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## The association of –627 interleukin-10 promoter polymorphism in Chinese patients with systemic lupus erythematosus

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**Abstract** The objective of the study is to examine whether interleukin-10 (IL-10) promoter polymorphism is a marker of susceptibility of systemic lupus erythematosus (SLE) in Chinese patients in Taiwan. The study included 119 Chinese patients with SLE. One hundred unrelated healthy individuals living in central Taiwan served as control subjects. Each polymorphism was detected as a result of a polymerase chain reaction (PCR)-based restriction analysis. The PCR product length was determined to be 412 bp (CC) whereas two fragments of 236 and 176 bp were determined to be excisable lengths (AA). The relationship between the IL-10 gene polymorphism and clinical manifestations of SLE was evaluated. For the genotype and allelic frequency, there were statistically significant

differences between the SLE patients and the normal control subjects ( $p=0.007$  and  $0.003$ , respectively). But we did not detect any association of carriage rate of the IL-10 polymorphism and the normal control subjects ( $p=0.077$ ). Furthermore, we did not detect any association of IL-10 genotype with antinuclear antibody, malar rash, photosensitivity, discoid lupus, mucosal ulcer, arthritis, serositis, hematology, immunology, involvement of central nervous system, and renal disease involvement in the SLE patients. The significant relation of –627 IL-10 genotype and allelic frequency with SLE implies that the IL-10 gene polymorphism can serve as a candidate gene marker for further study in patients with SLE in Taiwan.

**Keywords** Interleukin-10 (IL-10) · Systemic lupus erythematosus (SLE)

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### Introduction

Systemic lupus erythematosus (SLE) is a prototype for systemic autoimmune disease with a wide spectrum of laboratory and clinical manifestations. It is known to involve multisystems and the production of autoantibodies to intracellular antigens. The etiology is largely unknown although valuable knowledge was accomplished through intensive research in this field in recent years. Interleukin-10 (IL-10) is produced at a high level by the B lymphocytes and monocytes of patients with SLE. The high IL-10 production contributes to the abnormal production of immunoglobulins (Ig) and of autoantibodies in SLE [1]. It may be a risk factor for susceptibility and severity of rheumatoid arthritis (RA) and SLE [2–4]. The spontaneous in vitro production of IgM, IgG, and IgA by peripheral blood mononuclear cells from SLE patients was weakly increased by recombinant IL (rIL)-6, but strongly by rIL-10. IL-10 is an important pleiotropic cytokine with anti-inflammatory and stimulatory activities [5]. It exerts its anti-inflammatory effect by inhibiting the synthesis of proinflammatory cytokines such as IL- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-12, and TNF- $\alpha$  in activated macrophages [6]. IL-10

also enhances B cell proliferation, differentiation, and survival [7–9]. Levels of IL-10 production are critical in immunity regulation. Thus, the expression of IL-10 was implicated in a number of autoimmune disorders, including RA, Sjogren's syndrome, and SLE [4]. This study has examined the polymorphism of the gene IL-10 to determine whether it was a marker of susceptibility to SLE by focusing on single nucleotide polymorphisms (SNPs). We compared allelic and genotypic frequencies between 119 Chinese patients with SLE and 100 healthy individuals in Taiwan.

