

# Lack of Association between Interleukin 23R (IL-23R) rs10889677 Polymorphism and Inflammatory Bowel Disease Susceptibility In an Iranian Population

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## Abstract

**Background:** Inflammatory bowel diseases (IBDs), which include ulcerative colitis (UC) and Crohn's disease (CD), are inflammatory disorders that affect the gastrointestinal tract. A combination of inflammatory cytokines has an important role in IBD development. Genome-wide association studies have shown that polymorphisms in the interleukin-23R gene (*IL-23R*) increase susceptibility to IBD. The aim of this study was to investigate the *IL-23R* 3' UTR SNP to determine a potential association between genotype distribution and IBD.

**Methods:** The case group included 102 IBD patients and the control group included 107 healthy individuals. *IL-23R* polymorphisms rs10889677 were genotyped using PCR-RFLP analysis. RFLP results were confirmed by direct sequencing.

**Results:** The allele and genotype frequencies in patients and controls were evaluated and compared, and no significant association between this functional rs10889677 polymorphism and risk of IBD was observed ( $P=0.587$ ; adjusted OR: 0.89; 95% CI: 0.597-1.339). We also found no significant association between CD (14.71%) and UC (85.29%) patients in allele or genotype levels ( $P>0.05$ ).

**Conclusions:** Our results suggest that the rs10889677 A>C polymorphism is not a potential prognostic marker in Iranian patients with IBD.

**Keywords:** Crohn's disease, Inflammatory bowel diseases, Interleukin 23 receptor, rs10889677, Ulcerative colitis

## Introduction

Inflammatory bowel disease (IBD) is a general term for a group of chronic inflammatory diseases that involve the gastrointestinal tract and decrease the quality of patients' lives (1-3). It seems that IBD is caused by poor performance and constant activity in the mucosal immune system in response to normal intestinal bacteria, which cause intestinal epithelial barrier and mucosal immune system dysfunction (4).

The disease is categorized as either Crohn's disease (CD) or ulcerative colitis (UC) depending on the area of the gastrointestinal tract involved and also IBD is a one of the known gastrointestinal disorders in the world that fluctuates between remission and flare-up phases (5). Additionally, epidemiological studies have shown that the incidence and prevalence of IBD are based on geographical location and ethnic and

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racial backgrounds (6, 7). In general, genetic, immunologic, and environment factors play pivotal roles in the pathogenesis of IBD (8-10). The logical relationship between these three factors is such that immune system function is disrupted in individuals who are genetically prone to intestinal diseases, resulting in IBD (9).

The immune system plays a vital role in IBD with lymphoid, inflammatory, and hematopoietic cells affecting the formation of an inflammatory response (11, 12). The relationships between these cells are cytokine mediated. Cytokines act by binding to specific receptors on target cell surfaces to activate signaling pathways and ultimately alter gene expression in these cells (13, 14). Interleukins are a class of cytokines secreted by certain white blood cells that affect other white blood cells (15).

Interleukin-23 (IL-23) is a member of the IL-12 family of heterodimeric cytokines (16, 17). The IL-23 receptor (IL-23R) is a heterodimer composed of IL-12R $\beta$ 1 and IL-23R chains. IL-23 activates memory T cells via binding to IL-23R (18, 19). IL-23R is expressed by macrophages, monocytes, dendritic cells, T cells, and NK cells. IL-23R signaling includes Jak2/Tyk2 and causes the phosphorylation of Stat1 and Stat3 (20). Genome-wide association studies indicate that polymorphisms in IL-23R and components of its signaling pathways, such as Jak2 and Stat3, are considered as sensitivity and susceptibility factors for IBD (21). Additionally, rs10889677 SNP in the 3' untranslated region (3' UTR) of the IL-23R gene (*IL-23R*), located in the let-7f miRNA binding site, may influence IBD risk (22). The allelic frequencies of single nucleotide polymorphisms (SNPs) often differ markedly among populations; therefore, ethnic-specific association studies are necessary to identify genetic associations in different populations. The aim of this study was to investigate the association of the *IL-23R* 3' UTR SNP with IBD.

## Materials and methods

### Study population

This study included 102 IBD patients (15 with CD and 87 with UC) who were referred to the Research Center for Gastroenterology and Liver Diseases (RCGLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran, with positive

colonoscopy and pathology results for IBD. All the patients categorized with activity index in two phases; remission and flare up. Ulcerative colitis and crohn's disease activity index's score used for differentiated between two clinical phases in both UC and CD. Patients with IBD had a mean age of  $47.96 \pm 10.11$  years. The control group included 107 healthy individuals with no family histories of gastrointestinal disorders. Their mean age was  $42.01 \pm 12.51$  years. Control group subjects were selected based on family and personal histories and no symptoms of inflammatory diseases including gastritis, UC, or CD. The patients and healthy individuals were all Iranian. This study was approved by the Ethics Committee of the Research Center for Gastroenterology and Liver diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

### Genotyping

Peripheral blood mononuclear cells (PBMCs) were isolated from 5 ml of peripheral blood and their genomic DNA was extracted using the standard salting-out method (23). The quality of the extracted DNA was assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). Genotype determination was performed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. Primers and the restriction enzyme used for PCR and RFLP are presented in Table 1. The digested PCR products were electrophoresed on a 2.5% agarose gel, stained with DNA Green Viewer™ (Pars Tous Biotechnology, Iran), and visualized using an UV gel documentation instrument (Fig. 1).

