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Interleukin-6 promoter polymorphisms (-174 G/C) in Malaysian patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that involves the inflammation of various organs upon deposition of immune complexes and is characterized by uncontrolled B cell hyperactivity. Despite intensive research on the etiology of the disease, the exact cause of the onset of SLE is unknown. The pathogenesis of the disease has been proposed to be associated with the imbalance of T helper type 1 (Th1) and Th2 cytokine activities. Elevated serum levels of interleukin-6 (IL-6), a Th2 cytokine with various functions in the regulation of human biological systems, are observed in SLE patients. In the present study, 100 Malaysian SLE patients and 100 controls were evaluated in order to determine the association of polymorphisms existing in the promoter region of the IL-6 gene with the onset of SLE. The homozygous G genotype was found to be significant in SLE patients (χ^2 = 33.754; P = 0.00000000625), whereas the heterozygous G/C genotype was significant in the controls (χ^2 = 25.087; P = 0.000000548). We suggest that the C allele might have a masking effect on the G allele when both alleles are present in heterozygous individuals. However, we did not observe any significant association of the homozygous C allele with the onset of SLE or with protection from the disease (χ^2 = 1.684; P = 0.194366).

Key words: Interleukin-6; Systemic lupus erythematosus; Promoter; Polymorphisms

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Introduction

Systemic lupus erythematosus (SLE) is a well-known autoimmune disease that often involves multiple organ inflammation. Generally, the organs affected by SLE are the heart, the skin, the brain, joints, lungs, and blood vessels (1). These organs could be damaged upon deposition of immune complexes by the body's own immune system. During the early stage of the disease, SLE patients often present a variety of symptoms that are similar to those of other diseases, with difficulty in diagnosis by physicians, leading to a consequent delay in the onset of treatment. Thus, there is a great deal of interest in the identification of biomarkers that are responsible for the onset and progression of the disease. The common symptoms of SLE are extreme fatigue, painful or swollen joints, unexplained fever, skin rashes, and malaise (2). Photosensitivity is also present in some patients. The butterfly rash is one of the characteristic features of SLE. This rash usually occurs over the bridge of the patient's nose and resembles the shape of a butterfly. The fatal outcome of SLE patients is often due to infections and renal disorders. Despite much effort by scientists, there is still no cure for SLE. However, treatments are available to control the symptoms of the disease. For example, immunosuppressants can be used to prevent flares (3).

Interestingly, SLE occurs ten times more often in females than males, usually striking women of reproductive

age (4). This could possibly be due to differences between genders in terms of hormone levels and life styles. Ethnicity might also play a role in the predisposition to disease development (5). The exact etiology of the onset of SLE has yet to be confirmed. It is suggested that both environmental and genetic factors play a role in triggering the onset of the disease. Environmental factors such as ultraviolet light, external stress, and infectious agents interact with complex genes and ultimately lead to the uncontrolled production of autoantibodies (3). Genetic variation is also believed to have an effect on the susceptibility to SLE. Despite intensive investigations carried out by researchers worldwide, there is still no single gene shown to be responsible for the onset of the disease.

It has been proposed that the functional disequilibrium of T helper type 1 (Th1) and Th2 cells is possibly associated with immune responses in different kinds of immune disorders (6). Th1 cytokines [interferon gamma (IFN-γ) and interleukin-2 (IL-2)] activate macrophages and promote the production of antibodies that are responsible for complement fixing and opsonization. Th2 cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13), on the other hand, induce antibody production and mast cell activation (7). It has been reported that Th1 levels are decreased, whereas Th2 levels are elevated, in SLE patients (8). Thus, alteration of Th1 and Th2 lymphocyte functions can result in the up-regulation of autoantibody production by B cells that can lead to the pathogenesis of SLE. Additionally, it has been suggested that the ratio of Th1 and Th2 could serve as a strong determinant predicting the histopathology of lupus nephritis (9).

IL-6 is a multifunctional Th2 cytokine involved in acute inflammation by the production of acute phase proteins and by the modulation of specific immune responses. IL-6 is responsible for the transformation of IL-4-preactivated B cells to immunoglobulin-secreting plasma cells (10). In addition, IL-6 is also involved in the regulation of several biological processes such as bone metabolism and the production of platelets (11). Linker-Israeli and colleagues (12) have reported that elevated plasma levels of IL-6 messenger-RNA and proteins could be detected in SLE patients.

The IL-6 gene has been mapped to the short arm of chromosome 7 at location 21 to 15 (13). The entire IL-6 gene is organized into five exons and four introns. The translation of IL-6 RNA and post-translational processing result in the formation of a 21- to 28-kDa protein with 128 amino acids (14). In the present study, we investigated whether there is an association between SLE susceptibility and single nucleotide polymorphisms located in the promoter region of the IL-6 gene (-174 G/C) in our Malaysian SLE patients, although many negative correlation results have been observed in various studies in the world (10,15,16).