## Materials and methods

One hundred nineteen patients with definite SLE according to the 1982 revised American College of Rheumatology criteria for SLE [10] and 100 unrelated healthy individuals living in central Taiwan, who served as control subjects, were enrolled in this study. Informed consent was obtained from all patients involved. Clinical and serological data were available on these patients, including malar rash, photosensitivity, antinuclear antibody (ANA), involvement of central nervous system, and renal disease (defined as proteinuria >1 g/day). The involvement of the central nervous system was evaluated by a neurologist. The genomic DNA was prepared from peripheral blood by the use of a genomic DNA isolation reagent kit (Genomaker, Taiwan, Republic of China). The polymerase chain reaction (PCR) for IL-10 gene polymorphism was carried out on a total volume of 25  $\mu$ l containing genomic DNA (2–6 pmol of each primer), 1X *Taq* polymerase buffer (1.5 mM MgCl<sub>2</sub>), and 0.25 U of AmpliTaq DNA polymerase (Perkin-Elmer, Foster City, CA, USA). The primer for the IL-10 promoter gene at position –627 was 5'-CCTAGGTCACAGTGACGTGG-3' and 5'-GGTGAGCACTACCTGACTAGC-3. The PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin-Elmer). The cycling conditions for IL-10 polymorphism were set as follows: 1 cycle at 96°C for 5 min, 35 cycles at 96°C for 30 s, 35 cycles at 60°C for 30 s, 35 cycles at 72°C for 45 s, and 1 final cycle of extension at 72°C for 7 min. The PCR product of 412 bp was mixed with 2 U of *Rsa* I (New England Biolabs, Beverly, USA) and two fragments of 236 and 176 bp were present when the product could already be digested (AA homozygote). The reaction was incubated for 3 h at 37°C. Then 10  $\mu$ l of the product was loaded onto a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was divided into digestible (AA homozygote), indigestible (CC homozygote), and A/C heterozygote.

The carriage rate of an allele is the number of individuals carrying at least one copy of the allele relative to the total number of individuals. Allelic frequency was expressed as a percentage of the total number of alleles. Results from the control subjects and the SLE patients were compared using the  $\chi^2$  test (3×2 and 2×2 contingency tables) for statistical significance. When the assumption of the  $\chi^2$  test was violated and one cell had an expected count of <1, or >20%

of the cells had an expected count of <5, Fisher's exact test was used. The distributions of the IL-10 gene polymorphisms in each group were evaluated. A *p* value of less than 0.05 was considered statistically significant. The odds ratios (OR) were calculated from allelic frequency with a 95% confidence interval (95% CI) for the polymorphism of the IL-10 gene.

## Results

The frequencies of the genotype in the SLE and control groups are shown in Table 1. It shows that among the 119 SLE patients, 66 patients (55.5%) had IL-10 genotype AA, 48 patients (40.3%) had A/C, and 5 patients (4.2%) had CC. Among the 100 control volunteers, the IL-10 genotype AA was found in 40 (40.0%), A/C in 45 (45.0%), and CC in 15 (15.0%). There were significant differences in the distribution of the IL-10 gene polymorphism between the healthy control subjects and the SLE patients (*p*=0.007). In addition, there was a significant difference in the allelic frequency of the IL-10 between the SLE patients and healthy controls (*p*=0.003), giving an OR of 1.210 for A allele (95% CI 1.063–1.377). There was no significant association in the carriage rates of the IL-10 (*p*=0.077), giving an OR of 1.164 for A allele (95% CI 0.981–1.382).

Clinical manifestations and laboratory findings of the SLE patients are shown in Table 2. The associations of IL-10 with particular clinical features of SLE were examined in the 119 Chinese patients. We did not detect any association of IL-10 genotype with ANA, malar rash, photosensitivity, discoid lupus, mucosal ulcer, arthritis, serositis, hematology, immunology, involvement of central nervous system, and renal disease involvement in the SLE patients (all *p*>0.05).

**Table 1** Comparison of IL-10 genotype distributions, allele frequencies, and carriage rates observed in SLE patients and healthy control subjects

IL-10	SLE patients <i>n</i> =119 (%)	Controls <i>n</i> =100 (%)	<i>p</i> value	OR (95% CI)
<b>Genotype</b>				
AA	66 (55.5)	40 (40)	0.007	
A/C	48 (40.3)	45 (45)		
CC	5 (4.2)	15 (15)		
<b>Allelic frequencies</b>				
A	180 (75.6)	125 (62.5)	0.003	1.210 (1.063~1.377)
C	58 (24.4)	75 (37.5)		0.650 (0.488~0.865)
<b>Carriage rate</b>				
A	114 (95.8)	85 (85.0)	0.077	1.164 (0.981~1.382)
C	53 (44.5)	60 (60.0)		0.767 (0.571~1.030)

