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-1Ra 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 Msp-I 11100. Results: Results of the analyzed data revealed a remarkable, positive association for the promoter sequence of the IL-1Ra 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 Msp-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].
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 (Msp-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. 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.

Genotype frequencies

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

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>Alleles/Genotypes</th>
<th>Controls (n = 140) N (%)</th>
<th>Patients (n = 59) N (%)</th>
<th>Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1α</td>
<td>-889</td>
<td>C</td>
<td>186 (68.4)</td>
<td>77 (65.8)</td>
<td>0.8 (0.5-1.4)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>86 (31.6)</td>
<td>40 (34.1)</td>
<td>1.1 (0.6-1.8)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>62 (45.6)</td>
<td>26 (44.8)</td>
<td>0.9 (0.5-1.8)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>62 (45.6)</td>
<td>25 (43.1)</td>
<td>0.9 (0.4-1.7)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>12 (4.8)</td>
<td>7 (12)</td>
<td>1.4 (0.4-4.1)</td>
<td>0.6</td>
</tr>
<tr>
<td>IL1β</td>
<td>-511</td>
<td>C</td>
<td>154 (55.4)</td>
<td>58 (50.0)</td>
<td>0.8 (0.5-1.2)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>124 (44.6)</td>
<td>58 (50.0)</td>
<td>1.2 (0.7-1.9)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>36 (25.8)</td>
<td>17 (29.3)</td>
<td>1.1 (0.5-2.4)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>82 (59)</td>
<td>24 (41.3)</td>
<td>0.4 (0.2-0.9)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>21 (15.2)</td>
<td>17 (29.3)</td>
<td>2.3 (1.0-5.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>IL1R</td>
<td>Pst-1 1970</td>
<td>C</td>
<td>174 (62.1)</td>
<td>71 (60.6)</td>
<td>0.6 (0.5-1.5)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>106 (44.2)</td>
<td>46 (39.3)</td>
<td>1.5 (0.6-1.7)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>54 (38.6)</td>
<td>18 (31)</td>
<td>0.6 (0.3-1.4)</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>66 (47.1)</td>
<td>33 (56.8)</td>
<td>0.5 (0.7-2.8)</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>20 (14.3)</td>
<td>7 (12)</td>
<td>1.7 (0.3-2.2)</td>
<td>0.8</td>
</tr>
<tr>
<td>IL1Ra</td>
<td>Mspa-I 11100</td>
<td>C</td>
<td>64 (22.9)</td>
<td>18 (15.7)</td>
<td>0.6 (0.3-1.1)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>216 (77.1)</td>
<td>96 (84.2)</td>
<td>1.5 (0.8-2.9)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>4 (2.9)</td>
<td>1 (1.7)</td>
<td>0.6 (0.5-9.9)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>56 (40)</td>
<td>16 (28)</td>
<td>0.5 (0.2-1.2)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>80 (57.1)</td>
<td>40 (70.1)</td>
<td>1.7 (0.8-3.6)</td>
<td>0.1</td>
</tr>
<tr>
<td>IL-6</td>
<td>-174</td>
<td>G</td>
<td>177 (63.7)</td>
<td>73 (61.8)</td>
<td>0.9 (0.5-1.4)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>4 (2.9)</td>
<td>2 (3.3)</td>
<td>1.1 (0.1-7.8)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>93 (66.9)</td>
<td>41 (69.4)</td>
<td>1.1 (0.5-2.2)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>42 (30.2)</td>
<td>16 (27.1)</td>
<td>0.8 (0.4-1.7)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>50 (18)</td>
<td>30 (25.4)</td>
<td>1.5 (0.9-2.6)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>228 (82)</td>
<td>88 (74.5)</td>
<td>0.6 (0.3-1.1)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>4 (2.9)</td>
<td>2 (3.3)</td>
<td>1.1 (0.1-7.8)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG</td>
<td>42 (30.2)</td>
<td>26 (44.0)</td>
<td>1.8 (0.9-3.5)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>93 (66.9)</td>
<td>31 (52.5)</td>
<td>0.5 (0.2-1.0)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

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 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].

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

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