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Association between Interleukin-23 Receptor R381Q Gene Polymorphism and Asthma

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ABSTRACT

The SNP (rs11209026, Arg381Gln, R381Q) in the IL-23 receptor (IL23R) confers protection against multiple inflammatory diseases, representing one of the most significant human genetic polymorphisms in inflammatory diseases. We, therefore, investigated the association between IL-23 R R381Q gene polymorphism and asthma.

This case-control study was performed on 209 patients, and 200 healthy controls. Using PCR-RFLP, the R381Q variant was screened in the IL-23R gene of the patients and controls.

Serum IgE levels were measured using ELISA technique. Eosinophil absolute count was done with Sesmex cell counter.

Our results indicated that the genotype and allele frequencies of the IL-23R R381Q polymorphism is significantly different between asthmatic patients and control subjects ($p<$ 0.001; odd ratio= 0.266; 95%, CI=0.118-0.604. Moreover, the asthmatic patients had higher eosinophil count and total serum IgE levels than controls as expected ($p<0.001$).

The present study suggested that R381Q polymorphism in IL-23 receptor may be a predisposing allele for asthma.

Keywords: Asthma; IL-23 Receptor; Single Nucleotide Polymorphism

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INTRODUCTION

Asthma is a chronic inflammatory disease of the airways. It is suggested that IL-17A and IL-17F (Th 17 cytokines) recruit neutrophils into the airways and bronchi of animal models of asthma.[1,2] IL-17A expression in the airways of asthmatic patients is correlated with the severity of asthma.[3-5] IL-23 has been recognized as a new IL-12 family cytokine which consists of p19 and a p40 subunits.[6] It is known that IL-23 plays pathogenic roles in chronic inflammatory diseases such as inflammatory bowel diseases, arthritis, and psoriasis.[7,8] Furthermore, IL-23 is vital for the differentiation, maintenance and effector functions of Th17 cells.[9] that the role of IL-23/Th17 axis in the development of inflammatory diseases has been fully elucidated.[10-12] In addition, IL-23 can cause eosinophilic inflammation in the airways.[13] IL-23 signals through IL-23R[14] and IL-23R is located on the chromosome 1p31.[15]

Polymorphisms in the IL-23R chain may affect IL-23 responses. Recent studies have shown that a functional single-nucleotide polymorphism (SNP) (Arg381Gln;R381Q; rs11209026; 1142 G wild type A reduced function) in the IL-23R gene leads to decreasing IL-23-dependent IL-17 production.[16] It has been reported that the R381Q allele confers protection against inflammatory bowel disease (IBD),[17] psoriasis,[18] ankylosing spondylitis.[19]

In this study, we evaluated the role of IL-23R R381Q polymorphism in susceptibility to asthma. Maybe this study can help to better understand the immunopathogenic process of asthma.

MATERIALS AND METHODS

Study Population

This case-control study was performed on 209 patients, and 200 healthy controls. The subjects studied were asthmatic patients attending the Allergy Clinic of the Amin hospital, Isfahan, Iran. A full verbal and written explanation of the study was given to all patients who had been interviewed before participation in this study. Informed consent was obtained and for subjects younger than 18 years old, it was given by their parents. The study was conformed to the provisions of the Declaration of Helsinki and approved by Isfahan University of Medical Sciences Ethics Committee.[20] Asthma was diagnosed according to the criteria of the Global Initiative for Asthma (GINA).[21] Pregnancy or breast-feeding and presence of parasitic infection were exclusion criteria. The controls did not have any of the symptoms of allergic and respiratory disease in their previous history or past physical check-up.

Total Serum IgE Measurements

Serum was separated from 4 ml blood samples, and total serum IgE estimation was carried out for each subject using the Euroimmun IgE ELISA kit (Germany) according to the manufacturer's instructions.

Eosinophil Count

For counting Eosinophils in the peripheral blood, a Cell Counter (SESMEX-XT 1800, Japan) was used.

DNA Preparation

Genomic DNA was extracted from peripheral blood cells using a Prime Prep™ Genomic DNA isolation kit (Genet Bio, Korea) according to the manufacturer's instructions.

PCR- RFLP

For PCR, the primers were designed by Primer3 software. The sequence of the primer pairs was 5'-CTTTTCTGGCAGGGTCATTTTG-3'(forward primer) and 5'-AAGTTGTTTCCTGGGGTAGTTGTG-3'(reverse primer). The PCR conditions were optimized for R381Q SNP as follows: 95°C for 5 min; 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. PCR amplification was carried out in a programmable PCR in an Eppendorf thermal cycler (Eppendorf, Germany). The 508-bp PCR product was then resolved over a 1% agarose gel and the quality was confirmed using green viewer stain and a UV transilluminator.

For RFLP assay, Hpy188i restriction endonuclease (New England Biolabs, Beverly, MA) was applied. The RFLP was performed in a total volume of 20 µl containing 10µl PCR product, 7.8µl distillated water, 2µl related buffer and 0.2 µl restriction enzyme. The RFLP conditions were optimized in 37°c for 3 hours. The restriction-digested fragments were separated on 2% agarose gels and stained with DNA green viewer and visualized under UV illumination. Hpy188i cut the 508 bp PCR product into fragments 323, 288, 103, 82 and 35 bp in sizes. Fragments of 288, 103, 82 and 35

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bp indicated the presence of homozygous GG genotype, and Fragments of 323, 288, 103 and 82 bp represented the presence of heterozygous GA genotype in Figure 1.

