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# Tumor necrosis factor-alpha single nucleotide polymorphisms in juvenile systemic lupus erythematosus

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ABSTRACT

*Background:* Juvenile systemic lupus erythematosus (JSLE) is a multi-system autoimmune disorder of unknown origin. Given the importance of the contribution of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), towards the pathogenesis of JSLE, this study was performed to assess *TNFA* gene polymorphisms in a case-control study.
*Methods:* Fifty nine patients with JSLE were enrolled in this study as case group and compared with healthy control subjects. The frequency of alleles, genotypes, and haplotypes of *TNFA* single-nucleotide polymorphisms (SNPs) at positions −308 and −238 were evaluated, using polymerase chain reaction with sequence-specific primers method.
*Results:* The G allele at position −238 in *TNFA* promoter region was significantly more frequent in patients with JSLE than in the healthy controls ($P$ value < 0.001), while the frequency of A allele at the same position was significantly lower than controls. Furthermore, a significant positive association for G/G genotype at the same position was detected in patients' group compared with control subjects ($P$ value < 0.001). The GA haplotype of *TNFA* (positions −308, −238) was significantly less frequent in case group than in controls ($P$ value < 0.001), while GG was the most frequent haplotype for *TNFA* in the patient group, compared to controls ($P$ value < 0.01).
*Conclusions:* Pro-inflammatory cytokine gene polymorphisms may influence susceptibility to JSLE. Particular *TNFA* gene variants are associated with JSLE and could be used as a genetic marker for susceptibility to JSLE.
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## 1. Introduction

Juvenile systemic lupus erythematosus (JSLE) is a multi-system autoimmune disorder of unknown origin with a remarkably diverse spectrum of immunologic and clinical abnormalities which appears before the age of 16 years [1–3]. JSLE is primarily characterized by exuberant production of autoantibodies as well as enhanced immune complex deposition, in company with autoreactive T cells, which together culminate in subsequent pathology in multiple target organs [4–6].

A complex interplay between genetic risk factors and environmental events seems to contribute towards disease initiation and progression [7,8].

Given the importance of the role of cytokines in the pathogenesis of autoimmune and inflammatory diseases, deregulated cytokine production patterns have been reported as important actors in SLE [9].

Several circulating cytokine abnormalities result in impaired immune regulation and mediates tissue inflammation. Pro-inflammatory cytokines, like tumor necrosis factor-alpha (TNF-α), play a significant role in initiation and propagation of inflammatory and immune responses through enhancement of adhesion molecules expression, neutrophil activation, stimulation

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of cytokine production, in addition to acting as a co-stimulator for T-cell activation and antibody production [8,10–14].

Several genetic variations within the coding and promoter sequences of pro-inflammatory cytokine genes have already been identified to influence their expression together with subsequent cytokine production in different immunological disorders [15–20]. Indeed association of *TNFA* genetic variants with susceptibility to and outcome of SLE has also been investigated, but arriving at a consensus appears to be difficult as variable results are achieved by different studies [21]. Moreover, there is a conspicuous paucity of investigations exploring the immunopathology of JSLE, and much is deduced from studies of patients with adult-onset SLE. To the best of our knowledge, the contribution of cytokine gene polymorphisms for development of JSLE in Iranian patients has not been established so far.

The current study aimed to determine the associations between SNPs in *TNFA* at positions −238 and −308 and juvenile SLE in a number of pediatric patients.

## 2. Patients and methods

### 2.1. Study population

In the present study, 59 SLE patients (10 male, 49 female), with mean age of male 10.10 ± 4.38 and female 11 ± 3.47, were enrolled from the Rheumatology Clinic of the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Iran. The control group is consisted of 137 unrelated healthy subjects who were randomly selected from blood donors at Iranian blood transfusion organization [22].

Thorough history taking, comprehensive examination and relevant laboratory and radiological studies were conducted for all the patients. SLE was diagnosed based on the revised criteria of the American College of Rheumatology (ACR) for classification of SLE [23].

Written informed consents were taken from all participants according to the guidelines of the Ethics Committee of Tehran University of Medical Sciences prior to enrollment.

