

## Original Article

# Association of interleukin-10 promoter haplotypes with systemic lupus erythematosus susceptibility in Han Chinese

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**Abstract:** Objectives: The association between interleukin-10 (IL-10) promoter (-1082 A/G, -819 T/C, -592 A/C) polymorphism and haplotypes with systemic lupus erythematosus (SLE) has been investigated in recent years. However, the susceptibility to SLE in different ethnic subjects was various. This study aimed to analyze IL-10 promoter haplotypes in Han Chinese patient with SLE. Methods: 237 Han Chinese patients with SLE and 259 healthy controls (HC) were investigated. Polymerase chain reaction and restriction fragment length polymorphism analysis were used to analyze IL-10 promoter polymorphism (-1082 A/G, -819 T/C, -592 A/C). Results: The -1082 G allele frequency was significantly higher in SLE patients than that in HC ( $P < 0.05$ ,  $OR = 0.358$ ). A significantly higher distribution of -1082 A/G AG genotype was found in SLE patients compared with HC ( $P < 0.05$ ,  $OR = 0.335$ ). The haplotypes ATA, GTA, GCC exhibited higher prevalence in Han Chinese patients with SLE than that in HC. GCC carriers were significantly associated with SLE in Han Chinese population ( $P < 0.01$ ,  $OR = 2.882$ ). However, the haplotype ATC was a significant protective factor for SLE in Han Chinese population ( $P < 0.05$ ,  $OR = 0.675$ ). Our research indicated the contribution of IL-10 promoter GCC haplotype, -1082 A/G AG genotype and G allele to susceptibility to SLE in Han Chinese population, it was different with previous researchs performed in other races and countries. Conclusion: IL-10 promoter GCC haplotype was significantly associated with SLE in Han Chinese population, while the haplotype ATC was significantly protective from SLE in Han Chinese population.

**Keywords:** Interleukin-10, promoter, systemic lupus erythematosus, haplotypes

## Introduction

Systemic lupus erythematosus (SLE) is a prototype autoimmune disorder characterized by polyclonal B-cell activation, the presence of auto-antibody and immune complexes leading to multiple organ damage. Although the exact etiology of SLE is still not fully clearly illuminated, it is known that susceptibility genes and environmental factors have been linked to the initiation and promotion of this complex disease [1, 2]. IL-10 is an important pleiotropic cytokine that can be produced by almost all leukocytes and to a lesser extent by lymphocyte. It is a potent inhibitor of both T lymphocyte and antigen-presenting cell functions. On the other hand, it enhances B-cell survival, proliferation, differentiation and the production of auto-antibodies, these effects appear to play a pivotal role in autoimmune diseases including SLE [3].

It has been demonstrated that the level of IL-10 is significantly increased in SLE patients [4], in addition, a large amount of evidence indicated that Interleukin-10 (IL-10) is a strong candidate gene in SLE susceptibility [5].

The human IL-10 gene maps to chromosome 1 (1q31-1q32) and encodes for five exons [6]. The IL-10 promoter is polymorphic, containing two CA-repeat microsatellites (MS), IL10 R (-4.0 kb) [7] and IL10 G (-1.1 kb) [8] and six single nucleotide polymorphisms (SNPs): -3575 A/T, -2849 A/G, -2763 A/C, -1082 A/G, -819 T/C and -592 A/C [9]. It has been known that -1082 A/G, -819 T/C and -592 A/C combined to form three haplotypes: GCC, ACC and ATA, which are believed to affect the production of IL-10 [10]. Several studies have verified the influence of these nucleotide polymorphisms and haplotypes on the development of SLE, but their

**Table 1.** Clinical manifestations of Subjects with SLE

Manifestation	n	%
Hematological system damage	142	59.9
Renal impairment	135	56.9
Arthritis	123	51.9
Skin impairment	100	42.1
Positivity for anti-dsDNA antibodies	134	56.5
Alopecia	90	37.9
Photosensitivity	40	16.9
Oral ulcer	28	11.8
Nervous system change	10	4.2
Raynaud phenomenon	11	4.6

findings are still in controversial [11, 12]. In the present study, we attempt to investigate whether there was an association between IL-10 promoter haplotypes with the susceptibility to SLE in Han Chinese patients.

