

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

Sara Karimkhani¹, Vahid Chaleshi¹, Hedieh Balaii¹, Peyman Tarban¹, Mahyar Nourian¹, Shiva Irani², Shabnam Shahrokh³, Hamid Asadzadeh Aghdai¹, Amir Houshang Mohammad Alizadeh³, Mohsen Norouzinia^{*3}, Mohammad Reza Zali³

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

1: Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2: Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

3: Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

*Corresponding authors: Mohsen Norouzinia; Tel: +98 21 22432525; Fax: +98 21 22432514; E-mail: norouzinia@gmail.com.

Received: Aug 8, 2017; Accepted: Sep 23, 2017

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 β 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 ± 10.11 years. The control group included 107 healthy individuals with no family histories of gastrointestinal disorders. Their mean age was 42.01 ± 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 χ^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.

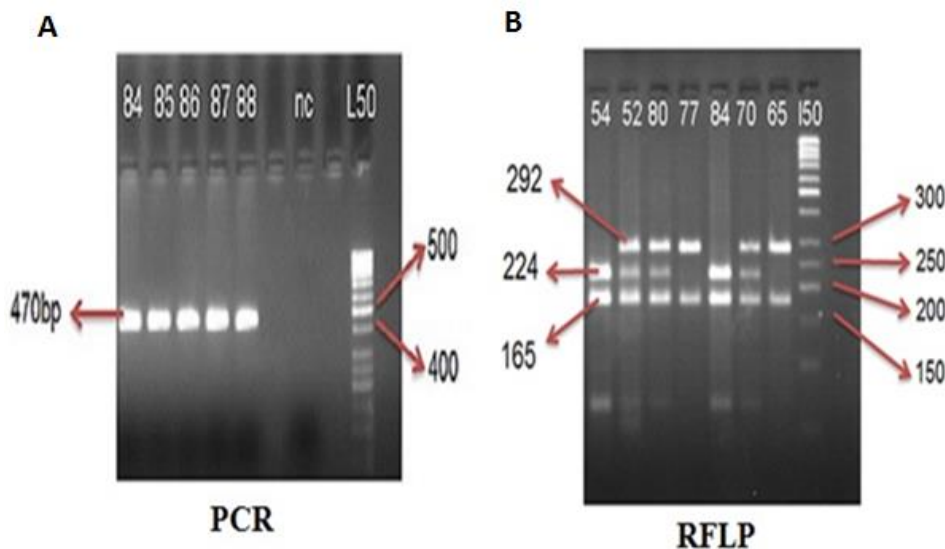


Fig.1. Agarose gels of PCR products before (A) and after (B) digestion with MnlI. A 50 base-pair marker was used (L50). A) PCR products from samples 84 – 88. B) PCR products after digestion with MnlI. Samples 54 and 84 are from CC genotype subjects, samples 52, 80, and 70 are from AC genotype subjects, 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
Age (mean ± SD)	47.96 ± 10.11	42.01 ± 12.51	>0/05
BMI^a	24.96 ± 3.65	25.39 ± 5.89	>0/05
Gender n (%)^b			>0/05
Female	59 (57.84%)	53 (49.53%)	
Male	43 (42.16%)	54 (50.47%)	
Smoking, n (%)^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
Genotypes				
-	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
Alleles				
-	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
Genotypes				
-	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
Alleles				
-	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
Genotypes				
-	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
Alleles				
-	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 α , 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).

References

1. Endo, K., et al., Inflammatory bowel disease: IBD. *Rinsho byori. The Japanese journal of clinical pathology*, 2009. 57(6): p. 527-532.
2. Balaii, H., et al., Time trend analysis and demographic features of inflammatory bowel disease in Tehran. *Gastroenterology and hepatology from bed to bench*, 2014. 8(4): p. 253-261.
3. Nourian, M., et al., Evaluation of tumor necrosis factor (TNF)- α mRNA expression level and the rs1799964 polymorphism of the TNF- α gene in peripheral mononuclear cells of patients with inflammatory bowel diseases. *Biomedical Reports*, 2017. 6(6): p. 698-702.
4. Gracie, D.J., et al., Poor correlation between clinical disease activity and mucosal inflammation, and the role of psychological comorbidity, in

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).

Acknowledgements

The authors thank the patients who participated in the study, which was conducted with the support of the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran. Grant No: 734. The authors declare no conflict of interest.

- inflammatory bowel disease. *The American journal of gastroenterology*, 2016.
5. Horje, C.S.H.T., et al., Prevalence of upper gastrointestinal lesions at primary diagnosis in adults with inflammatory bowel disease. *Inflammatory bowel diseases*, 2016. 22(8): p. 1896-1901.
 6. Ng, S.C., et al., Epidemiology of Inflammatory Bowel Disease from 1981 to 2014: Results from a Territory-Wide Population-Based Registry in Hong Kong. *Inflammatory Bowel Diseases*, 2016. 22(8): p. 1954-1960.
 7. Loftus, E.V., Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology*, 2004. 126(6): p. 1504-1517.

