RESEARCH ARTICLE

Interleukin-6, interleukin-1 gene cluster and interleukin-1 receptor polymorphisms in Iranian patients with juvenile systemic lupus erythematosus

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ABSTRACT. ***Background:*** **Juvenile systemic lupus erythematosus (JSLE) is a polygenic, autoimmune disorder of unknown origin. As proinflammatory cytokines, including interleukin-6 (IL-6) and the interleukin-1 (IL-1) family, seem to contribute to the pathogenesis of JSLE, this investigation was performed to assess the associations of particular single nucleotide polymorphisms (SNPs) of IL-6 and IL-1 genes in a case-control study.** ***Methods:*** **Fifty nine JSLE cases were recruited for this study as the patient group, and were compared against 140 healthy, unrelated, control subjects. Using the polymerase chain reaction with the sequence-specific primer method, genotyping was carried out for the IL-6 gene at positions -174 and nt565, as well as the IL-1α gene at position -889, the IL-1β gene at positions -511 and +3962, the interleukin-1 receptor (IL-1R) gene at position Pst-I 1970, and the interleukin-1 receptor antagonist (IL-1Ra) gene at position Mspa-I 11100.** ***Results:*** **Results of the analyzed data revealed a remarkable, positive association for the promoter sequence of the IL-1β gene at position -511 for T/T in the patient group compared with healthy controls (P value, 0.03). Furthermore, a significant negative association was found between the T/C genotype at the same position on the IL-1β gene in juvenile SLE (P value, 0.03).** ***Conclusions:*** **cytokine gene polymorphisms might play a role in the pathophysiology of JSLE. Particular IL-1 gene variants could affect individual susceptibility to JSLE.**

Key words: interleukin-1, interleukin-6, single nucleotide polymorphism, systemic lupus erythematosus, children

Systemic lupus erythematosus (SLE) is a chronic, inflammatory, autoimmune disorder with multi-organ involvement. Notwithstanding the unknown etiology of SLE, it has been suggested that a combination of hereditary, environmental and hormonal factors may contribute to the development of the disease. SLE is primarily characterized by autoantibody production and immune complex deposition, which subsequently results in the autoimmune phenomenon [1].

Proinflammatory cytokines including interleukin-1 (IL-1) and interleukin-6 (IL-6) are implicated in the initiation and progression of inflammation in a variety of infectious and autoimmune disorders, including SLE [2]. IL-6 is produced by various cell types including B and T lymphocytes, monocytes, endothelial cells and fibroblasts [3], and serves as an influential factor in widespread biological activities such as induction of B and T cell differentiation and proliferation, macrophage proliferation, immunoglobulin G (IgG) production, bone marrow stem cell maturation and activation of neutrophils [4-6]. It has been established that patients with active SLE have elevated serum IL-6 levels, which, in certain investigations, correlated positively with anti-DNA levels and disease activity [7, 8].

The IL-1 family of cytokines consists of two principal, agonist molecules (IL-1α and IL-1β), that have proinflammatory effects, together with an IL-1 receptor antagonist (IL-1Ra), which is the natural antagonist of IL-1β [9-12]. Binding of IL-1α and IL-1β to the IL-1 receptor (IL-1R) culminates in their corresponding biological effects through initiation of intracellular signaling pathways, while IL-1Ra does not elicit signal transduction when bound to the aforesaid receptor [13]. It has been speculated that the imbalance between IL-1 and IL-1Ra may be a significant factor in the dysregulation of inflammatory responses, leading to autoimmune reactions [13-15].

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The orthodox understanding of cytokine regulatory mechanisms encompasses the fact that proinflammatory cytokine production is affected by particular gene polymorphisms [16]. Associations of these cytokines single nucleotide polymorphisms (SNPs) have been examined in a number of immunological diseases [17-21]. Thus, gene polymorphisms may set the stage for the pathogenesis of SLE.