## Materials and methods

### *Patients and controls*

237 patients fulfilling the American College of Rheumatology (ACR) criteria for SLE [13] and 259 healthy controls (HC) were included for this study. Both SLE patients and healthy controls were Han Chinese population. All participants provided the written informed consent. This study was approved by the ethics committee of the Third Xiangya Hospital of Central South University.

### *Genotyping*

The genomic DNA was isolated from peripheral blood using a phenol-chloroform extraction method. The IL-10 gene promoter polymorphism of -592 A/C, -819 T/C and -1082 A/G was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses. PCR was performed on a Gene-Amp PCR System 9700 thermocycler (Applied Biosystems, USA). The primer sequences and other information were shown in **Table 2**. The PCR products were digested with restriction enzymes, and separated by agarose gel electrophoresis stained with ethidium bromide for visualization. 10% PCR-amplified DNA samples were examined by DNA sequencing by Shanghai biological engineering company to confirm the genotyping result.

### *Haplotype reconstruction and statistical analysis*

Polymorphism genotype frequencies and allelic frequency distributions in SLE patients and HC subjects were analyzed with chi-square tests to confirm that they conformed to Hardy-Weinberg equilibrium based on their frequencies. Statistical significance was considered if the *P*-value was <0.05. Odds ratios (OR) were calculated from genotype frequencies and allelic frequencies at a 95% confidence interval (CI). These statistical analyses were performed using the SPSS statistical software suite for Windows (SPSS Inc., Chicago, IL, United States). Haplotype construction, Linkage disequilibrium (LD), coefficient (*D'* and *r*-squared) for haplotypes and their frequencies were performed using the online genetic statistical software SHESIS (<http://analysis.bio-x.cn/myAnalysis.php>) [14]. Differences were assumed statistically significant if the two-sided *P*-value was <0.05.

## Results

### *Characteristics of patients and controls*

237 Chinese patients with SLE and 259 HC subjects were included in this study. The mean age of SLE patients was  $30.4 \pm 10.4$  years while the average age of control group was  $32.5 \pm 11.2$  years. The age distribution was similar between the two groups ( $P > 0.05$ ). Clinical manifestations in the SLE patient group were found including hematological system damage (59.9%), renal impairment (56.9%), arthritis (51.9%), skin impairment (42.1%), positive for anti-dsDNA antibodies (56.5%), alopecia (37.9%), photosensitivity (16.9%), oral ulcer (11.8%), nervous system change (4.2%) and raynaud phenomenon (4.6%) (**Table 1**).

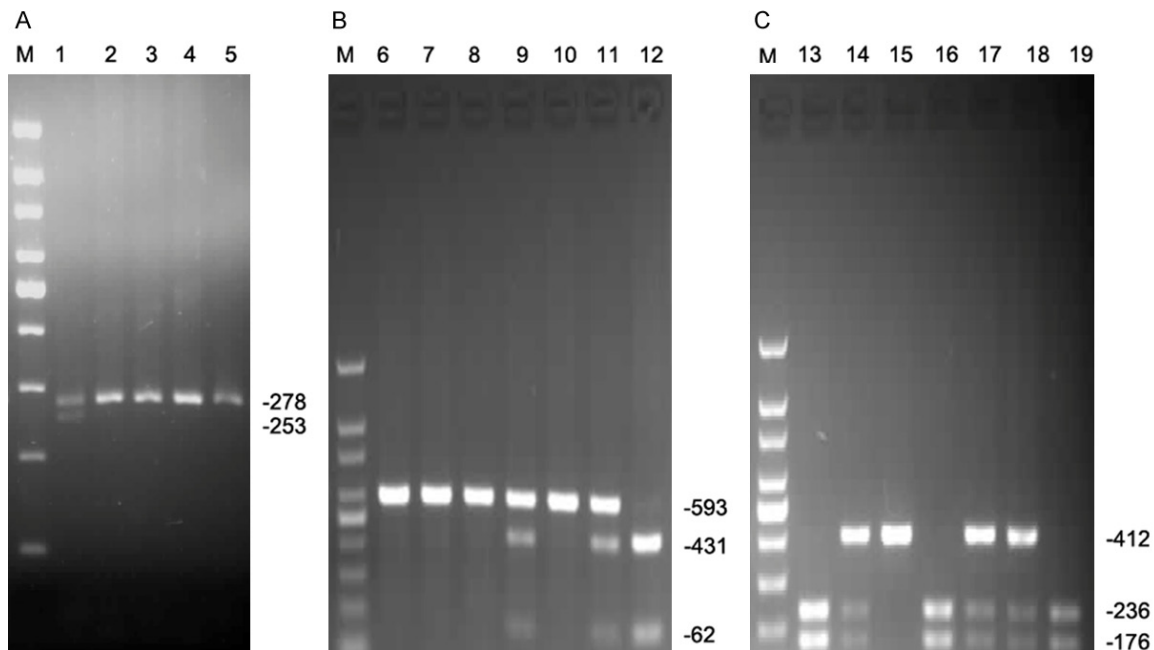
### *Analysis of IL-10 promoter polymorphism*