### 2.2. Sampling and genotyping

For all of the participants, amount of 5 milliliters (ml) of venous blood samples were collected and kept with ethylenediaminetetraacetic acid (EDTA) at −20 °C until analyzed. Genomic DNA was isolated using the "salting out" technique [24]. Cytokine gene typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany) [22]. Amplification of the extracted DNA was carried out using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK) under the following conditions: initial denaturation at 94 °C, 2 min; denaturation at 94 °C, 10 s; annealing + extension at 65 °C, 1 min (10 cycles); denaturation at 94 °C, 10 s; annealing at 61 °C, 50 s; extension at 72 °C, 30 s (20 cycles). The availability of PCR products was visualized by subjecting DNA fragments to electrophoresis in 2% agarose gel. After electrophoresis, the gel was placed on an ultraviolet transilluminator, and an image was taken for analysis and documentation. The frequencies of alleles, genotypes, and haplotypes of *TNFA* at positions −308 and −238 were evaluated.

### 2.3. Statistical analysis

The allele, genotype and haplotype frequencies were calculated by direct counting and compared with the controls using the chi-square test. In order to test the Hardy–Weinberg equilibrium, the frequencies of various genotypes were compared using the chi square test. The odds ratio (OR), 95% confidence interval (CI) and the probability of type 2 error ($\beta$) were estimated for each allele, genotype, and haplotype in the patient and control groups. The *P* value of less than 0.05 was considered to be statistically significant.

## 3. Results

### 3.1. Allele frequencies

Details of allelic frequencies in patients with juvenile SLE and healthy controls are depicted in Table 1.

We discovered a significant increment in the frequency of G allele at position −238 in *TNFA* among patients with juvenile SLE (94.7% vs. 78.5%; *P* value < 0.001; OR, 4.9; 95% CI, 1.9–13.1); while the frequency of A allele at the same position was remarkably lower than healthy controls (5.2% vs. 21.5%; *P* value < 0.001; OR, 0.2; 95% CI, 0–0.5). The allele frequencies of *TNFA* at position −308 were similar in two groups of patients and controls.

### 3.2. Genotype frequencies

Details of genotype frequencies in patients with juvenile SLE and healthy controls are demonstrated in Table 1.

We found a significant positive association for promoter sequence of *TNFA* gene at position −238 for G/G in patients' group in comparison with healthy controls (91% vs. 57.7%; *P* value < 0.001; OR, 7.4; 95% CI, 2.6–22.8). Furthermore, an outstanding negative association was observed between the G/A genotype at the same position of *TNFA* gene with juvenile SLE (7.1% vs. 41.6%; *P* value < 0.001; OR, 0.1; 95% CI, 0–0.3).

No significant differences were found between the two groups for the −308 position.

### 3.3. Haplotype frequencies

Details of haplotype frequencies in patients with juvenile SLE and healthy controls are shown in Table 2.

A noteworthy negative haplotypic association for juvenile SLE was noticed for *TNFA* (positions −308, −238) GA haplotype (5.3% vs. 21.5%; *P* value < 0.001; OR, 0.2; 95% CI, 0.08–0.5).

The GG haplotype for *TNFA* (positions −308, −238) was observed in 85.5% of patients with JSLE, which was a prominently higher frequency than that noted in control group (64.2%) (*P* value < 0.01; OR, 3.3; 95% CI, 1.8–6.2).

## 4. Discussion

Systemic lupus erythematosus is a multifactorial disease with a wide variety of immunological and clinical manifestations, as well as heterogeneity of underlying mechanisms, which can affect multiple organs. The fact that individual susceptibility to SLE exists even with identical environmental exposure is well recognized. Polymorphisms in the pro-inflammatory cytokines genes, resulting in variations in cytokine production, may have accounted for this difference among individuals at least to some degree [25–27]. Hence genetic vulnerability to SLE has been a topic of intensive research over the past two decades.

TNF-α is a potent pro-inflammatory cytokine which serves as a pivotal actor in inflammatory and immune responses [28,29]. A number of studies have investigated the role of TNF-α in the pathogenesis of SLE. It has been hypothesized that genetic variants affecting cytokine transcription exist in the regulatory sequences of *TNFA* genes, and that such polymorphisms may correlate with

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