In the current study, we investigated single nucleotide polymorphisms (SNPs) of IL-6, the IL-1 family and IL-1 receptor in Iranian patients with JSLE and in healthy individuals. Cytokine gene polymorphisms including IL-1α (-889 C/T; rs1800587), IL-1β (-511 C/T; rs16944 and +3962 C/T; rs1143634), IL-1R (Pst-1 1970 C/T; rs2234650), IL-1Ra (Mspa-I 11100 C/T; rs315952) and IL-6 (-174 C/G and nt565 A/G) were determined.

PATIENTS AND METHODS

Subjects

Fifty-nine, Iranian pediatric patients with JSLE, who had been referred to the Rheumatology Clinic of the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Iran, were enrolled in this study. The diagnosis of SLE was established according to the revised criteria of the American College of Rheumatology (ACR) for classification of SLE [22]. One hundred and forty, unrelated, healthy subjects, who were randomly selected from blood donors at Iranian blood transfusion organizations, participated as the control group [23].

Before blood sampling, written, informed consent was obtained from all participants according to the guidelines of the Ethics Committee of Tehran University of Medical Sciences.

Sampling and genotyping

For all of the participants, 5 ml peripheral blood samples were collected over ethylenediaminetetra-acetic acid (EDTA), and kept at -20°C until used. Genomic DNA was extracted using the "salting out" technique [24]. Cytokine gene typing was accomplished using the polymerase chain reaction with a sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany) [23]. Amplification of the isolated DNA was performed using a thermal cycler Techne Flexigene apparatus (Roche, Cambridge, UK) under the following conditions: initial denaturation at 94°C for 2 min; denaturation at 94°C for 10 sec; annealing + extension at 65°C for 1 min (10 cycles); denaturation at 94°C for 10 sec; annealing at 61°C for 50 sec; extension at 72°C for 30 sec (20 cycles). Visualization of PCR products was realized by subjecting DNA fragments to electrophoresis in 2% agarose gel. Subsequently, the gel was positioned on an ultraviolet transilluminator, and a picture was captured for analysis and documentation.

Statistical analysis

Allele, genotype, and haplotype frequencies were calculated by direct counting, and compared with the controls using the chi-square test. We estimated the odds ratio (OR) and 95% confidence interval (CI) for each allele, genotype, and haplotype in both subjects and controls. The P value of less than 0.05 was considered to be statistically significant. Adherence to the Hardy–Weinberg equilibrium constant was established using chi-square test.

RESULTS

Allele frequencies

The allele frequency, P value, odds ratio and its 95% CI for both subjects and controls are shown in *table 1*.

The allele frequencies for IL-1α at position -889, IL-1β at positions -511 and +3962, IL-1R at position Pst-I 1970, IL-1Ra at position Mspa-I 11100 as well as IL-6 at positions -174 and nt565 were similar in two groups of patients with JSLE and healthy, unrelated controls.

Genotype frequencies

The genotype frequency, P value, odds ratio and its 95% CI for both subjects and controls are shown in *table 1*.

We discovered a remarkable, positive association for the promoter sequence of the IL-1β gene at position -511 for T/T in the patient group compared with healthy controls (29.3% *versus* 15.2%; P value, 0.03; OR, 2.3; 95% CI, 1.0-5.1). Moreover, a notable negative association was found between the T/C genotype at the same position of the IL-1β gene with juvenile SLE (41.3% *versus* 59%; P value, 0.03; OR, 0.4; 95% CI, 0.2-0.9).

No significant differences were observed between the two groups for IL-1α at the -889 position, IL-1β at the +3962 position, IL-1R at the Pst-I 1970 position, IL-1Ra at the Mspa-I 11100 position, or IL-6 at the -174 and nt565 positions.

Haplotype frequencies

The haplotype frequency, P value, odds ratio and its 95% CI for both subjects and controls are shown in *table 2*.

We found no significant difference between the two groups for GG, CG, CA, and GA haplotypes at positions -174 and nt565 of the IL-6 gene.