The PCR product of IL-10 promoter -1082 A/G, -819 T/C and -592 A/C digested by restriction enzyme *B*si I, *M*si I and *R*sa I respectively. The electrophoresis of digested fragment was as follow: for -1082 A allele, 278 bp+37 bp, G allele, 253 bp+37 bp+25 bp (**Figure 1A**); for -819 T allele, 593 bp, C allele, 431 bp+62 bp (**Figure 1B**); for -592 A allele, 176 bp+236 bp, C allele, 412 bp (**Figure 1C**). The results of electrophoresis were totally consistent with the results of sequence analysis.

## IL-10 promoter haplotypes in SLE

**Table 2.** Primer, annealing temperature, PCR product, restriction enzyme, digested fragments and concentration of agarose gel

	-1082 A/G	-819 T/C	-592 A/C
Forward primer	5'-GACAACACTACTAAGGCTCCTTTGGGA-3'	5'-AAACTTTAGACTCCAGCCACA-3'	5'-GGTGAGCACTACCTGACTAGC-3'
Reverse primer	5'-TGAGCAAAGTGGGACAGAAAT-3'	5'-TGCCCATCCAGAATACAA-3'	5'-CCTAGGTCACAGTGACGTGG-3'
Annealing temperature	53°C	59°C	59°C
Length of PCR product	315 bp	593 bp	412 bp
Restriction enzyme	Bsl I	Msl I	Rsa I
Digested fragments	A: 278 bp+37 bp; G: 253 bp+37 bp+25 bp	T: 593 bp; C: 431 bp+62 bp	A: 176 bp+236 bp; C: 412 bp
Agarose gel	4%	2%	2%



**Figure 1.** Gel analysis of PCR products digested with restriction enzymes. A: -1082 A/G digested with Bsl I restriction enzyme ( M: 100 bp ladder, lane 1: AG, lane 2, 3, 4, 5: AA); B: -819 T/C digested with Msl I restriction enzyme (lane 6, 7, 8, 10: TT, lane 9, 11: TC, lane 12: CC); C: -592 A/C digested with Rsa I restriction enzyme (lane 15: CC, lane 14, 17, 18: AC, lane 13, 16, 19: AA).

### *Distribution of IL-10 promoter polymorphism in Han Chinese patients with SLE*

Analysis of IL-10 (-1082 A/G, -819 T/C, -592 A/C) promoter polymorphism were performed in Han Chinese SLE patients and HC subjects. Observed frequencies were in Hardy-Weinberg equilibrium ( $P > 0.05$ ). The AA genotype was more frequent in SLE patients and HC for IL-10 -1082 A/G promoter polymorphism (82.7% and 93.4%, respectively). For IL-10 -819 T/C promoter polymorphism, frequency of TT genotype was higher in SLE patients and HC (52.7% and 54.1%, respectively). For IL-10 -592 A/C promoter polymorphism, AA genotype was higher in SLE patients and HC subjects (46.0% and 44.4%, respectively) (Table 3).

The -1082 G allele frequency was significantly higher in SLE patients than in HC subjects ( $P < 0.05$ , OR=0.358, 95% CI 0.200-0.639) and amounted 8.6% and 3.3%, respectively. We also observed a higher distribution of -1082 A/G AG genotype in SLE patients compared to HC subjects ( $P < 0.05$ , OR=0.335, 95% CI 0.185-0.609), amounted 17.3% and 6.6%, respectively. No other statistical significant differences were found between Han Chinese SLE patients and HC subjects.