Material and Methods

Our study included 100 SLE patients (6 males and 94 females) and 100 matched healthy control individuals, who were recruited from the University Malaya Medical Center, located in Kuala Lumpur (Ethics Approval No. 380.1). In general, the patients ranged in age from 16 to 50 years and their clinical manifestations were renal disorder with proteinuria (>0.5 g/day), malar rash, arthritis, and photosensitivity with production of anti-dsDNA at >200 IU/mL. Blood samples were collected into tubes containing EDTA. A conventional phenol-chloroform genomic DNA extraction method was employed as described previously (17). Primer sequences (forward primer G: 5'-GCACTTTT CCCCCTAGTTGTGTCTTACG-3'; forward primer C: 5'-GACGACCTAAGCTTTACTTTTCCCCCTAGTTGTGT CTTGAC-3'; reverse primer: 5'-ATAAATCTTTGTTGG AGGGTGAGG-3') and PCR cycling parameters (95°C, 10 min [1 cycle]; 94°C, 30 s, 66°C, 45 s, 72°C, 45 s [40 cycles]; 72°C, 7 min [1 cycle]) were used based on the study of Schotte et al. (10). Each PCR assay contained 10 ng genomic DNA in a final volume of 20 µL containing 10 pmol of each primer (1st Base Pte. Ltd., Singapore), 1 U *Taq* DNA polymerase (Fermentas, USA), 0.1 mM dNTP mix (Promega, USA), 2.0 µL *Taq* buffer with KCl, and 1.0 mM $MgCl_2$. PCR was carried out in a thermal cycler (Mastercycler gradient, Eppendorf, Germany). The amplified products were then visualized by 18% polyacrylamide gel electrophoresis (PAGE). Genotype distribution and allelic frequencies of the polymorphisms were generated in all samples. In addition, χ^2 and odds ratio (OR) tests with 95% confidence intervals (95%CI) were performed to test for any significant association.

Results

Figure 1 shows the band patterns observed for the PCR products in the study of the IL-6 promoter polymorphism (-174 G/C) on 18% (w/v) polyacrylamide gel. Three genotypic combinations were observed in the study, i.e., homozygous G, heterozygous G/C, and homozygous C. A 121-bp fragment denotes the G allele, whereas the C allele is indicated by a 136-bp band on the polyacrylamide gel.

The genotypic distribution and allelic frequencies of the study are summarized in Table 1. The homozygous G genotype was observed significantly in our Malaysian SLE patients. On the other hand, the heterozygous G/C genotype was significantly associated with the healthy control

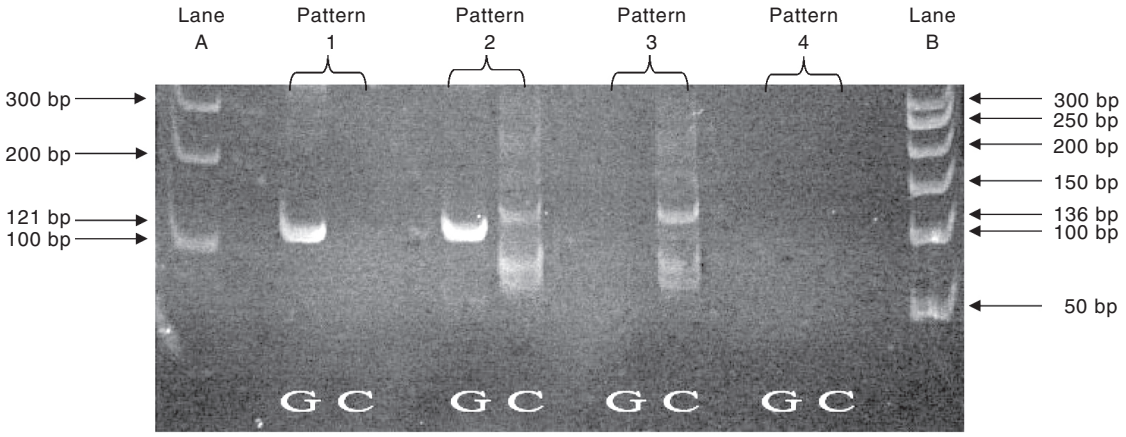

Figure 1. PCR products of IL-6 promoter polymorphism (-174 G/C) were shown by ethidium bromide-stained 18% polyacrylamide gels run at 140 V for 2 h. *Lane A*, 100-bp DNA marker (Fermentas Life Sciences, USA). *Pattern 1*: Homozygous G (121 bp). *Pattern 2*: Heterozygous G/C (121 and 136 bp). *Pattern 3*: Homozygous C (136 bp). *Pattern 4*: DNA blank. *Lane B*, 50-bp DNA marker (Fermentas Life Sciences, USA).

