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ORIGINAL ARTICLE

Association of interleukin-1 family gene polymorphisms with juvenile idiopathic arthritis in Iranian population

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KEYWORDS
Interleukin-1;
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Abstract
Background: Cytokines, including interleukin-1 (IL-1), seem to contribute towards the pathogenesis of juvenile idiopathic arthritis (JIA), so this study was designed to evaluate the associations of *IL-1* gene cluster and IL-1 receptor (*IL-1R*) gene single nucleotide polymorphisms (SNPs) with JIA proneness in Iranian population.
Materials and methods: Genomic DNA of 55 Iranian patients with JIA and 140 controls were extracted and typed for *IL-1α* gene at position −889, *IL-1β* gene at positions −511 and +3962, *IL-1R* gene at position Pst-I 1970, and interleikin-1 receptor antagonist (*IL-1Ra*) gene at position Mspa-I 11100, using polymerase chain reaction with sequence-specific primers method, and compared between patients and controls.
Results: The CC genotype of *IL-1Ra* at Mspa-I 11100 position was found to be more frequent in patients with JIA compared to healthy individuals ($P = 0.03$), although the CT genotype at the same position was significantly higher in the control group in comparison with patients with JIA ($P = 0.02$). No significant differences were observed between the two groups of case and control for *IL-1α* (−889 C/T), *IL-1β* (−511 C/T and +3962 C/T) and *IL-1R* (Pst-1 1970 C/T).
Conclusion: The results of the present investigation suggest that certain *IL-1Ra* gene variants are associated with individuals' susceptibility to JIA. Nevertheless, further studies are required to establish the results of the current study.

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Introduction

Juvenile idiopathic arthritis (JIA) is a common autoimmune disease, characterised by chronic arthritis of unknown aetiology with onset prior to the age of 16 years. It has been previously propounded that numerous risk alleles from various susceptibility genes, including a multitude of major histocompatibility complex and non-major histocompatibility complex regions, predispose individuals to development of JIA following exposure to as yet unravelled environmental factors.[10] Given the increased efficacy of the early prescription of disease-modifying anti-rheumatic drugs, it stands to reason that identification of other genetic markers associated with individual susceptibility to JIA would be valuable to initiate therapy at an early stage of the disease.

Cytokines such as interleukin-1 (IL-1) are known to be the central mediators of joint inflammation, found in both the synovial fluid and sera of patients with JIA. The aforementioned immune mediators with polymorphic gene sequences have been suggested as potential markers of individuals' susceptibility to JIA.[5,9,18]

To date, various single nucleotide polymorphisms (SNPs) in different cytokines genes, influencing their level of synthesis, have been studied in the context of rheumatologic disorders, such as juvenile-onset systemic lupus erythematosus.[14,20,22,24] However, to the best of our knowledge, no association study has been conducted on interleukin-1 gene cluster and interleukin-1 receptor polymorphisms in an Iranian population with JIA.

The aim of the current investigation was to study possible genetic contributions of selected cytokine polymorphisms (*IL-1α* at position −889, *IL-1β* at positions −511 and +3962, interleukin-1 receptor (*IL-1R*) at position Pst-1 1970 and interleukin-1 receptor antagonist (*IL-1Ra) at position* Mspa-I 11100 C/T) on JIA vulnerability in a population of Iranian patients with JIA.

Patients and methods

Subjects

A total of 55 consecutive JIA patients, recruited from the Rheumatology Clinic of the Children's Medical Centre Hospital, the Paediatrics Centre of Excellence in Iran, were enrolled in the present study as the case group and compared to 140 healthy unrelated controls, randomly selected from blood donors at Iranian blood transfusion organisations.[3] The ILAR classification criteria for JIA were used to establish the diagnosis of JIA.[19] Our patients' group consisted of 25 individuals with oligoarticular JIA, 19 with polyarticular JIA, and 11 with systemic disease subtype.

Written informed consents were obtained from all participants according to the guidelines of the Ethical Committee of Tehran University of Medical Sciences prior to enrolment.

Sampling and genotyping

For all of the entrants to this study, 5 ml of peripheral blood samples were obtained and kept with EDTA at −20 °C until the extraction of genomic DNA using the ''salting out'' technique.[16] Polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany) was employed for cytokine gene typing as discussed previously.[3] This assay uses the self-same amplification and detection conditions, resulting in speedy and cost-efficient analysis of polymorphisms. Amplification of the extracted DNA was carried out by a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK) under the following conditions: prime 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, followed by ultraviolet transillumination. Additionally, photography for interpretation and documentation was performed. Each of the primer mixes contained a control primer pair that amplified either a part of the C-reactive protein (CRP) gene or a part of the β-globin gene. The β-globin control primers produce an 89 bp fragment, while the primer pairs amplifying the CRP gene produced a 440 bp amplicon.[13] The five single nucleotide polymorphisms (SNPs) investigated were *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) and *IL-1Ra* (Mspa-I 11100 C/T; rs315952).

Statistical analysis

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

Results

Allelic and genotype frequencies in Iranian patients with JIA and healthy individuals are depicted in Table 1.

The CC genotype of *IL-1Ra* at Mspa-I 11100 position was shown to be more frequent in patients with JIA, compared to healthy individuals (11.3% vs. 2.9%; *P* value, 0.03), although the CT genotype at the same position was significantly higher in the control group in comparison with patients with JIA (40% vs. 20.8%; *P* value, 0.02). The allelic frequencies of *IL-1Ra* at Mspa-I 11100 position were similar in the two groups of patients and controls. Additionally, no significant difference was observed between the two groups of case and control for *IL-1α* (−889 C/T), *IL-1β* (−511 C/T and +3962 C/T) and *IL-1R* (Pst-1 1970 C/T).

Furthermore, we observed no significant difference between the aforementioned gene variants and individuals' susceptibility to different JIA subtypes, including systemic, polyarticular, and oligoarticular JIA.

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