All the *p* values represent  $\chi^2$  test results

**Table 2** Relationship between IL-10 genotype and clinical signs and findings in patients with SLE

	AA n=66 (%)	A/C n=48 (%)	CC n=5 (%)	Total n=119 (%)	<i>p</i> value
Malar rash	33 (50.0)	30 (62.5)	4 (80.0)	67 (56.3)	0.228
Photosensitivity	32 (48.5)	29 (60.4)	4 (80.0)	65 (54.6)	0.229
Renal involvement	27 (40.9)	17 (35.4)	2 (40.0)	46 (38.7)	0.836
CNS involvement	11 (16.7)	4 (8.3)	1 (20.0)	16 (13.4)	0.396
ANA(+)	64 (97.0)	47 (98.0)	5 (100)	116 (97.5)	0.449

## Discussion

SLE is a prototype of autoimmune diseases that affects practically every organ in the body. There are strong epidemiological evidences that genes contribute to the risk of developing many common diseases. However, the genetic background of patients with SLE is still mostly unknown. To date, genetic researches of multifactorial diseases were studied with difficulty due to the vast uncertainty surrounding the presence of a polygenic trait. In the present study, we chose the IL-10 gene polymorphism to examine whether IL-10 gene polymorphism was a marker of susceptibility to SLE in Chinese patients in Taiwan.

IL-10 is a 36-kDa homodimeric cytokine, which is mainly produced by macrophage, monocytes, and lymphocytes. The IL-10 gene maps to the junction of 1q31–q32 [11]. IL-10 production appears to be controlled at the transcriptional level [12]. The IL-10 5' flanking region, which controls transcription, is polymorphic, with two microsatellites between –4,000 and –1,100 and three single base pair substitutions that were described in the IL-10 promoter at positions –1,082 (G/A), –819 (C/T), and –592 (C/A) [13]. Reports from Lazarus et al. [14] found that IL-10-1082G, IL-10-819C, and IL-10-592C haplotypes were associated with Ro autoantibodies and renal involvement in white patients with SLE. In Chinese patients, a different IL-10-1087\*A/-824\*T/-597\*A haplotype was also associated with renal involvement but not Ro autoantibodies [15]. These studies found no association with disease susceptibility. In contrast, Gibson et al. [16] found novel SNPs in the distal region of the IL-10 promoter significantly associated with SLE susceptibility in African-Americans. The study of Lim et al. [17] showed that –627\*A allele is associated with severe asthma, which is detected with low levels of IL-10 [18]. Grove et al. [19] also showed that the –627\*A IL-10 promoter gene is related to advanced alcoholic liver disease. They proposed that the –627\*A allele is associated with low IL-10 expression, which will favor the inflammatory, immune-mediated, and profibrotic mechanisms of an alcohol-related liver injury. Guseva et al. [20] found that polymorphism of genes FcγRIIIA and IL-10 is associated with predisposition to development of SLE in the Kazakh population. The analysis of combined genotypes of the studied genes suggests a synergic action of genes FcγRIIIA and IL-10-627 with the risk to develop SLE [20].

To our knowledge, an association between SLE and the gene polymorphism of –627 IL-10 including genotype,

allelic frequencies, and carriage rate has not been demonstrated before. In the present study, significant differences were observed in the allelic frequencies and genotype of IL-10 gene polymorphism between the patients with SLE and the healthy control subjects. We were unable to find significant differences in the carriage rate of IL-10 gene polymorphism between the normal controls and SLE patients. However, the possibility of a type II error must be kept in mind in view of the relatively small sample sizes. Recently, SNPs were used as tools for mapping the complex disease genes responsible for SLE [21–29]. SNPs provide a method for defining patients at risk from disease. In addition, they assist in determining the exact prognosis of patients, which in turn leads to the selection of a specific therapy based on specific genetic variations [30]. This study provides a useful method for the further study of genes in patients with SLE.

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