DISCUSSION

SLE is a multigenic, autoimmune disorder with a wide spectrum of immunological and clinical manifestations, in addition to heterogeneity of underlying mechanisms. The role of proinflammatory cytokine gene polymorphisms in individual susceptibility to diverse diseases has been documented [25-27]. Nevertheless, there is a paucity of data concerning cytokine gene polymorphisms that may influence individual vulnerability to JSLE. Here, we evaluated the associations between certain SNPs in the IL-1 gene cluster, Il-1R and IL-6 and disease susceptibility in patients with JSLE, and, to our knowledge, this is the first study to examine these genetic variants in relation to this disease Iranian pediatric patients. Our results suggest a positive association between the TT genotype at IL-1β -511. Furthermore, heterozygosity in this region was a preventive factor in our study group, being associated with a decreased risk of JSLE.

The IL-1 gene cluster comprises a 430-kb region with three genes encoding IL-1α, IL-1β, and IL-1Ra; it is

Table 1
Allele and genotype frequencies in patients with juvenile systemic lupus erythematosus, and healthy controls

Gene	Position	Alleles/Genotypes	Controls (n = 140) N (%)	Patients (n = 59) N (%)	Odds Ratio (95% CI)	*P*-value
IL1α	*-889*	C	186 (68.4)	77 (65.8)	0.8 (0.5-1.4)	0.7
		T	86 (31.6)	40 (34.1)	1.1 (0.6-1.8)	0.7
		CC	62 (45.6)	26 (44.8)	0.9 (0.5-1.8)	0.9
		TC	62 (45.6)	25 (43.1)	0.9 (0.4-1.7)	0.8
		TT	12 (8.8)	7 (12)	1.4 (0.4-4.1)	0.6
IL1β	*-511*	C	154 (55.4)	58 (50.0)	0.8 (0.5-1.2)	0.3
		T	124 (44.6)	58 (50.0)	1.2 (0.7-1.9)	0.3
		CC	36 (25.8)	17 (29.3)	1.1 (0.5-2.4)	0.7
		TC	**82 (59)**	**24 (41.3)**	**0.4 (0.2-0.9)**	**0.03**
		TT	**21 (15.2)**	**17 (29.3)**	**2.3 (1.0-5.1)**	**0.03**
	+3962	C	198 (70.7)	87 (77.6)	1.4 (0.8-2.4)	0.2
		T	82 (29.3)	25 (22.3)	0.6 (0.4-1.1)	0.2
		CC	70 (50)	34 (61.8)	1.6 (0.8-3.2)	0.1
		TC	58 (41.4)	17 (30.9)	0.6 (0.3-1.2)	0.2
		TT	12 (8.6)	4 (7.2)	0.8 (0.2-2.9)	1.0
IL1R	Pst-I 1970	C	174 (62.1)	71 (60.6)	0.6 (0.5-1.5)	0.8
		T	106 (44.2)	46 (39.3)	1.5 (0.6-1.7)	0.8
		CC	54 (38.6)	18 (31)	0.6 (0.3-1.4)	0.4
		TC	66 (47.1)	33 (56.8)	0.5 (0.7-2.8)	0.2
		TT	20 (14.3)	7 (12)	1.7 (0.3-2.2)	0.8
IL1Ra	Mspa-I 11100	C	64 (22.9)	18 (15.7)	0.6 (0.3-1.1)	0.1
		T	216 (77.1)	96 (84.2)	1.5 (0.8-2.9)	0.1
		CC	4 (2.9)	1 (1.7)	0.6 (0-5.9)	1.0
		CT	56 (40)	16 (28)	0.5 (0.2-1.2)	0.1
		TT	80 (57.1)	40 (70.1)	1.7 (0.8-3.6)	0.1
IL-6	-174	C	101 (36.3)	45 (38.1)	1.0 (0.6-1.7)	0.8
		G	177 (63.7)	73 (61.8)	0.9 (0.5-1.4)	0.8
		CC	4 (2.9)	2 (3.3)	1.1 (0.1-7.8)	1.0
		CG	93 (66.9)	41 (69.4)	1.1 (0.5-2.2)	0.8
		GG	42 (30.2)	16 (27.1)	0.8 (0.4-1.7)	0.7
	nt565	A	50 (18)	30 (25.4)	1.5 (0.9-2.6)	0.1
		G	228 (82)	88 (74.5)	0.6 (0.3-1.1)	0.1
		AA	4 (2.9)	2 (3.3)	1.1 (0.1-7.8)	1.0
		AG	42 (30.2)	26 (44.0)	1.8 (0.9-3.5)	0.08
		GG	93 (66.9)	31 (52.5)	0.5 (0.2-1.0)	0.08

