Allergol Immunopathol (Madr). 2016;44(4):303–306

Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica

www.elsevier.es/ai

ORIGINAL ARTICLE

Association of *Interleukin-2*, but not *Interferon-Gamma*, single nucleotide polymorphisms with juvenile idiopathic arthritis

M. Maddah[a], S. Harsini[b], A. Rezaei[b], M. Sadr[c], S. Zoghi[d], M.H. Moradinejad[a], V. Ziaee[a,e], N. Rezaei[b,c,d,f,*]

[a] *Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
[b] *Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Molecular Immunology Research Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[d] *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[e] *Pediatric Rheumatology Research Group, Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[f] *Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran*

Received 24 May 2015; accepted 7 October 2015
Available online 31 March 2016

KEYWORDS
Interleukin-2;
Interferon-gamma;
Single nucleotide polymorphism;
Juvenile idiopathic arthritis;
Children

Abstract
Background: Cytokines, including interleukin-2 (IL-2) and interferon-gamma (IFN-γ), seem to play a role in the pathogenesis of juvenile idiopathic arthritis (JIA). The aim of this study was to investigate the associations of *IL-2* and *IFN-γ* single nucleotide polymorphisms (SNPs) with susceptibility to JIA in an Iranian population.
Methods: Genomic DNA of 54 Iranian patients with JIA and 139 healthy unrelated controls were typed for *IL-2* (G/T at −330 and +166) as well as *IFN-γ* gene (A/T at +874), using polymerase chain reaction with sequence-specific primers method, and compared between patients and controls.
Results: A significantly higher frequency of the *IL-2* −330 GG genotype ($p < 0.01$) was found in the JIA patients compared to the controls. However, the GT genotype at the same position was notably lower than in controls ($p < 0.01$). Moreover, *IL-2* (−330, +166) GT haplotype was more frequent in patients with JIA in comparison with controls. No significant differences was observed between the two groups of case and control for *IL-2* (G/T at +166) and *IFN-γ* (A/T at +874) SNPs.
Conclusion: The results of the current study suggest that certain SNPs of *IL-2* gene have association with individuals' susceptibility to JIA. However, further investigations are required to confirm the results of this study.

* Corresponding author.
E-mail address: rezaei_nima@tums.ac.ir (N. Rezaei).

http://dx.doi.org/10.1016/j.aller.2015.10.005

Introduction

Juvenile idiopathic arthritis (JIA) is the most prevalent chronic arthritic condition of childhood with its prevalence varying worldwide among different ethnicities,[14] which comprises seven distinct categories as demarcated by the International League of Associations for Rheumatology (ILAR) classification criteria.[16] JIA encompasses all forms of arthritis of unknown aetiology that commence before the age of 16 years and persists for more than 6 weeks.[16] Notwithstanding the heterogeneity between the JIA subgroups, they do appear to have genetic risk factors in common.[7] The common feature of all JIA subtypes is the presence of chronic inflammation within synovial joints, in which cytokines play pivotal roles. It has been previously suggested that certain single nucleotide polymorphisms (SNPs) within the promoter and coding sequences of various cytokines' genes, which affect their level of production, could be associated with the vulnerability to a group of autoimmune disorders.[4,6,10,12,21]

In the current study, we have selected two of the cytokines, including interferon-gamma (IFN-γ) and interleukin-2 (IL-2), that have been reported previously to have a role in JIA aetiopathogenesis,[3,19] to determine whether certain SNPs in these cytokines' genes are associated with susceptibility to JIA.

It has been postulated that these genes may also confer susceptibility to JIA. Therefore, the aim of this study was to assess whether *IL-2* (G/T at −330 and +166) as well as *IFN-γ* (A/T at +874) gene polymorphisms are also associated with susceptibility to JIA in Iranian patients.

Patients and methods

Study population

A total of 54 consecutive JIA patients, recruited from the Rheumatology Clinic of the Children's Medical Center Hospital, the Paediatrics Center of Excellence in Iran, were included in the case group and compared to 139 healthy unrelated individuals who were randomly selected from blood donors at Iranian blood transfusion organisations.[1] Diagnosis of JIA was based on the ILAR classification criteria for JIA.[17] Our patients' group comprised 24 individuals with oligoarticular JIA, 19 with polyarticular JIA, and 11 with systemic disease subtype.

Written informed consents were obtained from all entrants to this investigation, and the study protocol was approved by the Ethical Committee of Tehran University of Medical Sciences.

Sampling and genotyping

To obtain genomic DNA, 5 mL of peripheral blood was collected from each patient and stored at −20 °C in EDTA tubes until DNA extraction using the ''salting out'' technique.[13] Polymerase chain reaction, with sequence-specific primers method (PCR-SSP assay kit; Heidelberg University, Germany) was employed for cytokine gene typing according to the manufacturer's instructions.[1] The three single nucleotide polymorphisms (SNPs) studied were *IL-2* (G/T at −330 and +166) and *IFN-γ* (A/T at +874).

Statistical analysis

The allele, genotype and haplotype frequencies were evaluated by direct gene counting and compared with the healthy controls using the chi square test. The odds ratio (OR) and 95% confidence interval (CI) were estimated for each allele, genotype, and haplotype in the patient and control groups. Adherence to the Hardy–Weinberg equilibrium for each genotype was assessed for both case and control groups using the chi square test. *P* values of less than 0.05 were considered statistically significant.

Results

Allele, genotype and haplotype frequencies

The allelic and genotype frequencies in patients with JIA and healthy controls are demonstrated in Table 1.

A positive genotypic association was detected for *IL-2* −330/GG with JIA susceptibility (28.8% vs. 5.8%, $p < 0.01$). Meanwhile, the GT genotype frequency at *IL-2* −330 in JIA patient was notably lower than in healthy individuals (33.3% vs. 67.6%, $p < 0.01$). No significant difference was discovered between the two groups of case and control for *IL-2* (G/T at +166) and *IFN-γ* (A/T at +874) SNPs.

At the haplotypic level, *IL-2* (−330, +166) GT haplotype was found to be more frequent in patients with JIA in comparison with healthy controls (3.6% vs. 0.3%, $p = 0.04$). We observed no significant differences between the two groups for GG, TG and TT haplotypes at the same positions (Table 2).

In addition, no significant difference was detected between the aforementioned gene polymorphisms and individuals' susceptibility to different JIA subtypes, including systemic, polyarticular, and oligoarticular JIA.

Discussion

This study aims at evaluating the association of *IL-2* (−330 G/T, +166 G/T) and *IFN-γ* (+874 A/T) single nucleotide polymorphisms in Iranian patients with JIA. To the best of our knowledge, ours is the first study to analyse the role of the above-said gene variants in susceptibility to JIA in an Iranian population.

Interleukin-2 (IL-2) is recognised as a typical T helper 1 (Th1) cytokine, which exerts forceful modulatory effect on various immune cells.[11] It has been proposed that dysfunction of the IL-2 could culminate in functional or pathological alterations in the immune system, resulting in autoimmunity.[8] It is widely accepted that *IL-2* expression is reduced in peripheral blood and both the synovial fluid and the synovium of patients with rheumatoid arthritis (RA) compared to healthy individuals.[11,20] Furthermore, IL-2 protein has previously been identified to be decreased in the sera of patients with systemic-onset JIA.[19] *IL-2* SNPs are the probable underlying causes of the variations found in IL-2 concentrations between patients and controls.[11] Two