Statistical Analysis

The SPSS 20 software package (SPSS Company, Chicago, IL, USA) was used to carry out statistical analysis. Fisher exact test was first applied to compare the frequency distribution of gender, and smoking status between case and control groups. In addition, this test was used to compare the genotype distributions, allele frequencies between two groups of patients and controls. Association between this polymorphism and asthma was declared as odds ratios (OR) estimates with 95% confidence intervals (95% CI). Logistic regression analysis was used to evaluate the association between the IL23R rs11209026 polymorphism and asthma. Furthermore, age, eosinophil count and total serum IgE levels between the two groups were compared by means of Independent T-Test and we also used analysis of covariance (ANCOVA) method to eliminate confounding factors. *P*-value <0.05 was considered significant in all of these tests. In order to have a normal distribution for the analysis, IgE levels were change to log10 values.

RESULTS

Selected features of the two groups and their relationship with asthma are shown in Table 1. As it is shown in Table1, the two variables, age and gender, were not significantly different between patients and controls. Compared to the controls, the cases had higher eosinophil counts and total serum IgE levels (p<0.001). In contrast smoking status was not statistically different between the two groups (p<0.726).

In the present study, rs11209026 polymorphism of IL-23R in asthmatic patients and healthy controls from normal population were analyzed. Statistically significant differences were found in frequency of rs11209026 G>A polymorphism (numerical name of IL23R R381Q) between asthmatic patients and controls (p< 0.001, Crude Odds Ratio= 0.266, 95%, CI= 0.118-0.604).

In all studied groups, GG and GA genotypes were observed but AA genotype was not observed in any of the groups. A successful genotyping for the SNP in the all individuals was performed and verified by sequence analysis (reverse sequencing) of PCR products (Figure 2). The genotype distribution in all of the subjects was in agreement with that expected by Hardy-Weinberg equilibrium.

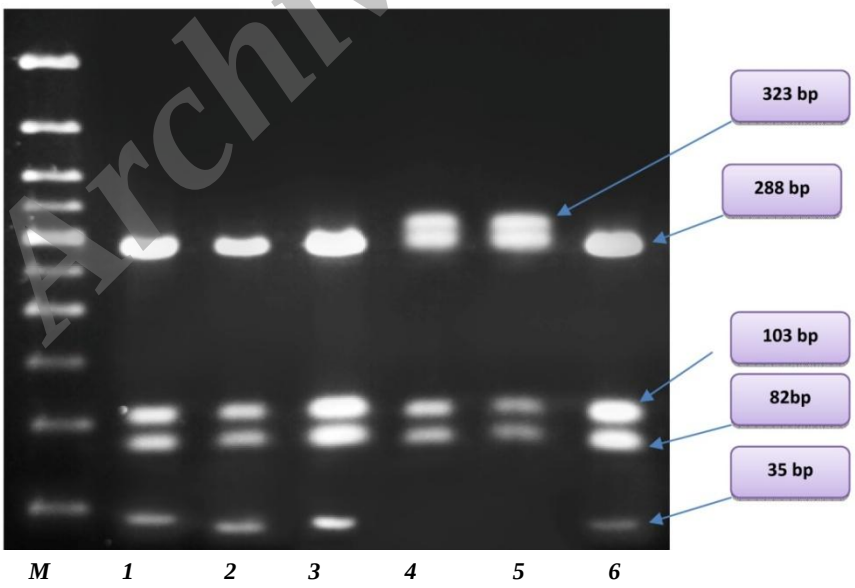

Figure 1. Genotyping of the IL23R R381Q polymorphism by PCR-RFLP method in six unrelated subjects. Subject 1,2,3 and 6 homozygote for the G allele; Subject 4 and 5 heterozygote for the A/G allele. M=marker 50bp

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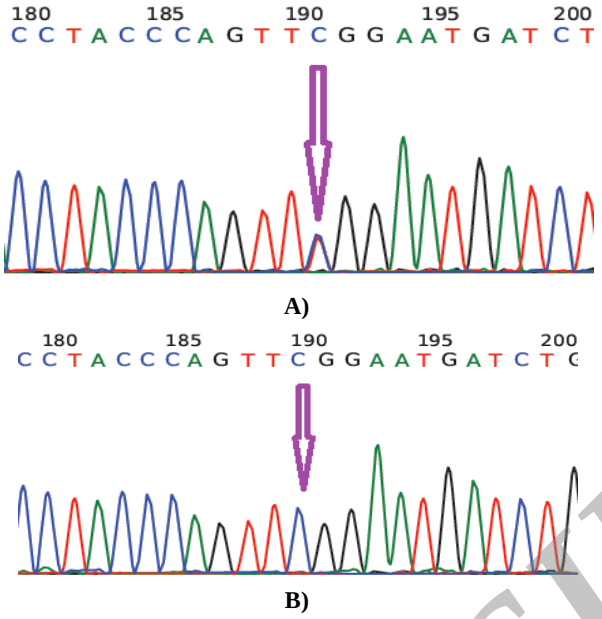

Figure 2- A) Electropherogram showing the location of the SNP as a double peak in the heterozygous condition for G/A genotype (arrow). B) Electropherogram showing the location of the SNP as a one peak in the homozygous condition for G/G genotype (arrow). The sequence that has been shown, is the reverse sequence, so instead of G, it is shown C & instead of A, it is shown T.

