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R202Q Mutation of Mediterranean Fever Gene in Iranian patients with Systemic-onset Juvenile Idiopathic Arthritis

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Abstract

Background: Systemic-onset Juvenile Idiopathic Arthritis (SoJIA) is an autoinflammatory disease with complex genetic trait starts in children less than 16 years of age with fever and cutaneous rash. Despite, the main genetic factors that may play a role in SoJIA have not yet been identified. High level of interleukin-1beta in the blood of SoJIA patients has been reported. The production and secretion of IL-1β is related to pyrin coded by mediterranean fever gene (*MEFV* gene). Therefore, mutation in *MEFV* may be associated with SoJIA diseases. This study aimed to identify the association between R202Q mutation in exon 2 of *MEFV* gene and SoJIA disease.

Materials and Methods: This study was done in 30 SoJIA patients and 30 controls. DNA was extracted from blood cells and analyzed by RFLP-PCR. The PCR product was digested with *Pvu*II and then separated by gel electrophoresis.

Results: R202Q mutation was found in 3.3% of control and 43.3% of patient group. Significant statistical differences were observed between cases and controls in the R202Q mutation.

Conclusion: The present study showed that the mutation in *MEFV* gene is a susceptible factor in development of SoJIA disease in Iranian patients.

Keywords: Mediterranean fever; Pyrin; R202Q; RFLP-PCR; Juvenile Idiopathic Arthritis

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Introduction

Systemic onset Juvenile Idiopathic Arthritis (SoJIA) is a subset of juvenile idiopathic arthritis disease (JIA). HLA (human leukocyte antigen) associations are significant genetic factors in most arthritis subtypes and our previous research prove this, but there are few or no associations with systemic JIA (1-3). SoJIA is an autoinflammatory disease with complex genetic trait starts in children less than 16 years of age with symptoms such as fever, cutaneous rash, lymphadenopathy, hepatosplenomegaly or serositis. The onset of disease in adolescence is rare, and only a few cases are reported. Activated monocytes from patients with SoJIA secrete significantly higher amounts of interleukin-1beta (IL-1β) compared with monocytes of healthy controls (4).

High level of IL-1β in blood is associated with systemic inflammation, fever, and rash. While specific genetic cause for the disease has not been identified yet, high level of IL-1β in the blood of patient; and relation between its complications, and clinical signs of disease; mutation in regulator of this cytokine can be a susceptible factor for SoJIA. Pyrin is one of these factors interfering in function of IL-1β production pathway and can inhibit its excessive production. Pyrin is coded by Mediterranean fever (*MEFV*) gene. The first time mutation in that was reported in association with familial Mediterranean fever disease (FMF). Also, Mutation in *MEFV* gene is related to many autoinflammatory diseases. *MEFV* consists of 10 exons; exons 2 and 10 are hot spots for

mutation in *MEFV* gene (5). R202Q is a mutation in exon 2 which has been previously reported by Ozturk *et al*. (6). This study investigated the association between R202Q mutation and SoJIA disease in Iranian children.

Materials and Methods

Blood samples were collected, with consent, from thirty pediatric SoJIA Iranian patients that were diagnosed by pediatric rheumatologist.Genomic DNA was extracted using previously described method (7). R202Q (605G>A) mutation of exon 2 was detected with restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) in patients and control samples. For this, exon2 fragments were amplified with primers: 5´-AGATGATTCCGCAGCG TCC-3´ and 5´-GGGGT-TCTGTTGCCGAGTCC-3´ based on PCR program as following: A predenaturation step of 3min at 94 °C-was followed by 35 cycle (94 °C for 30s, 58 °C for 36s, 72 °C for 50s) and final extension of 3 min at 72 °C. PCR products made 500bp fragments (Figure1).

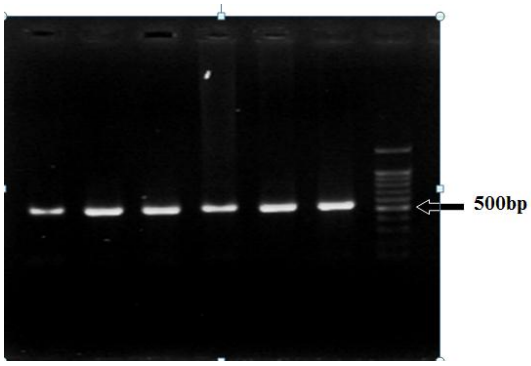

Figure 1. SSP-PCR results of amplification in exon2

R202Q mutation created a *Pvu*II restriction site in the PCR products of mutant allele. After digestion with *Pvu*II enzymes, the mutant allele produced–two fragments with 196bp and 304bp lengths while the normal allele gives 500bp uncut fragment (Figure 2).
At the end of the study 5 samples were randomly sequenced to confirm the results. Thus, exon 2 with mentioned primers were amplified then sequencing with ABI sequencer was done.

