

Lack of Association between Interleukin 12 C(-1188)A Polymorphism and Irritable Bowel Syndrome

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Abstract

Irritable Bowel Syndrome (IBS) is a functional gastrointestinal disorder, characterized by recurrent abdominal pain and altered bowel habits. This study was performed to investigate the important role of interleukin-12 (IL-12) in intestinal inflammation. For this study seventy one patients with IBS and 140 controls were investigated. The allele and genotype frequencies of IL-12 C(-1188)A were determined using polymerase chain reaction with sequence-specific primers. The allele A was more common than the allele C in both groups of patients and controls. There was not any significant difference on IL-12 alleles and genotypes between patients and controls. The AA genotype was the most common genotypes, which was seen in 57.4% of the patients and 51.4% of the controls ($p=0.53$). Although frequency of the CC genotype in the control group was lower than the patient group, this difference was not significant (5.7% *vs.* 11.5%, respectively, $p=0.16$). Considering the lack of association between IL-12 C(-1188)A polymorphism and IBS, this cytokine gene polymorphism may not have significant role in the pathophysiology of disease.

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Introduction

Irritable bowel syndrome (IBS) is a multifactorial functional gastrointestinal disorder. The affected patients usually suffer from recurrent abdominal pain and altered bowel habits^(1,2). Although the exact etiology of IBS is still uncertain, several mechanisms have been proposed for the pathophysiology of disease⁽³⁻⁸⁾. Genetic factors could have also a major role in this disease⁽⁹⁻¹¹⁾.

Interleukin-12 (IL-12), as a cytokine of T helper 1 (Th1)-mediated immunity in humans, can play an important role in intestinal inflammation⁽¹²⁻¹⁴⁾. IL-12 production is under genetic control and secretion of this cytokine could be affected by genetic polymorphisms^(15,16). Genetic predisposition to produce high or low amounts of IL-12 may affect the disease susceptibility and clinical outcome^(17,18).

We previously showed that IL-6 and TNF- α proinflammatory cytokine gene polymorphisms as well as IL-4 and IL-10 gene polymorphisms could affect individual's susceptibility to IBS^(19,20). This study was performed in the group of patients with IBS to analyze the genotype and allele frequencies of a polymorphic gene coding for IL-12 compared to healthy control subjects.

Materials and Methods

Seventy one unrelated Iranian patients with IBS (22 males, 49 females) were enrolled in this study. The diagnosis of IBS in this patient population was made based on the Rome III criteria, as described in our previous reports^(19,20). One hundred and forty healthy control subjects were also enrolled in this study⁽²¹⁾. This study was approved by the Ethics Committee of Tehran University of Medical Sciences. Written informed consent was obtained from all subjects before sampling.

After DNA extraction from whole blood, using salting out method, cytokine genes typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany)^(19,20).

Amplification was done using a thermal cycler Techne Flexigene apparatus (Rosche, Cambridge, UK). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on a UV transilluminator and a picture for interpretation and documentation was taken.

The allele and genotype frequencies of IL-12 C(-1188)A were determined and compared using the chi-square test. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for allele/ genotype in the patient and control groups. P-value of less than 0.05 was considered significant.

Results

The allele A at position -1188 IL-12 was detected in 72.9% of the patients, similar to what was detected in the control group. There

was not any significant difference on IL-12 alleles between the patient and the control groups ($p=0.92$).

The AA genotype was the most common genotype, which was seen in 57.4% of the patients and 51.4% of the controls ($p=0.53$, OR=1.27, 95% CI: 0.66-2.44). Although frequency of the CC genotype in the control group was lower than the patient group, this difference was not significant (5.7% vs. 11.5%, respectively, $p=0.16$, OR=2.14, 95% CI: 0.66-6.91). There was not any significant difference on IL-12 CA genotype between the patient and the control groups (31.1% vs. 42.9%, respectively, $p=0.16$, OR=0.60, 95% CI: 0.30-1.19).

Discussion

Cytokine gene polymorphisms could change the individual susceptibility to IBS and might have a role in the pathophysiology of disease. We have recently shown the associations of variety of cytokine gene polymorphisms (IL-4, IL-10, IL-6, TNF- α) and IBS^(19,20). In this study, we have genotyped a sample of IBS patients for IL-12.

It should be noted that there is no single pathophysiologic mechanism that explains the clinical manifestations of IBS. Although no active inflammation is expected in IBS, transient mucosal inflammation could be an important factor for the manifestation of IBS. Therefore, as cytokines are involved in the regulation of the immune and inflammatory reaction, their genetic polymorphisms, which could be associated with high or low cytokine production, may predispose individual to IBS, or affect its clinical manifestation⁽²²⁾.

IL-12 is secreted by antigen-presenting dendritic cells and phagocytes and activates CD4+ T cells to differentiate to Th1- and is associated with cellular immune responses⁽²³⁾. An A-to-C exchange has been found in the 3'-UTR of IL-12 gene, in position -1188⁽²⁴⁾ that correlates with decreased protein secretion⁽¹⁴⁾. A single nucleotide polymorphism situated at positions -1188 (A/C) in the promoter region of IL-12 gene was investigated in this study

that did not showed any significant difference on IL-12 alleles and genotypes between patients and controls. Although it was previously reported that the ratio of IL-10 secretion to IL-12 was significantly lower in IBS patients compared to controls ⁽²⁵⁾, there is not any evidence of the role of IL-12 gene polymorphisms in IBS patients, to our best knowledge.