### Sequencing

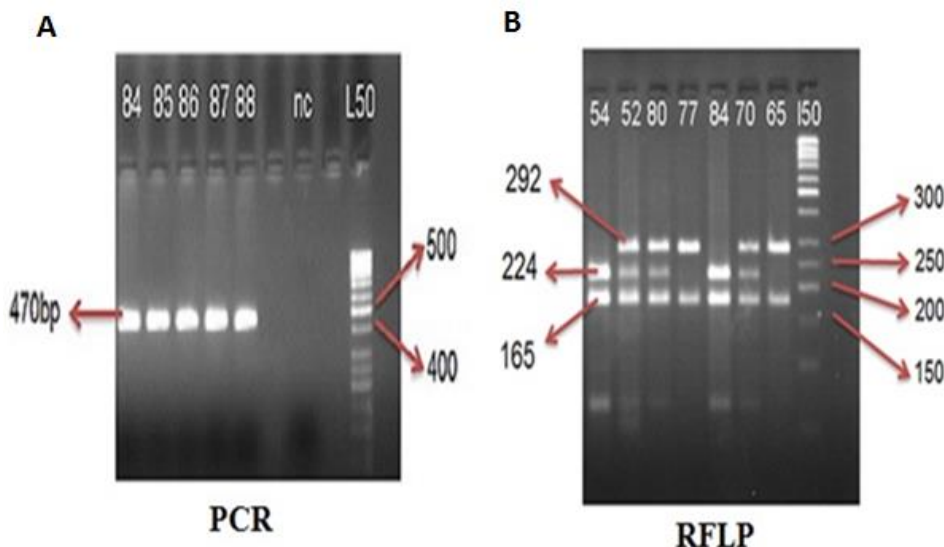
To confirm the RFLP genotyping results, the PCR products were sequenced on an ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems®, Invitrogen Life Technologies, and Carlsbad, CA, USA).

### Statistical analysis

Pearson's  $\chi^2$  and Student's t-tests were used to calculate the P values, with  $P < 0.05$  considered statistically significant. The data were analyzed using SPSS statistical software version 13

(SPSS, Inc, Chicago, IL, USA). Each polymorphism was tested to ensure the fitting

with Hardy–Weinberg equilibrium with an alpha threshold of 0.05.



**Fig.1.** Agarose gels of PCR products before (A) and after (B) digestion with MnlI. A 50 base-pair marker was used (L50). A) PCR products from samples 84 – 88. B) PCR products after digestion with MnlI. Samples 54 and 84 are from CC genotype subjects, samples 52, 80, and 70 are from AC genotype subjects, and samples 77 and 65 are from AA genotype subjects.

## Results

### *Characteristics of case and control populations*

The IBD patients included 43 males (42.16%) and 59 females (57.84%). The control group included 54 males (50.47%) and 53 females (49.53%). The difference in percentage of female vs. male subjects

in the two groups was not significant ( $P>0.05$ ). The mean ages of the IBD and healthy control groups were not significantly different, nor were their body mass indexes, genders, or smoking behaviors ( $P>0.05$  for all) (Table 2).

**Table 1.** Primer sequences and resulting fragments after PCR and digestion with MnlI.

SNP	Primer sequence	PCR Product (bp)	Restriction Enzyme	Digestion fragments (bp)
rs10889677	F:5'-ATCGTGAATGAGGAGTTGCC-3' R:5'-TGTGCCTGTATGTGTGACCA-3'	470	MnlI	AA: 292+165+13 CA: 292+224+165+68+13 CC: 224+165+68+13

### *Genotyping*

PCR of genomic DNA from all study subjects using the primers shown in Table 1 amplified a single 470 bp product. Five PCR samples are shown in Figure 1A. Digestion of the PCR products with MnlI resulted in three different fragment patterns. Fragments from A/A genotype subjects were 292, 165, and 13 bp, fragments from A/C genotype subjects were 292, 224, 165, 68, and 13 bp, and fragments from C/C genotype subjects were 224, 165, 68, and 13 bp. The RFLP patterns of seven digested samples are shown in Figure 1B. The rs10889677 polymorphism genotype frequency

percentages in the IBD patients were 19.6% for A/A, 42.2% for C/A, and 38.2% for C/C, and the genotype frequency percentages for the controls were 21.5% for A/A, 43.0% for C/A, and 35.5% for C/C. No significant differences were found between the patients and healthy controls for any of the rs10889677 polymorphisms. Further details and frequency percentages of the C and A allele for the patients and controls are shown in Table 3. In addition, no significant differences were found between CD and UC patients for allele and genotype levels (Table 4) or between IBD patients in remission or flare-up phases (Table 5).