Table 1. Genotypic distribution and allelic frequencies of the IL-6 promoter polymorphism (-174 G/C) in patients with systemic lupus erythematosus (SLE) and controls.

IL-6 Promoter	Frequency		χ^2 (P value)	OR (95%CI)
	SLE patients (N = 100)	Controls (N = 100)		
Genotype				
G/G	50 (50%)	12 (12%)	33.754 (0.00000000625)	7.3333 (3.5719-15.0559)
G/C	47 (47%)	81 (81%)	25.087 (0.000000548)	0.2080 (0.1102-0.3927)
C/C	3 (3%)	7 (7%)	1.684 (0.194366)	0.4109 (0.1032-1.6367)
Allele				
G	147 (73.5%)	105 (52.5%)	18.919 (0.0000136)	2.5094 (1.6503-3.8158)
C	53 (26.5%)	95 (47.5%)		0.3985 (0.2621-0.6060)

group. The homozygous C genotype, however, did not present any significant association.

Both the homozygous G and heterozygous G/C genotypes are present at almost equal frequencies in SLE patients, i.e., 50 and 47%, respectively. In contrast, the heterozygous G/C genotype was observed most frequently in the control group (81%) compared to the homozygous G genotype (12%). However, the homozygous C genotype was the least commonly observed in both the SLE patients (3%) and the control group (7%).

The allelic frequencies are noted to be significant in our study. The OR value for allele C was 0.3985, a 95%CI from 0.2621 to 0.6060. In SLE patients, the G allele was up to three times more frequent than the C allele. However, both alleles were observed equally in the healthy control group.

Discussion

IL-6 is a multifunctional cytokine produced in response to inflammatory stimuli to regulate the human immune response against infection (18). IL-6 levels are increased in patients with SLE, especially in those with active disease (18). It was believed that the over-production of IL-6 in SLE patients led to the pathogenesis of the disease (18). The IL-6 gene expression products may play a role in the onset of SLE. Thus, the polymorphic gene can be an important biomarker for predisposition to SLE.

In the present study, the homozygous G genotype was observed significantly in SLE patients, whereas the heterozygous G/C genotype was significant in the controls. The gene expression products from the G allele may affect

the susceptibility of an individual to SLE. Individuals with the homozygous G genotype were observed to be predisposed to SLE development. Individuals with the heterozygous G/C genotype could be protected from developing SLE. Thus, it is suggested that the C allele has a masking effect over the G allele in the heterozygous G/C genotype, which may be due to a complex interaction of both alleles when present co-dominantly. Under such conditions, it is postulated that the C allele induces a protective response that prevents the individuals from developing SLE. However, we did not observe any significant association of the homozygous C genotype with the onset of SLE or with protection from developing SLE. This observation further suggests the protective role initiated by the interaction of both G and C alleles in heterozygous individuals. Overall, the suppressor effect of the C allele can only be suggested for female SLE patients since the number of male SLE patients screened in this study was small.

In a study of Caucasian German SLE patients carried out by Schotte et al. (10) in 2001, the IL-6 promoter polymorphism (-174 G/C) did not reveal any significant association with susceptibility to SLE. However, the G allele was found to be responsible for the presence of discoid skin lesions and for the production of anti-histone antibodies in SLE patients (10). Based on their study, we suggest that the G allele may be involved in the pathogenesis of SLE and in the development of certain clinical manifestations in SLE patients.

Another study by Fishman et al. (19) showed that IL-6 promoter polymorphism is significantly associated with chronic juvenile arthritis, an autoimmune disease that affects the joints. They observed that the C allele was significantly less common in systemic juvenile chronic arthritis in the Anglo-Saxon Caucasian population studied (19). It was then reported that the C allele is responsible for the suppression of IL-6 transcriptional activity. Thus, the homozygous C genotype is suggested to reduce the levels of IL-6 in plasma and to play a role against the development of systemic juvenile chronic arthritis. The homozygous C genotype has also been linked to other clinical conditions, for example Gaucher disease (20). Gaucher disease is an autosomal recessive lysosomal storage disorder caused by mutations in the gene coding for β-glucocerebrosidase, which involves a characteristic increase of plasma IL-6 levels in patients (20). It was reported that patients with Gaucher disease carrying the homozygous C genotype can have low plasma levels of IL-6 (20).

In the present study, however, the homozygous C genotype did not show a significantly protective role against SLE. This observation again suggests the role of the complex interaction of multiple genes and the involvement of other factors in the development of SLE. Overall, our results do not agree with several other studies carried out on other world populations (10,15,16) and these contradictory results could be due to the genetic heterogeneity of SLE in different ethnicities.

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