8. Lakatos, P.L., et al., Current concept on the pathogenesis of inflammatory bowel disease-crosstalk between genetic and microbial factors: pathogenic bacteria and altered bacterial sensing or changes in mucosal integrity take " toll"? *World journal of gastroenterology*, 2006. 12: p. 1829-1840.
9. Xavier, R. and D. Podolsky, Unravelling the pathogenesis of inflammatory bowel disease. *Nature*, 2007. 448(7152): p. 427-434.
10. Loddo, I. and C. Romano, Inflammatory bowel disease: genetics, epigenetics, and pathogenesis. *Frontiers in immunology*, 2015. 6: p. 551.
11. Fiocchi, C., Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology*, 1998. 115(1): p. 182-205.
12. Welte, T., et al., STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. *Proceedings of the National Academy of Sciences*, 2003. 100(4): p. 1879-1884.
13. Seiderer, J., et al., Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p. His161Arg polymorphism in IBD. *Inflammatory bowel diseases*, 2008. 14(4): p. 437-445.
14. Dmowska-Chalaba, J. and E. Kontny, AB0043 Serum Cytokine Concentrations in Inflammatory Bowel Disease (IBD) and IBD-Related Spondyloarthritis (SPA). *Annals of the Rheumatic Diseases*, 2016. 75 (Suppl 2): p. 911-912.
15. Abraham, C. and J. Cho, Interleukin-23/Th17 pathways and inflammatory bowel disease. *Inflammatory bowel diseases*, 2009. 15(7): p. 1090-1100.
16. Trinchieri, G., S. Pflanz, and R.A. Kastelein, The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity*, 2003. 19(5): p. 641-644.
17. Zwirner, N.W. and A. Ziblat, Regulation of NK cell activation and effector functions by the IL-12 family of cytokines: the case of IL-27. *Frontiers in Immunology*, 2017. 8.
18. Oppmann, B., et al., Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity*, 2000. 13(5): p. 715-725.
19. Schmitt, H., et al., Expansion of IL-23 Receptor Bearing TNFR2+ T Cells Drives Resistance to Anti-TNF Therapy in Crohn's Disease. *Gastroenterology*, 2017. 152(5): p. S759.
20. Zundler, S. and M.F. Neurath, Integrating immunologic signaling networks: the JAK/STAT pathway in colitis and colitis-associated cancer. *Vaccines*, 2016. 4(1): p. 5.
21. Ghoreschi, K., A. Laurence, and J.J. O'Shea, Janus kinases in immune cell signaling. *Immunological reviews*, 2009. 228(1): p. 273-287.
22. Zhou, S., et al., Functional IL-23R rs10889677 genetic polymorphism and risk of multiple solid tumors: a meta-analysis. *PloS one*, 2013. 8(11): p. e80627.
23. MWER, S., D. Dykes, and H. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic acids res*, 1988. 16: p. 1215.
24. Hanauer, S.B., Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. *Inflammatory bowel diseases*, 2006. 12(5): p. S3-S9.
25. Ananthakrishnan, A.N., Epidemiology and risk factors for IBD. *Nature reviews Gastroenterology & hepatology*, 2015. 12(4): p. 205-217.
26. Aghazadeh, R., et al., Inflammatory bowel disease in Iran: a review of 457 cases. *Journal of gastroenterology and hepatology*, 2005. 20(11): p. 1691-1695.
27. Mirkov, M.U., B. Verstockt, and I. Cleynen, Genetics of inflammatory bowel disease: beyond NOD2. *The Lancet Gastroenterology & Hepatology*, 2017. 2(3): p. 224-234.
28. Horowitz, J., et al., Mutation spectrum of NOD2 reveals recessive inheritance as a main driver of Early Onset Crohn' s Disease. *bioRxiv*, 2017: p. 098574.
29. Duerr, R.H., et al., A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *science*, 2006. 314(5804): p. 1461-1463.

30. Sanchez-Muñoz, F., A. Dominguez-Lopez, and J.K. Yamamoto-Furusho, Role of cytokines in inflammatory bowel disease. *World journal of gastroenterology: WJG*, 2008. 14(27): p. 4280.
31. Zwiers, A., et al., Cutting edge: a variant of the IL-23R gene associated with inflammatory bowel disease induces loss of microRNA regulation and enhanced protein production. *The Journal of Immunology*, 2012. 188(4): p. 1573-1577.
32. Rueda, B., et al., The IL23R Arg381Gln non-synonymous polymorphism confers susceptibility to ankylosing spondylitis. *Annals of the rheumatic diseases*, 2008. 67(10): p. 1451-1454.
33. Daryani, N.E., et al., Interleukin-23 receptor single nucleotide polymorphisms in ulcerative colitis. A study in Iranian populations. *Clinics and research in hepatology and gastroenterology*, 2014. 38(3): p. 360-365.
34. Ferguson, L.R., et al., IL23R and IL12B SNPs and haplotypes strongly associate with Crohn's disease risk in a New Zealand population. *Gastroenterology research and practice*, 2010. 2010.
35. Okazaki, T., et al., Contributions of IBD5, IL23R, ATG16L1, and NOD2 to Crohn's disease risk in a population-based case-control study: Evidence of gene-gene interactions. *Inflammatory bowel diseases*, 2008. 14(11): p. 1528-1541.
36. Chaleshi, V., et al., Lack of Association between NOD2 rs3135500 and IL12B rs1368439 microRNA Binding Site SNPs and Colorectal Cancer Susceptibility in an Iranian Population. *Microna*, 2016. 5(2): p. 152-156.