**Table 2.** Demographic characteristics of the IBD study population.

Variable	Patients (n=102)	Controls (n=107)	P value
<b>Age (mean <math>\pm</math> SD)</b>	47.96 $\pm$ 10.11	42.01 $\pm$ 12.51	>0/05
<b>BMI<sup>a</sup></b>	24.96 $\pm$ 3.65	25.39 $\pm$ 5.89	>0/05
<b>Gender n (%)<sup>b</sup></b>			>0/05
Female	59 (57.84%)	53 (49.53%)	
Male	43 (42.16%)	54 (50.47%)	
<b>Smoking, n (%)<sup>b</sup></b>			>0/05
Smokers	17 (16.6%)	11 (10.28%)	
Non-smokers	85 (83.4%)	96 (89.72%)	

a: Student's t-test; b: chi square test.

**Table 3.** Genotype and allele distribution of rs10889677 SNP in IBD patients and healthy controls.

P value	Adjusted* OR (95% CI)	Controls 107 (%)	Patients 102 (%)	SNP rs10889677
<b>Genotypes</b>				
-	1.00 (Reference)	38 (35.5%)	39 (38.2%)	CC
0.652	0.862, 0.454-1.640	46 (43.0%)	43 (42.2%)	CA
0.751	0.743, 0.339-1.627	23 (21.5%)	20 (19.6%)	AA
<b>Alleles</b>				
-	1.00 (Reference)	99 (46.3%)	98 (48.0%)	C
0.587	0.894, 0.597-1.339	115 (53.7%)	106 (52.0%)	A

\* Adjusted for age and gender as confounder variables

**Table 4.** Genotype and allele distribution of rs10889677 SNP in UC and CD patients.

P value	Adjusted* OR (95% CI)	CD 15 (14.7%)	UC 87 (85.3%)	SNP rs10889677
<b>Genotypes</b>				
-	1.00 (Reference)	6 (40.0%)	33 (37.9%)	CC
0.912	1.088 (.243-4.878)	6 (40.0%)	37 (42.5%)	CA
0.855	1.121 (.329-3.817)	3 (20.0%)	17 (19.5%)	AA
<b>Alleles</b>				
-	1.00 (Reference)	18 (60.0%)	103 (59.2%)	C
934	459 (0.469-2.279)	12 (40.0%)	71 (40.8%)	A

\* Adjusted for age and gender as confounder variables

**Table 5.** Genotype and allele distribution of rs10889677 SNP in patients in flare-up and remission phases

P value	Adjusted* OR (95% CI)	Remission 62 (60.8%)	Flare-Up 40 (39.2%)	SNP rs10889677
<b>Genotypes</b>				
-	1.00 (Reference)	26 (41.9%)	13 (32.5%)	CC
0.561	0.765 (0.310-1.888)	26 (41.9%)	17 (42.5%)	CA
0.217	0.500 (0.166-1.503)	10 (16.1%)	10 (25.0%)	AA
<b>Alleles</b>				
-	1.00 (Reference)	78 (62.9%)	43 (53.8%)	C
0.195	1.459 (0.824-2.582)	46 (37.1%)	37 (46.3%)	A

\* Adjusted for age and gender as confounder variables.

## Discussion

Inflammatory bowel disease is a chronic inflammatory disease that includes UC and CD (8). The number of IBD patients in Iran and developing nations has increased in recent years. Although the cause of IBD is unknown, genetics, environment, immunity, and intestinal flora can all contribute to development of the disease (24-26). Several gene products including CARD15/NOD2, TNF $\alpha$ , ATG16L1, and IL23R have been reported to be associated with this disease (27, 28). Duerr et al. reported a strong connection between *IL23R* polymorphisms and IBDs. *IL-23R* has also been shown to be associated with autoimmune diseases (29). Indeed, past studies confirmed that IL23 and IL23R activate the JAK2/STAT3 signaling pathway leading to TH17 induction and contributing to IBD pathogenesis. Overexpression of IL23 in tissue and serum of IBD patients is an important factor in IBD pathogenesis (30). According to a previous study, the rs10889677 variant is associated with increased levels of *IL-23R* mRNA and protein production (31). Furthermore, another study found that IL12 and IL23 function in other inflammatory diseases including ankylosing spondylitis and psoriasis, and several analyses, indicate that *IL23R* polymorphism is related to these two inflammatory diseases (32).

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We observed no association between this polymorphism and increased risk for IBD among Iranian patients. Similar to our results, Daryani et al. found no significant association between the rs10889677 polymorphism and UC (33). Although we found no differences between study subjects, some studies show that the rs10889677 polymorphism was more prevalent in IBD patients than in healthy controls. For example, Ferguson and colleagues found that the rs10889677 SNP A/C genotype was associated with significantly increased risk for CD, while the C/C genotype was more common in healthy controls (34). Similarly, a study by Okazaki et al. showed the strongest association with CD risk and rs10889677 SNP (35). The reason for these contradictory findings is not clear; however, ethnic heterogeneity, genotype distributions, gene environment interactions, and different sample sizes may contribute to this discrepancy (36).

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