140 (35.8)   | 1.22 (0.81–1.83)     |                      |
|                | AA genotype          | 40 (5.1)           | 25 (6.4)     | 1.53 (0.71–2.38)     |                      |
|                | C allele             | 1226 (78.1)        | 592 (75.7)   | 1.00 (ref)           |                      |
|                | A allele             | 344 (21.9)         | 190 (24.3)   | 1.26 (0.78–1.97)     |                      |
| rs1800871 -819C/T | Codominant           |                    |              |                      |
|                | CC genotype          | 530 (67.5)         | 225 (57.5)   | 1.00 (ref)           | 0.275                |
|                | CT genotype          | 225 (28.7)         | 142 (36.3)   | 1.41 (1.07–1.81)     |                      |
|                | TT genotype          | 30 (3.8)           | 24 (6.1)     | 1.92 (1.23–2.86)     |                      |
|                | C allele             | 1285 (81.8)        | 592 (75.7)   | 1.00 (ref)           |                      |
|                | T allele             | 285 (18.2)         | 190 (24.3)   | 1.47 (1.12–1.94)     |                      |
| rs3024498 +4529(A/G) | Codominant           |                    |              |                      |
|                | AA genotype          | 460 (58.6)         | 230 (58.8)   | 1.00 (ref)           | 0.416                |
|                | AG genotype          | 277 (35.3)         | 138 (35.3)   | 1.23 (0.93–1.60)     |                      |
|                | GG genotype          | 48 (6.1)           | 23 (5.9)     | 1.32 (0.85–1.85)     |                      |
|                | A allele             | 1197 (76.2)        | 598 (76.5)   | 1.00 (ref)           |                      |
|                | G allele             | 373 (23.8)         | 184 (23.5)   | 1.27 (0.90–1.65)     |                      |

SNPs: single nucleotide polymorphisms; SLE: systemic lupus erythematosus; OR: odds ratio; CI: confidence interval; HWE: Hardy-Weinberg equilibrium.
aAdjusted for age, gender, BMI, smoking, and alcohol drinking.

Table 4. GMDR analysis for the best interaction combination models.

| Locus no. | Best combination | Cross-validation consistency | Testing balanced accuracy | P-valuesa |
|-----------|------------------|------------------------------|---------------------------|-----------|
| Gene–gene interactionsa | 2, 1 | 8/10 | 0.518 | 0.172 |
| 3, 1, 2 | 7/10 | 0.513 | 0.377 |
| 4, 1, 2, 3, 4 | 7/10 | 0.518 | 0.426 |
| Gene–alcohol drinking interactionsb | 2, 1, alcohol drinking | 8/10 | 0.601 | 0.182 |
| 3, 1, 2, alcohol drinking | 7/10 | 0.540 | 0.213 |
| 4, 1, 2, 3, alcohol drinking | 5/10 | 0.540 | 0.256 |
| 5, 1, 2, 3, 4, alcohol drinking | 6/10 | 0.496 | 0.426 |
| Gene–smoking interactionsc | 2, 1, smoking | 10/10 | 0.632 | 0.001 |
| 3, 1, 2, smoking | 8/10 | 0.532 | 0.172 |
| 4, 1, 2, 3, smoking | 6/10 | 0.532 | 0.256 |
| 5, 1, 2, 3, 4, smoking | 6/10 | 0.512 | 0.532 |

aAdjusted for age, gender, BMI, alcohol drinking, and smoking.
bAdjusted for age, gender, BMI, smoking.
cAdjusted for age, gender, BMI, alcohol drinking. SNPs named with 1, 2, 3, and 4 were rs1800896, rs1800872, rs1800871, and rs3024498, respectively.
with SLE risk. Human IL-10 gene directly controls the synthesis of IL-10 in human body by influencing the level of IL-10 in serum. The gene is located in the Q31-Q32 region of the chromosome 1 and consists of five exons and four introns. Previous study has shown a positive association between SNPs in IL-10 gene and SLE, but conflicting results were also reported in the other literature regarding the correlation between IL-10 gene SNPs and susceptibility to SLE. Schotte et al. suggested that SNPs (rs1800896, rs1800872, and rs1800871) alone were not significantly associated with susceptibility to SLE. SNP-rs1800896 has been more studied in the previous studies. A meta-analysis provided evidence for the association between the IL10-rs1800896 polymorphism and the increased SLE risk in overall and Asian populations, but this relationship was not found in Caucasian populations. Another meta-analysis suggested a positive association between the IL-10 rs1800896 and susceptibility to SLE in Europeans, and the IL-10 rs1800871 polymorphism is positive associated with susceptibility to SLE in Asians. Mohammadi et al. concluded that the three IL10 SNPs—rs1800896, rs1800872, and rs1800871—were associated with higher SLE disease activity and elevated IL-10 levels in an Iranian population. Another study also concluded that IL-10 expression was up-regulated in active juvenile SLE (JSLE) in Thai children; the rs1800872 and rs1800871 mutant genotypes are associated with increased susceptibility to JSLE. In terms of rs3024498, which was not well studied previously, Lv et al. considered that IL-10 rs3024498 played an important role in the pathogenesis of SLE. However, we obtained negative results on this relationship in current study. The aforementioned inconsistencies addressing the genetic risk involved in relationship between SLE and IL-10 SNPs may be attributable to different races, different genetic heterogeneity, population admixture, gene–environment and gene–gene interactions. The mechanism of the effect of IL-10 gene on the risk of SLE is not clear. A study has shown that deficiencies in IL-10 secretion lead to increased activation of CD4+ T cells by immature pre-B cells, which may enhance autoimmune function in the body.

According to the results reported by previous studies, the pathogenesis of SLE is very complex and is influenced by environmental factors, genetic factors, and the interaction between various factors. Some studies suggest that not only smoking is positively correlated with the risk of SLE, but also the synergistic effect between gene loci and smoking has an important influence on the susceptibility of SLE. In current study, we first investigated the impact of interaction between IL-10 SNPs and smoking on susceptibility to SLE risk in Chinese population. The results also indicated a significant interaction involving rs1800896 and smoking, smokers with rs1800896-AG or GG within IL-10 gene have the highest SLE risk, compared with never smokers with rs1800896-AA. The detailed mechanism of interaction between IL-10 gene and smoking is not clear. We speculate that there is a common pathway in the mechanism of interaction between IL-10 and smoking and SLE that affects the occurrence and development of SLE.

However, there were also some limitations in this study: first, more extra environmental factors ought to be further identified; second, more SNPs should be included in the analysis; finally, because of limited information on the time and amount of smoking, we could not present the results on smoking duration and total amount, so the results obtained in this study should been checked in future studies with larger sample size in different populations.

**Conclusions**

Our data demonstrated that the rs1800896-G and rs1800871-T alleles and interaction between
rs1800896 and current smoking were all risk factors of SLE.

Acknowledgements
We appreciate the cooperation of the families and individuals who cooperated in this study.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethical approval
Ethical approval for this study was obtained from “the local ethical committee of the Anqing Hospital Affiliated to Anhui Medical University (20180013).”

Informed consent
Written informed consent was obtained from all subjects before the study.

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