Conclusion

Considering lack of association between IL-12 C(-1188)A polymorphism and IBS, this cytokine gene polymorphism may not have significant role in the pathophysiology of disease.

References

1. Jones R, Lydeard S. Irritable bowel syndrome in the general population. *BMJ* 1992;304:87-90.
2. Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Müller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999;45 (Suppl 2):1143-1147.
3. Chey WJ, Jin HO, Lee MH, Sun SW, Lee KY. Colonic motility abnormality in patients with irritable bowel syndrome exhibiting abdominal pain and diarrhea. *Am Gastroenterol* 2001;96(5):1499-1506.
4. Azpiroz F, Bouin M, Cammilleri M, Mayer EA, Poitras P, Serra J, et al. Mechanisms of hypersensitivity of IBS and functional disorder. *Neurogastroenterol Motil* 2007;19(Suppl 1):62-88.
5. Barbara G, DE Giorgio R, Stanghellini V, Cremon C, Corinaldesi R. A role for inflammation in irritable bowel syndrome? *Gut* 2002;51(Suppl 1):41-44.
6. Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, et al. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002;122(7):1778-1783.
7. Drossman DA, McKee DC, Sandler RS, Mitchell CM, Cramer E, Lowman BC, et al. Psychosocial factors in the irritable bowel syndrome. A multivariate study of patients and nonpatients with irritable bowel syndrome. *Gastroenterology* 1988;95 (3):701-708.
8. Whitehead WE, Palsson OS. Is rectal pain sensitivity a biological marker for irritable bowel syndrome: psychological influences on pain perception. *Gastroenterology* 1998;115(5):1263-1271.
9. Hotoleanu C, Popp R, Trifa AP, Nedelcu L, Dumitrascu DL. Genetic determination of irritable bowel syndrome. *World J Gastroenterol* 2008;14(43): 6636-6640.
10. Adam B, Liebrechts T, Holtmann G. Mechanisms of disease: genetics of functional gastrointestinal disorders-searching the genes that matter. *Nat Clin Pract Gastroenterol Hepatol* 2007;4(2):102-110.
11. Talley NJ. Genes and environment in irritable bowel syndrome: one step forward. *Gut* 2006;55 (12):1694-1696.
12. Monteleone G, Biancone L, Marasco R, Morrone G, Marasco O, Luzzza F, et al. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* 1997;112(4):1169-78.
13. Parronchi P, Romagnani P, Annunziato F, Sampognaro S, Becchio A, Giannarini L, et al. Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am J Pathol* 1997;150(3):823-832.
14. Chikano S, Sawada K, Shimoyama T, Kashiwamura SI, Sugihara A, Sekikawa K, et al. IL-18 and IL-12 induce intestinal inflammation and fatty liver in mice in an IFN- γ dependent manner. *Gut* 2000; 47(6):779-786.
15. Stanilova S, Miteva L. Taq-I polymorphism in 3' UTR of the IL12B and association with IL-12p40 production from human PBMC. *Genes Immun* 2005;6:364-366.
16. Seegers D, Zwiers A, Strober W, Peña AS, Bouma G. A TaqI polymorphism in the 3'UTR of the IL-12 p40 gene correlates with increased IL-12 secretion. *Genes Immun* 2002;3(7):419-423.
17. Shokrgozar MA, Sarial S, Amirzargar AA, Shokri F, Rezaei N, Arjang Z, et al. IL-2, IFN- γ , and IL-12 gene polymorphisms and susceptibility to multiple sclerosis. *J Clin Immunol* 2009;29(6):747-751.
18. Hall MA, McGlinn E, Coakley G, Fisher SA, Boki K, Middleton D, et al. Genetic polymorphism of IL-12 p40 gene in immune-mediated disease. *Genes Immun* 2000;1(3):219-224.
19. Barkhordari E, Rezaei N, Ansari-pour B, Larki P, Alighardashi M, Ahmadi-Ashtiani HR, et al. Proinflammatory cytokine gene polymorphisms in irritable bowel syndrome. *J Clin Immunol* 2010;30(1): 74-79.
20. Barkhordari E, Rezaei N, Mahmoudi M, Larki P, Ahmadi-Ashtiani HR, Ansari-pour B, et al. T-helper 1, T-helper 2, and T-regulatory cytokines gene polymorphisms in irritable bowel syndrome. *Inflammation* 2010;33(5):281-286.

21. Amirzargar AA, Naroueynejad M, Khosravi F, Dianat S, Rezaei N, Mytilineos J, et al. Cytokine single nucleotide polymorphisms in Iranian populations. *European Cytokine Network* 2008;19:104-112.
22. Hotoleanu C, Popp R, Trifa AP, Nedelcu L, Dumitrascu DL. Genetic determination of irritable bowel syndrome. *World J Gastroenterol* 2008;14(43):6636-6640.
23. Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, et al. The interleukin-12/interleukin-12 receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 1998;16:495-521.
24. Huang D, Cancilla M, Morahan G. Complete primary structure, chromosomal localization, and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit. *Genes Immun* 2000;1(8):515-520.
25. O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005;128(3):541-551.