The frequency of GA and GG genotypes in patients was 3.8 % and 96.2%, respectively and the frequency of A and G alleles were 1.9% and 98.1%, respectively. The multivariate logistic regression analysis was applied to investigate the association between the rs11209026 G>A polymorphism genotypes and asthma. As shown in Table 3, there is an association between GA genotype and asthma (adjusted OR= 0.274, 95%, CI = 0.114-0.656, p=0.004, crude OR = 0.266, 95%, CI = 0.118-0.604, p<0.001).

Table 1. Frequency distributions of selected variables and characteristics of the study population in cases and controls

	Case (N=209)	control (N =200)	*P*- value
Age (mean±SD)	43.167±14.89	41.96±14.11	0.399
Gender			
male	69(33%)	80(40%)	0.142
female	140(67%)	120(60%)	
Smoking			
no	202(96.7%)	192(96%)	0.726
yes	7(3.3%)	8(4%)	
Total serum IgE log10 (mean±SD)	1.73±0.65	0.75±0.38	0.001
Eosinophils (mean±SD) 10^3 per µl	0.236 ±0.26	0.085±0.05	<0.001

Table 2. Genotype and allele frequencies of IL23R rs11209026 G>A polymorphism

Allel	Control (N/%)	Patients (N/%)	CrudeOdds Ratio (95% CI)	*P*-value
A	26(6.5%)	8(1.9%)	0.266(0.118-0.604)	<0.001
G	374(93.5)	410(98.1)	3.56(1.59-7.97)	<0.001
Genotype				
GA	26(13%)	8(3.8%)	0.266(0.118-0.604)	<0.001
GG	174(87%)	201(96.2)	3.754(1.657-8.507)	<0.001
AA	0 (0%)	0 (0%)		

Table 3. Adjusted Odds Ratios with 95% Confidence Interval (CI) in IL23R R381Q genotypes with adjustment for age, gender, smoke status, eosinophil count and total IgE.

Groups	adjusted Odds Ratios	*P*-value
GA	**0.274(0.114-0.656)**	**0.004**
GG	3.651(1.523-8.749)	0.004

DISCUSSION

The previous studies have shown that population of Th17 cells are more frequent in circulation of asthmatic patients than in normal controls.[22] In fact, relatively enriched levels of Th17 cells in circulation of asthmatic patients, suggest that these cells may play key roles in pathogenesis of asthma.[22] IL-17, secreted by Th17 cells, is suggested to have a major role in stimulating bronchial fibroblasts, epithelial cells, and smooth muscle cells and inducing the expression of a variety of cytokines and chemokines, which are important for granulopoiesis and neutrophil recruitment.[23] IL-23 is crucial for the maintenance, differentiation, and effectors' function of Th17 cells.[12,24] The different genetic variants of the IL-23 receptor (IL-23R) have been studied in a number of inflammatory diseases.[25]

Di Meglio et al showed that Th17 cells generated from A allele of IL23R R381Q had significantly reduced IL-23-induced IL-17A production compared to G allele.[25] Considering that asthma is a common chronic inflammatory disease of the airways, in this study, the association between asthma and IL23R R381Q gene variant was shown.

We undertook this study to determine the presence and prevalence of this gene mutation in asthma. In the present study, the frequency of the A allele in Iranian asthmatic patients and in normal controls was 1.9% and 6.5%, respectively.

Several studies have analyzed the A allele and various inflammatory diseases such as inflammatory bowel disease (IBD), crohn's disease (CD), ulcerative colitis (UC),[26] psoriasis,[27] multiple sclerosis (MS) and ankylosing spondylitis (AS)[28] in different populations.

Richard H et al studied the frequency of IL23R R381Q gene variant in CD on a European non-Jewish population. Their results showed that frequency of CD patients having the A allele was 1.9% while it was 7% for controls.[26]

P.L. Lakatos et al showed that the frequency of the A allele in UC patients was 2.68% while in controls it was 4.69%. Although, allele frequency in patients was lower than in control in their study, the difference did not reach significance (OR=0.56, 95% CI= 0.23-1.35).[29] Our result was consistent with these findings.

In conclusion, although the allele frequencies of this polymorphism in different diseases and populations were different, all previous studies have supported protective role of R381Q polymorphism in IL-23R gene against inflammatory diseases. Therefore, we suggest functional analysis of R381Q polymorphism in IL-23R.

Probable protective effect of R381Q polymorphism against asthma in Persian population may guide us towards interfering IL-23R functions as a potential therapeutic approach for asthmatic patients in future works.

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