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ORIGINAL ARTICLE

Association of interleukin-2 and interferon-γ single nucleotide polymorphisms with Juvenile systemic lupus erythematosus☆

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Abstract
Purpose: Juvenile systemic lupus erythematosus (JSLE) is a severe and chronic autoimmune disease of unknown origin. Inflammatory cytokines can play a pivotal role in the pathogenesis of JSLE, while their secretion is under genetic control. The current investigation was performed to analyse the associations of particular single nucleotide polymorphisms (SNPs) of interleukin-2 (IL-2) and interferon-gamma (IFN-γ) genes in a case control study.
Materials and methods: The allele, genotype and haplotype frequencies of the polymorphic *IL-2* (G/T at −330, rs2069762, and G/T at +166, rs2069763) and *IFN-γ* (A/T at +874, rs2430561) genes were estimated in 59 patients with JSLE by contrast with 140 healthy controls using polymerase chain reaction with sequence-specific primers method.
Results: Results of the analysed data revealed a negative allelic association for JSLE in IL-2 −330/T ($P=0.02$), as well as a positive allelic association for IL-2 −330/G ($P=0.02$).

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IL-2 GG genotype (−330) in the patient group was also significantly overrepresented ($P < 0.001$), while IL-2 GT genotype (−330) was notably decreased in the patients with JSLE ($P < 0.001$). Additionally, the frequency of IL-2 (−330, +166) GT haplotype was significantly higher in the patient group ($P < 0.001$).
Conclusion: IL-2 cytokine gene polymorphisms could affect individual susceptibility to JSLE and can take on the role of possible genetic markers for vulnerability to JSLE.

Introduction

Systemic lupus erythematosus (SLE) is a severe and chronic autoimmune disease of unknown origin, characterised by widespread inflammation and the production of a multitude of autoantibodies against native DNA and other cellular components. SLE is a prototypic autoimmune disease with a diverse spectrum of clinical manifestations that may affect virtually every organ in the human body.[21,22] Ten to twenty percent of all SLE patients are categorised as patients with juvenile SLE (JSLE) with a disease onset prior to the age of 16 years.[27,32] A plausible genetic predisposition along with environmental factors culminate in the expression of disease pathology.[17,29] The earlier onset of JSLE, along with its more active entity at diagnosis and over time, compared to the adult-onset SLE, could be possibly rationalised by the presence of different SLE susceptibility loci in JSLE.

Given the pivotal role of cytokines in the pathogenesis of inflammatory and autoimmune disorders, it stands to reason that cytokines contribution to the initiation and progression of multiple diseases including SLE have been a topic of intensive research recently.[31] Some such cytokines include interleukin-2 (IL-2) and interferon-gamma (IFN-γ). IL-2 is a multifunctional cytokine primarily secreted by T cells. This cytokine plays an important role in the processes leading to T cell activation, proliferation, and contraction. It has been speculated that production of IL-2 is decreased in patients with SLE and this deregulation exerts influence on several aspects of host immunity.[12,13] IFN-γ is produced by the immune cells, particularly NK cells and T cells, which are involved in both innate and acquired immune systems. This cytokine is known to exert antiviral capacities, contribute to cytotoxic T-cell activity, activate macrophages, and be associated with T helper 1 responses. IFN-γ production has been demonstrated to be diminished in SLE.[20,28]

There is some evidence concerning the probable association of multiple cytokine gene polymorphisms with serum levels of the cytokines,[1,3,4,14,24] but analyses of such loci have been limited to specific cytokines and few disorders, and as far as we know, only a few association studies have ever been established concerning Iranian paediatric patients with JSLE.[15,16,23,30,34]

The primary objective of this study was to determine the associations between certain SNPs in both *IL-2* at positions −330 and +166 and *IFN-γ* at position +874 and juvenile SLE in a number of Iranian paediatric patients.

Patients and methods

Study population

Fifty-nine Iranian children diagnosed with juvenile SLE according to the revised criteria of the American College of Rheumatology (ACR) for classification of SLE,[10] were recruited consecutively during routine visits at the Rheumatology Clinic of the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Tehran, Iran. One hundred and forty healthy subjects who were randomly selected from blood donors at Iranian blood transfusion organisations were enrolled as the control group.[4]

The study was approved by the ethical committee of Tehran University of Medical Sciences. Written informed consent was obtained from all guardians and, as appropriate, assent was taken from the participants, before blood sampling.

Sampling and genotyping

Amount of five millilitres of peripheral blood was collected from all of the entrants to this study and kept with ethylenediaminetetraacetic acid (EDTA) as anticoagulant, at −20 °C until investigation. Genomic DNA was extracted from blood samples using the ''salting out'' technique.[18] Genotyping of the polymorphisms in cytokine genes was carried out using polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), as discussed previously.[4] Amplification of the isolated DNA was performed by a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK) under the following conditions: initial denaturation at 94 °C for 2 min; denaturation at 94 °C for 10 s; annealing + extension at 65 °C for 1 min (10 cycles); denaturation at 94 °C for 10 s; annealing at 61 °C for 50 s; extension at 72 °C for 30 s (20 cycles). The availability of polymerase chain reaction (PCR) products was visualised by 2% agarose gel electrophoresis and subsequent ultraviolet transilluminator. The frequencies of alleles, genotypes, and haplotypes of *IL-2* at positions −330 and +166 and *IFN-γ* at position +874 were assessed.

Statistical analysis

We evaluated allele, genotype, and haplotype frequencies for all cytokine gene polymorphisms by direct counting. Using the chi-square test, frequencies of alleles, genotypes,