G/T polymorphism in the interleukin-2 exon 1 region among Han Chinese systemic lupus erythematosus patients in Taiwan

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Abstract Interleukin-2 (IL-2), one of the crucial immunoregulatory cytokines required for T lymphocyte activation, plays an important role in autoimmune diseases. An IL-2 genetic G/T polymorphism (rs2069763) has been linked with multiple sclerosis and rheumatoid arthritis. We tested a hypothesis that this polymorphism confers systemic lupus erythematosus (SLE) susceptibility. Study participants were Han Chinese SLE patients and a healthy control group in Taiwan. Our results indicate (a) a significantly higher G allele frequency in SLE patients (P = 1.91 × 10^{-14}; OR = 3.94; 95% CI = 2.74–5.66), (b) a significantly higher G allele frequency in SLE patients with antinuclear antibodies (ANA) (P = 0.033; OR = 4.21; 95% CI = 1.01–17.51) and (c) a significantly lower G allele frequency in SLE patients with discoid rash (P = 0.019; OR = 0.41; 95% CI = 0.19–0.88). Our results suggest that this polymorphism may be involved in the genetic background of Taiwanese SLE.

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KEYWORDS Systemic lupus erythematosus; Interleukin-2; Single nucleotide polymorphism
Introduction

Systemic lupus erythematosus (SLE), a multisystemic disorder of autoimmune disease, damages multiple organ systems which causes clinical manifestations [1–4]. Arthritis, serum autoantibodies, glomerulonephritis, joint pain, skin rash and vasculitis are developed in combination with one or more symptoms observed in SLE patients [5]. Cytokines are potent immunomodulatory molecules that mediate immune response and inflammation. Investigators have looked at several cytokines involved in SLE pathogenesis [6,7].

Interleukin-2 (IL-2) is a T lymphocyte produced molecule [8,9]. The T lymphocytes from SLE produce decreased amounts of IL-2 [10,11]. Deficient production of IL-2 leads to an increased rate of infections and increased numbers of activated autoreactive cells [12,13]. Genetic variants in IL-2 have been discussed in the susceptibility of autoimmune diseases including multiple sclerosis and rheumatoid arthritis in Japanese and Spanish populations [14–16].

To test our hypothesis that IL-2 genetic variants confer SLE susceptibility we examined and compared IL-2 genotype distribution in a group of Han Chinese SLE patients and a non-SLE control group in Taiwan. An attempt was also made to clarify the association between IL-2 and SLE severity.

Methods

DNA samples

The study subjects including SLE patients and healthy controls were unrelated Han Chinese descendants which were recruited from China Medical University Hospital in Taiwan. The Han Chinese forms the largest ethnic group in Taiwan, making up over 98% of the population. None of the participants was aboriginal Taiwanese, which account for the remaining 2% of the Taiwan's population. The 116 individuals of SLE patients were diagnosed according to the American College of Rheumatology criteria [5]. The 258 healthy individuals from the general population were also enrolled. Informed consent was obtained from each patient and control subjects involved. DNA collection was approved by China Medical University Hospital's Ethics Board.

IL-2 G/T genetic polymorphism genotyping

The genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Genomic DNA kit; Qiagen). The IL-2 G/T genetic variant (rs2069763) was genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Briefly, PCR was performed in a volume of 25 μl containing 30 ng of DNA, 5 pmol of each primer, 1 U of Amplitaq DNA polymerase (Perkin Elmer) and 2 mmol of dNTP mix, with the buffer containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2 mM MgCl₂. The primers for exon 1 at position 114 of the IL-2 gene were 5′-ATGTACAGGATGCAACTCCT-3′ and 5′-TGGTGAGTTTGG-GATTCTTGG-3′. After denaturation for 5 min at 95 °C, 40 cycles of 95 °C for 60 s, 50 °C for 60 s and 72 °C for 60 s were performed. The last step was extended to 72 °C for 7 min. The PCR product of 262 bp was mixed with two units of Mwo I (New England Biolabs, Beverly, USA) and two fragments of 151 bp and 111 bp were present when the product was digested (GG genotype) (Fig. 1). The reaction was incubated for 3 h at 37 °C. Then, 10 μl of the product was loaded into a 3% agarose gels. The polymorphism was divided into digestible (GG genotype), indigestible (TT genotype) and GT heterozygote (Fig. 1).

Statistical analysis

The genotype frequency and allelic frequency distributions in the polymorphisms in both SLE patients and controls were analyzed by the χ² method. SPSS Version 10.0 software was used to analyze the data. A P-value less than 0.05 was considered statistically significant. Allelic frequencies were expressed as a percentage of the total number of alleles. Odds ratios (OR) were calculated from genotype frequencies and allelic frequencies with 95% confidence interval (95% CI). Adherence to the Hardy–Weinberg equilibrium constant was tested using the χ² test with a one degree of freedom.