### *IL-10 promoter haplotypes in Han Chinese SLE susceptibility*

Linkage disequilibria analyzed by SHEsis between IL-10 (-1082 A/G, -819 T/C, -592 A/C) pro-

## IL-10 promoter haplotypes in SLE

**Table 3.** Distribution of IL-10 (-1082 A/G, -819 T/C, -592 A/C) promoter polymorphism in Han Chinese SLE patients and HC

	SLE (%), n=237	HC (%), n=259	Chi-square	P value	OR (95% CI)
<b>-1082 A/G</b>					
Allele					
A	433 (0.914)	501 (0.967)			
G	41 (0.086)	17 (0.033)	12.955	<0.05	0.358 [0.200-0.639]
Genotype					
AA	196 (0.827)	242 (0.934)			
AG	41 (0.173)	17 (0.066)	13.813	<0.05	0.335 [0.185-0.609]
<b>-819 T/C</b>					
Allele					
T	346 (0.730)	389 (0.751)			
C	128 (0.270)	129 (0.249)	0.569	0.450	1.115 [0.839-1.482]
Genotype					
TT	125 (0.527)	140 (0.541)			
TC	96 (0.405)	109 (0.421)			
CC	16 (0.068)	10 (0.039)	2.086	0.352	
Dominant model					
TT	125 (0.527)	140 (0.541)			
TC+CC	112 (0.473)	119 (0.459)	0.086	0.770	0.949 [0.666-1.351]
Recessive model					
TT+TC	221 (0.932)	249 (0.962)			
CC	16 (0.068)	10 (0.039)	2.081	0.149	0.555 [0.247-1.248]
<b>-592 A/C</b>					
Allele					
A	315 (0.665)	335 (0.647)			
C	159 (0.335)	183 (0.353)	0.348	0.554	1.082 [0.832-1.406]
Genotype					
AA	109 (0.460)	115 (0.444)			
AC	97 (0.409)	105 (0.405)			
CC	31 (0.131)	39 (0.151)	0.416	0.811	
Dominant model					
AA	109 (0.460)	115 (0.444)			
AC+CC	128 (0.540)	144 (0.556)	0.126	0.722	1.066 [0.748-1.519]
Recessive model					
AA+AC	206 (0.869)	220 (0.849)			
CC	31 (0.131)	39 (0.151)	0.399	0.527	1.178 [0.708-1.959]

moter (**Table 5**). Linkage of -819 and -592 was stronger than that between -819 and -1082, or -592 and -1082. And we tested SLE susceptibility association with the IL-10 promoter haplotypes in Han Chinese patients (**Table 4**). The haplotypes ATA, GTA, GCC exhibited higher prevalence in Han Chinese SLE patients than in HC subjects. Furthermore, GCC carriers were significantly associated with SLE in Han Chinese population (P=0.001613, OR=2.882, 95% CI 1.453-5.718). However, the haplotype

ATC has a significant protective effect for the development of SLE in Han Chinese population (P=0.038077, OR=0.675, 95% CI 0.464-0.980).

### Discussion

SLE is an autoimmune disease associated with genetic contribution [15]. There are a variety of immune disorders in SLE patient. Anti-DNA autoantibodies are the hallmark of human

## IL-10 promoter haplotypes in SLE

**Table 4.** Distribution of IL-10 (-1082 A/G, -819 T/C, -592 A/C) promoter haplotypes in Han Chinese SLE patients and HC

Haplotype	SLE (%), n=237	HC (%), n=259	Chi-square	P value	OR (95% CI)
ACA	21.59 (0.046)	27.86 (0.054)	0.295	0.587069	0.852 [0.479-1.517]
GCA	0.48 (0.001)	1.33 (0.003)	-	-	-
ATA	283.14 (0.597)	304.22 (0.587)	0.363	0.547104	1.082 [0.837-1.399]
GTA	9.79 (0.021)	1.59 (0.003)	-	-	-
ACC	76.32 (0.161)	87.93 (0.170)	0.072	0.788008	0.955 [0.682-1.336]
GCC	29.61 (0.062)	11.89 (0.023)	9.954	0.001613	2.882 [1.453-5.718]
ATC	51.95 (0.110)	81.00 (0.156)	4.302	0.038077	0.675 [0.464-0.980]
GTC	1.12 (0.002)	2.19 (0.004)	-	-	-