Results

R202Q mutations were screened in 30 Iranian SoJIA patients and 30 healthy control samples. Mutation frequency was 43% which is significantly higher than control population ($p<0.01$).
Thirteen patients had R202Q mutation that six of them were homozygote and the others were heterozygote; whereas, just one control sample was heterozygote for R202Q mutation.

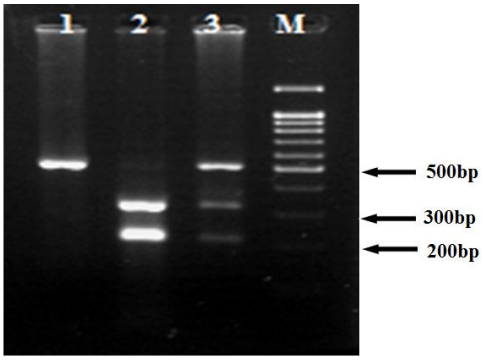

Figure 2. RFLP-PCR of R202Q mutation: lane1; Normal homozygote,
Lane 2; Mutated homozygote, lane3; Heterozygote

Mutation frequency in patients was more significant than in control population ($p<0.01$) (Table 1).

Table 1. R202Q mutant genotype frequencies in patients and controls

Genotype	Patient		Control		p-value
	Count	Percent	Count	Percent	
AA	6	20	0	0	0.001
AG	7	23.3	1	3.3	
GG	17	56.7	29	96.7	
Total	30	100	30	100	

The consequence of sequencing was consistent with RFLP-PCR results and confirmed them. Also, some SNPs such as G138G, A165A in exon 2 in sequencing results have been detected (Figure 3).

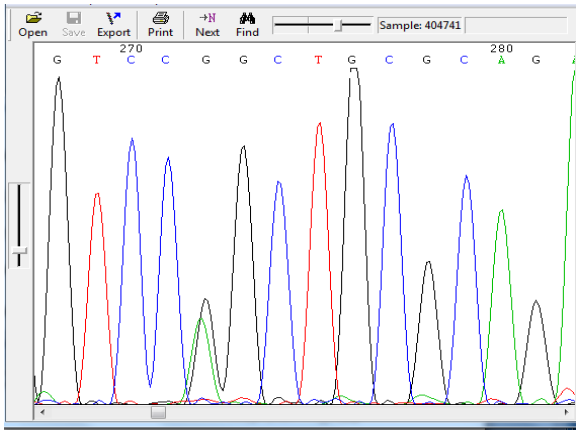

Figure 3. Electropherogram of the R202Q mutation in the MEFV gene revealed by DNA Sequencing.

Discussion

The International League of Associations for Rheumatology classified SoJIA as an arthritis in children starting before 16 years of age with symptoms like daily quotidian fever of 39 °C (or

more) and evanescent rash, lymphadenopathy, hepatosplenomegaly or serositis (8, 9). The outstanding clinical feature that distinguishes SoJIA from other subtypes of JIA is fever up to and greater than 39 °C. The second important clinical sign in SoJIA is cutaneous rash. Both of these symptoms can be due to high level of proinflammatory cytokine, IL-1β, in the blood which is the consequence of mutation in regulatory factor of IL-1β production pathway.[4] Based on this hypothesis, we decided to study Pyrin as an important regulatory factor in IL-1β production pathway which was first identified as the main cause of FMF disease (another autoinflammatory disease with similar symptoms to SoJIA). We screened R202Q mutation in exon 2 of *MEFV* gene as a candidate gene for susceptibility to SoJIA in Iranian patients. Thirteen patients had R202Q mutation and mutation frequency in patients was more significant than in population ($p<0.01$).

Although previous studies reported R202Q mutation as a frequent polymorphism (10), genotype frequency of this mutation (both of heterozygote and homozygote) were significantly associated with SoJIA disease in our patients. Our results are coordinated with Villani *et al* results in ulcerative colitis disease (11). We screened R202Q mutation in SoJIA patients for the first time.

MEFV mutation influences the IL-1β production and is probably an important cause of high level of IL-1β in SoJIA patients. Therefore, genetic diagnosis of *MEFV* mutations could help pediatric rheumatologist in choosing the best method for treatment. In general, mutation in *MEFV* gene can act as a susceptible factor in the development of SoJIA disease in Iranian patients, but sequencing of whole gene with more number of cases is suggested to verify the results.

Conflict of Interest

The authors declare that they have no conflict of interest in this work.

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