located on chromosome 2q13 [28]. IL-1β is a proinflammatory, pleiotropic cytokine with the capacity to stimulate the expression of genes involved in inflammation and the immune response, such as inducible nitric oxide synthase, type 2 phospholipase A and cyclooxygenase type 2 [29]. Additionally, IL-1β possesses the ability to increase the expression of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), on endothelial and other cell surfaces [29]. Notwithstanding that the role of IL-1β in SLE has not been fully documented, there is evidence

Table 2
IL-6 haplotype (-174, nt565) polymorphism in patients with juvenile systemic lupus erythematosus, and healthy controls

Haplotype	Controls (n = 140) N (%)	Patients (n = 59) N (%)	Odds Ratio (95% CI)	*P*-value
GG	173 (62.2)	73 (61.8)	0.9 (0.6-1.5)	0.9
CG	55 (19.8)	15 (12.7)	0.5 (0.3-1.1)	0.1
CA	**46 (16.6)**	**30 (25.4)**	**1.7 (0.9-2.9)**	**0.05**
GA	4 (1.4)	0 (0)	0 (0-3.9)	0.5

propounding that IL-1 is a determining factor for the synthesis of IgG autoantibodies in patients with SLE [30]. It has been speculated that IL-1β secretion by peripheral blood mononuclear cells (PBMC) is greater in patient groups than in healthy individuals [14]. We considered the contribution of SNPs in the -511 C/T and +3962 C/T regions of IL-1β to the development of SLE. Our results added weight to the hypothesis that the TT genotype in the-511 region of IL-1β increases the risk of SLE, while the TC genotype at the same region may serve as a protective factor. The TT genotype of this polymorphism appears to correlate with decreased IL-1β production [31]. One possible explanation is that it is the imbalance of mediators such as the IL-1 receptor and the IL-1 receptor antagonist rather than the level of IL-1β production that contributes to the development of JSLE. Studies investigating the association between the IL-1β -511 C/T polymorphism and SLE have produced inconsistent results [28, 32-35]. A recently performed meta-analysis by Wang *et al.* revealed that the IL-1β -511C/T polymorphism was not, overall, associated with an increased risk of SLE, although a subgroup analysis for Asian ethnicity indicated that the IL-1β -511C/T polymorphism was associated with SLE, but only for TT *versus* CT+CC, and was not for T *versus* C, TT+CT *versus* CC or TT *versus* CC [36]. In this study, variations at the IL-1β +3962 C/T region showed no association with SLE.

We also evaluated the polymorphisms in the IL-1α -889 C/T region in both SLE subjects and healthy individuals. Accordingly, no association was detected between SNPs in this region and SLE. Our results are in contrast with a meta-analysis that suggested a probable association of the IL-1α -889 C/T polymorphism with SLE [36].

In the current study, an IL-1 receptor antagonist (IL-1Ra) polymorphism in SLE was also examined. Accordingly, polymorphisms in IL-1Ra (Mspa-I 11100 C/T) did not show a significant association with JSLE. Concordantly, studies in a Taiwanese population showed no association between IL-1Ra SNPs and SLE [34, 37], even though Tsai *et al.* [37] found that the frequencies of the IL-1Ra Mspa-I 11100 alleles in SLE patients without renal disorder were remarkably different from those in patients with renal disorder and healthy controls. Our findings are in contrast with another study performed by Tahmasebi *et al.* [32] in which the frequency of the IL-1Ra Mspa-I 11100 CT genotype was significantly lower among patients with SLE in comparison with healthy controls.