**Table 5.** Linkage disequilibria between IL-10 (-1082 A/G, -819 T/C, -592 A/C) promoter

Loci	D'	r-squared
-592, -819	0.694	0.320
-819, -1082	0.652	0.039
-592, -1082	0.573	0.076

which are believed to be a key pathogenic factor for renal tissue and to initiate immune glomerulonephritis [16]. IFN-gamma is a major effector molecule in SLE [17, 18]. Besides, genetic factors including Fc-gamma receptors, interleukin-6 and tumor necrosis factor-alpha also play a role in SLE susceptibility and clinical phenotype [19]. Various cytokines have also been found to be important in the pathogenesis of SLE in previous studies [18, 20, 21]. Among these cytokines, interleukin-10 (IL-10) is a substantial component to SLE, and IL-10 production may be genetically determined [22]. Furthermore, IL-10 promoter polymorphism is linked to IL-10 production [23]. The level of serum IL-10 influences severity of SLE and is related to clinical manifestations of SLE patients.

Our research showed that IL-10 promoter -1082 A/G AG genotype and -1082 G allele significantly contributed to the susceptibility to SLE in Han Chinese population ( $P < 0.05$ ,  $OR = 0.335$ ,  $95\% \text{ CI } 0.185-0.609$  and  $P < 0.05$ ,  $OR = 0.358$ ,  $95\% \text{ CI } 0.200-0.639$ , respectively). However, in southern Chinese, Hong Kong, IL-10 promoter -1082 AG genotype and -1082 A allele frequency showed no differences between SLE patients and healthy subjects [24, 25], which indicating that even in the same country, the contribution of IL-10 promoter -1082 A/G polymorphism for SLE susceptibility may not be identical. In addition,

IL-10 promoter -1082 G allele's contribution to the susceptibility of SLE is different with us in some previous studies performed in various other nations [26-30], these research showed that there was no significant differences between SLE patients and HC subjects of the IL-10 promoter -1082 G allele frequency. It is interesting that the frequency of IL-10 promoter -1082 G allele in Asia is generally lower than that in Europe and South America, suggesting that IL-10 level may be distinct in Asian and non-Asians. Whether it can influence the prevalence of SLE among different nations and different races need to be investigated in future study.

IL-10 promoter -1082 A/G, -819 T/C, and -592 A/C form three kinds of haplotypes including GCC, ACC, and ATA normally [10]. Unlike the common situation, we find two more haplotypes, ACA and ATC, and the ATC haplotype has a significant protective effect against SLE in Han Chinese population ( $P = 0.038$ ,  $OR = 0.675$ ,  $95\% \text{ CI } 0.464-0.980$ ). However, the relationship between IL-10 promoter haplotypes and SLE susceptibility has not yet in-depth studied in previous studies, a study performed by Schotte et al. showed that IL-10 promoter (-2489 G/A, -1082 A/G, -819 T/C, -592 C/A) GGCC haplotype appeared to be protective from SLE in Germany ( $OR = 0.67$ ,  $95\% \text{ CI } 0.48-0.94$ ) [29]. It indicating that some type of IL-10 promoter haplotypes could prevent individuals suffering from SLE. Which patient who carry special IL-10 promoter haplotypes are at risk and which are not for developing of SLE is not clear yet and will require further research.

Our study showed that there existed an association of IL-10 gene polymorphisms with SLE risk. In Han Chinese population IL-10 promoter

## IL-10 promoter haplotypes in SLE

GCC haplotype is significantly associated with SLE susceptibility, GCC haplotype associated with 2.882 times increased risk of SLE, in contrast with this, Hirankarn et al. found that ACC haplotype was increased in SLE patients compared with healthy controls ( $P=0.03$ ,  $OR=1.47$ ) in Thailand [26]. In addition, a meta-analysis showed the haplotype GCC/ATA polymorphism of IL-10 promoter is not likely to be involved in SLE susceptibility in Asian and Caucasian [31]. The above listed data showed that the association between IL-10 promoter haplotypes and SLE susceptibility might be different among various races and countries.

In conclusion, we found that IL-10 promoter -1082 A/G AG genotype and -1082 G allele significantly contributed to SLE susceptibility in Han Chinese population, in addition, IL-10 promoter GCC haplotype was significantly associated with SLE risk in Han Chinese population, while the haplotype ATC could significantly protect individuals suffering from SLE in Han Chinese population. However, due to the included sample size was small, additionally, the pathogenesis of SLE is still unclear up to now. Therefore, our results should be settled rationally, and more well designed further study is necessary.

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### Disclosure of conflict of interest

None.

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## IL-10 promoter haplotypes in SLE

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