Results

Allele and genotype frequencies of IL-2 genetic polymorphism

The allele and genotype frequencies were shown in Table 1. The genotype distributions were consistent with the existence of Hardy–Weinberg equilibrium. The allele frequencies were found to have different distribution in patients and controls (P-value = 1.91×10⁻¹⁴) (Table 1). The frequency of G allele was significantly higher in SLE patients than in control subjects (P-value = 1.91×10⁻¹⁴; Odds ratio = 3.94; 95% CI = 2.74–5.66). In addition, the genotype frequencies were also found to have different distribution in patients and controls (P-value = 5.19×10⁻¹³) (Table 1). The GG genotype frequency was significantly increased in SLE patients (P-value = 5.19×10⁻¹³; Odds ratio = 24.00; 95% CI = 7.19–80.15). The GT genotype frequency was significantly decreased in SLE patients (P-value = 5.19×10⁻¹³; Odds ratio = 0.04; 95% CI = 0.01–0.35).

![Figure 1](image-url)
These results suggest that the IL-2 genetic variant is associated with SLE patients.

**IL-2 genetic polymorphism and clinical features of SLE**

The association between the clinical manifestations of SLE patients with various alleles and genotypes was shown in **Table 2**. The frequency of G allele was significantly increased in patients with ANA (P-value = 0.033, Odds ratio = 3.94; 95% CI = 2.01–5.66). However, the frequency of G allele was significantly decreased in patients with discoid rash (P-value = 0.019, Odds ratio = 0.41; 95% CI = 0.19–0.88). The genotype frequency was significantly different in patients in the presence of ANA, discoid rash and oral ulcers (P-value < 0.05). Together, our data suggest that the IL-2 genetic variant is associated with the clinical manifestation of SLE patients.

**Discussion**

We have shown the significant association between SLE and an IL-2 polymorphism previously investigated in multiple sclerosis and rheumatoid arthritis in Japanese and Spanish populations [14–16]. Our results provide the evidence for genetic association conferred by this polymorphism with the clinical features of Taiwanese SLE.

This IL-2 genetic polymorphism is silent and does not produce any amino acid change in the leader peptide [17,18]. Furthermore, no functional significance about this polymorphism has been reported. However, there is a genetic polymorphism at position –384 of the promoter region of IL-2 gene near this +114 polymorphism [16]. The homozygous for the G allele (G/G) at site –384 in the promoter region of the IL-2 gene produced more than three times than their G/T and T/T counterparts [19]. The polymorphism at position –384 in the promoter region has an influence on IL-2 production. The +114 polymorphism may be in linkage disequilibrium with the functional promoter polymorphism at position –384.

Our results showed that this IL-2 genetic polymorphism associated with ANA and discoid rash in SLE patients. Abnormal secretion of IL-2 results in adverse reactions ranging from aberrant T cell activation to immunodeficiencies [20,21]. The IL-2 has been associated with clinical manifestations of SLE [22–25]. These clinical manifestations are anti-dsDNA antibody and discoid rash. Our genetic association finding has also shown that the IL-2 genetic polymorphism associated with clinical manifestations of SLE.

### Table 1 Allele and genotype frequencies of the IL-2 genetic polymorphism in SLE patients and controls

<table>
<thead>
<tr>
<th>Polymorphism (rs2069763)</th>
<th>SLE Number (%)</th>
<th>Controls Number (%)</th>
<th>P-value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>185 (79.7)</td>
<td>258 (50.0)</td>
<td>1.91 × 10^{-14}</td>
<td>3.94 (2.74–5.66)</td>
</tr>
<tr>
<td>T</td>
<td>47 (20.3)</td>
<td>258 (50.0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Genotype frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>72 (62.1)</td>
<td>64 (24.8)</td>
<td>5.19 × 10^{-13}</td>
<td>24.00 (7.19–80.15)</td>
</tr>
<tr>
<td>GT</td>
<td>41 (35.3)</td>
<td>130 (50.4)</td>
<td>6.73 (2.01–22.56)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>3 (2.6)</td>
<td>64 (24.8)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Cl = Confidence interval.

### Table 2 Allele and genotype frequencies of IL-2 genetic polymorphism of SLE patients with various clinical features

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Allele frequency</th>
<th>P-value</th>
<th>Genotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G allele (n=185)</td>
<td>T allele (n=47)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>ANA</td>
<td>181 (97.8)</td>
<td>43 (91.5)</td>
<td>0.033</td>
</tr>
<tr>
<td>Malad rash</td>
<td>99 (53.5)</td>
<td>29 (61.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>25 (13.5)</td>
<td>13 (27.7)</td>
<td>0.019</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>97 (52.4)</td>
<td>27 (57.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>56 (30.3)</td>
<td>16 (34.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Arthritis</td>
<td>94 (50.8)</td>
<td>22 (46.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Serositis</td>
<td>43 (23.2)</td>
<td>7 (14.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Renal disorder</td>
<td>71 (38.4)</td>
<td>19 (40.4)</td>
<td>NS</td>
</tr>
<tr>
<td>CNS disorder</td>
<td>24 (13.0)</td>
<td>8 (17.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Hematological disorder</td>
<td>79 (42.7)</td>
<td>27 (57.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Immunologic disorder</td>
<td>140 (75.7)</td>
<td>38 (80.9)</td>
<td>NS</td>
</tr>
</tbody>
</table>

n = number of SLE patients in genotype analysis; NS = not significant; ANA = antinuclear antibodies.
In conclusion, our findings illustrated the 114 position in the exon 1 region of IL-2 genetic variant in SLE patients from Taiwanese population in association with disease susceptibility. These observations suggest that this polymorphism may be involved in the genetic background of SLE.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.clim.2008.05.011.

References