In addition to the previously stated loci, we also evaluated polymorphisms at the IL-1R Pst-I 1970 C/T region, which showed no association with SLE. Comparably, Tahmasebi and colleagues were unable to discover an association between SNPs at this position and SLE in Iranian patients [32].

IL-6 is a pleiotropic cytokine with its encoding gene being located on the long arm of human chromosome 7, at 7q21 [38]. It is believed that the concentration of IL-6 is raised in SLE. Increases in serum IL-6 levels in patients with SLE correlated with disease activity and anti-dsDNA titers [7]. In this study, there was no significant difference in allele, genotype and haplotype frequencies for IL-6 (-174 and nt565) between patients and controls. Similarly, other authors have been unable to find an association between allelic and genotype distributions at position –174 and SLE in Iranian patients [38] or in Caucasian German patients [39] and patients from China [40]. Moreover, Guarnizo-Zuccardi *et al.* [41] found no association between SLE and SNPs at position –174 G/C in IL6 in a group of Colombian patients, and Santos *et al.* [42], who also examined IL6 -174, observed no significant association in Portuguese Caucasian cases. These results are in contrast with some previous studies, which indicated a significant association between IL-6 and SLE. Hrycek *et al.* showed an increased frequency of the IL-6 G allele at position of -174 in their SLE patients. They also detected a significantly higher percentage of GG and GC genotypes in patient groups [43]. In another study, Asano and colleagues detected a raised level of the IL-6 G allele at position -174 in subjects with SLE as well as an increased frequency of the CC genotype at the same position in healthy controls [44]. The investigation conducted by Chua *et al.* revealed a marked increase in the GG genotype in Malaysian patients with SLE, compared with an increased level of the GC genotype in controls [45]. In the study performed by Hristova *et al.* the G allele and the GG genotype at the aforementioned position appeared to be associated with SLE in Bulgarian patients [46]. All of the previously presented, inconsistent results involve the relationship between the IL-6 gene polymorphism at position -174 and SLE. This study is the first report to suggest a role for the IL-6 gene polymorphism at position nt565 in susceptibility to JSLE.

The current study has certain limitations including the relatively small number of participants in the patient category, which could diminish the statistical power of our analysis, together with our difficulties in measuring serum levels of IL-1 and IL-6. As a result, the relevance of the gene variants, in terms of cytokine levels, cannot be confirmed in patients with JSLE.

However, in summary, we found that the IL-1β T/T genotype at position -511 was positively associated with JSLE in Iranian patients: the Il-1β T/C genotype at the same position was a protective factor. These genetic variants might be possible genetic risk factors for the development of JSLE. However, in order to confirm the involvement of these polymorphisms in JSLE, further studies, in other populations, with different ethnicities, are necessary.

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REFERENCES

1. Yap DYH, Lai KN. The role of cytokines in the pathogenesis of systemic lupus erythematosus–from bench to bedside. *Nephrology* 2013; 18: 243.

2. Tsao BP. Update on human systemic lupus erythematosus genetics. *Curr Opin Rheumatol* 2004; 16: 513.

3. Hirano T. Interleukin 6 and its receptor: ten years later. *Int Rev Immunol* 1998; 16: 249.

4. Muraguchi A, Hirano T, Tang B, *et al*. The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. *J Exp Med* 1988; 167: 332.

5. Lotz M, Jirik F, Kabouridis P, *et al*. B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. *J Exp Med* 1988; 167: 1253.

6. Sachs L, Lotem J, Shabo Y. The molecular regulators of macrophage and granulocyte development. *Ann N Y Acad Sci* 1989; 557: 417.

7. Gröndal G, Gunnarsson I, Rönnelid J, Rogberg S, Klareskog L, Lundberg I. Cytokine production, serum levels and disease activity in systemic lupus erythematosus. *Clin Exp Rheumatol* 1999; 18: 565.

8. Linker-Israeli M, Deans R, Wallace D, Prehn J, Ozeri-Chen T, Klinenberg J. Elevated levels of endogenous IL-6 in systemic lupus erythematosus. A putative role in pathogenesis. *J Immunol* 1991; 147: 117.

9. Alheim K, Bartfai T, Interleukin-1 System: Receptors T. Ligands, and ICE in the Brain and Their Involvement in the Fever Response. *Ann N Y Acad Sci* 1998; 840: 51.

10. Bresnihan B, Cunnane G. Interleukin-1 receptor antagonist. *Rheum Dis Clin North Am* 1998; 24: 615.

11. Arend WP. Interleukin-1 receptor antagonist. *Adv Immunol* 1993; 54: 167.

12. Brugos B, Kiss E, Dul C, *et al*. Measurement of interleukin-1 receptor antagonist in patients with systemic lupus erythematosus could predict renal manifestation of the disease. *Hum Immunol* 2010; 71: 874.

13. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* 1996; 87: 2095.

14. Sturfelt G, Roux-Lombard P, Wollheim F, Dayer J. Low levels of interleukin-1 receptor antagonist coincide with kidney involvement in systemic lupus erythematosus. *Rheumatology* 1997; 36: 1283.

15. Aringer M, Smolen J. Cytokine expression in lupus kidneys. *Lupus* 2005; 14: 13.

16. Barkhordari E, Rezaei N, Ansaripour B, *et al*. Proinflammatory cytokine gene polymorphisms in irritable bowel syndrome. *J Clin Immunol* 2010; 30: 74.

17. Amirzargar A, Shahram F, Nikoopour E, *et al*. Proinflammatory cytokine gene polymorphisms in Behcet's disease. *Eur Cytokine Netw* 2010; 21: 292.

18. Mahdaviani SA, Rezaei N, Moradi B, Dorkhosh S, Amirzargar AA, Movahedi M. Proinflammatory cytokine gene polymorphisms among Iranian patients with asthma. *J Clin Immunol* 2009; 29: 57.

19. Rezaei N, Amirzargar AA, Shakiba Y, Mahmoudi M, Moradi B, Aghamohammadi A. Proinflammatory cytokine gene single nucleotide polymorphisms in common variable immunodeficiency. *Clin Exp Immunol* 2009; 155: 21.

20. Amirzargar AA, Bagheri M, Ghavamzadeh A, *et al*. Cytokine gene polymorphism in Iranian patients with chronic myelogenous leukaemia. *Int J Immunogenet* 2005; 32: 167.

21. Amirzargar AA, Rezaei N, Jabbari H, *et al*. Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. *Eur Cytokine Netw* 2006; 17: 84.

22. Hochberg MC. Updating the American College of *Rheumatology* revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.

23. Amirzargar AA, Naroueynejad M, Khosravi F, *et al*. Cytokine single nucleotide polymorphisms in Iranian populations. *Eur Cytokine Netw* 2008; 19: 104.

24. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.

25. Amirzargar A, Bagheri M, Ghavamzadeh A, *et al*. Cytokine gene polymorphism in Iranian patients with chronic myelogenous leukaemia. *Int J Immunogenet* 2005; 32: 167.

26. Amirzargar A, Shahram F, Nikoopour E, *et al*. Proinflammatory cytokine gene polymorphisms in Behcet's disease. *Eur Cytokine Netw* 2010; 21: 292.

27. Amirzargar AA, Rezaei N, Jabbari H, *et al*. Cytokine single nucleotide polymorphisms in Iranian patients with pulmonary tuberculosis. *Eur Cytokine Netw* 2006; 17: 84.

28. Camargo J, Correa P, Castiblanco J, Anaya J. Interleukin-1β polymorphisms in Colombian patients with autoimmune rheumatic diseases. *Genes Immun* 2004; 5: 609.

29. Dinarello C. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol* 2002; 20: S1.

30. Jandl RC, George JL, Dinarello CA, Schur PH. The effect of interleukin 1 on IgG synthesis in systemic lupus erythematosus. *Clin Immunol Immunopathol* 1987; 45: 384.

31. Iacoviello L, Di Castelnuovo A, Gattone M, *et al*. Polymorphisms of the interleukin-1β gene affect the risk of myocardial infarction and ischemic stroke at young age and the response of mononuclear cells to stimulation in vitro. *Arterioscler Thromb Vasc Biol* 2005; 25: 222.

32. Tahmasebi Z, Akbarian M, Mirkazemi S, *et al*. Interleukin-1 gene cluster and IL-1 receptor polymorphisms in Iranian patients with systemic lupus erythematosus. *Rheumatol Int* 2013; 33: 2591.

33. Parks C, Cooper G, Dooley M, *et al*. Systemic lupus erythematosus and genetic variation in the interleukin 1 gene cluster: a population based study in the southeastern United States. *Ann Rheum Dis* 2004; 63: 91.

34. Tsai LJ, Hsiao SH, Tsai JJ, Lin CY, Tsai LM, Lan JL. Higher genetic susceptibility to inflammation in mild disease activity of systemic lupus erythematosus. *Rheumatol Int* 2009; 29: 1001.

35. Huang CM, Wu MC, Wu JY, Tsai FJ. Lack of association of interleukin-1ß gene polymorphisms in Chinese patients with systemic lupus erythematosus. *Rheumatol Int* 2002; 21: 173.

36. Wang B, Zhu JM, Fan YG, *et al*. The association of IL1α and IL1β polymorphisms with susceptibility to systemic lupus erythematosus: A meta-analysis. *Gene* 2013; 527: 95.

37. Tsai LJ, Lan JL, Lin CY, Hsiao SH, Tsai LM, Tsai JJ. The different expression patterns of interleukin-1 receptor antagonist in systemic lupus erythematosus. *Tissue Antigens* 2006; 68: 493.

38. Godarzi EM, Sarvestani EK, Aflaki E, Amirghofran Z. Interleukin-6 gene polymorphism in Iranian patients with systemic lupus erythematosus. *Clin Rheumatol* 2011; 30: 179.

39. Schotte H, Schlüter B, Rust S, Assmann G, Domschke W, Gaubitz M. Interleukin-6 promoter polymorphism (− 174 G/C) in Caucasian German patients with systemic lupus erythematosus. *Rheumatology* 2001; 40: 393.

40. Huang CM, Huo AP, Tsai CH, Chen CL, Tsai FJ. Lack of association of interleukin-6 and interleukin-8 gene polymorphisms in Chinese patients with systemic lupus erythematosus. *J Clin Lab Anal* 2006; 20: 255.

41. Guarnizo-Zuccardi P, Lopez Y, Giraldo M, *et al*. Cytokine gene polymorphisms in Colombian patients with systemic lupus erythematosus. *Tissue Antigens* 2007; 70: 376.

42. Santos MJ, Fernandes D, Capela S, da Silva JC, Fonseca JE. Interleukin-6 promoter polymorphism −174 G/C is associated with nephritis in Portuguese Caucasian systemic lupus erythematosus patients. *Clin Rheumatol* 2011; 30: 409.

43. Hrycek A, Siekiera U, Cieslik P, Szkrobka W. HLA-DRB1-DQB1 alleles and gene polymorphisms of selected cytokines in systemic lupus erythematosus. *Rheumatol Int* 2005; 26: 1.

44. Asano NM, Angelo HD, da Silva HA, Maia MM, Lins OG, Souza PE. Interleukin-6 promoter polymorphisms −174 GC in Brazilian patients with systemic lupus erythematosus. *Hum Immunol* 2013; 74: 1153.

45. Chua K, Kee B, Tan S, Lian L. Interleukin-6 promoter polymorphisms (-174 G/C) in Malaysian patients with systemic lupus erythematosus. *Braz J Med Biol Res* 2009; 42: 551.

46. Hristova M, Dourmishev L, Kamenarska Z, *et al*. Role of the Promoter Polymorphism IL-6 −174 G/C in Dermatomyositis and Systemic Lupus Erythematosus. *Biomed Res Int* 2013; 2013.