



Association of Tumor Necrosis Factor Alpha Gene Polymorphisms with Inflammatory Bowel Disease in Iran

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Abstract

Background: Inflammatory bowel disease (IBD) is a chronic disease of unknown etiology, in which genetic factors, seem to play an important role in the disease predisposition and course. Assessment of tumor necrosis factor (TNF- α) gene polymorphisms in many populations showed a possible association with IBD. Considering the genetic variety in different ethnic groups, the aim of the present study was to investigate the association of five important single nucleotide polymorphisms (SNPs) in the promoter region of (TNF- α) gene with IBD in Iran.

Methods: In this case-control study, 156 Ulcerative colitis (UC) patients, 50 Crohn's disease (CD) patients and 200 sex and age matched healthy controls of Iranian origin were enrolled. The study was performed during a two year period (2008-2010) at Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. DNA samples were evaluated for (TNF- α) gene polymorphisms (including -1031, -863, -857, -308 and -238) by PCR and RFLP methods.

Results: The frequency of the mutant allele of -1031 polymorphism was significantly higher in Iranian patients with Crohn's disease compared to healthy controls ($P=0.01$, OR=1.92; 95% CI: 1.14-3.23). None of the other evaluated polymorphisms demonstrated a significant higher frequency of mutant alleles in Iranian IBD patients compared to controls.

Conclusion: Among the five assessed (SNPs), only -1031 polymorphism of (TNF- α) gene may play a role in disease susceptibility for Crohn's disease in Iran. This pattern of distribution of (TNF- α) gene polymorphisms could be specific in this population.

Keywords: TNF- α gene, SNPs, Crohn's disease, Ulcerative colitis

Introduction

Inflammatory bowel disease (IBD) is a chronic, complex inflammation of gastrointestinal tract which includes at least two major disorders: Crohn's disease (CD) and Ulcerative colitis (UC). Ulcerative colitis, with a slightly higher incidence than CD, affects only the colon and inflammation is typically confined to the mucosa whereas CD can affect any part of the gastrointestinal tract and

inflammation is often transmural. Despite extensive research, the etiology of IBD is unknown, but accumulating evidences suggest that it results from an interaction between genetic and environmental factors, leading to an abnormal immune response of the intestinal mucosa to intra luminal antigens (1). Ethnic and familial aggregations of IBD and greater concordance for IBD in

monozygotic twins are major evidences for a central role of genetic factors in the disease pathogenesis. Over the past years, these evidences were supplemented by molecular data from genome-wide linkage studies of multiply affected IBD families; which are likely to play a more prominent role in Crohn's disease than in ulcerative colitis (2). Genome wide association studies have been shown that several chromosomal loci are associated with IBD. These loci are located on chromosomes 16q12 (IBD1), 12q13 (IBD2), 6P13 (IBD3), 14q11 (IBD4), 5q31-33 (IBD5), 19P13 (IBD6), 1P36 (IBD7), 16P (IBD8), 3P (IBD9) and 7q. Among these loci many genes are being evaluated as candidate genes contributing in the disease pathogenesis. IBD3 region, located on chromosome 6p21, encompasses the tumor necrosis factor- α (TNF- α) gene (3). TNF- α gene, due to its position and function, is a strong positional and functional candidate for IBD susceptibility. TNF- α is a multi-functional pro-inflammatory cytokine and a primary mediator that is involved in the early phase of the cytokine cascade and plays an important role in the initiation and regulation of the immune response. Data from a number of studies has confirmed that TNF- α is increased in the serum, stool, intestinal tissue and peripheral blood monocytes of IBD patients and the efficacy of TNF antibody in IBD patients added further evidence (4).

Several single nucleotide polymorphisms (SNPs) have been described in TNF- α gene, which five in the promoter region are of great concern; including -1031 [thiamine (T) to cytosine (C) substitution] -863 and -857 [both (C) to adenine (A) substitutions], -308 and -238 [both guanine (G) to adenine (A) substitutions]. These SNPs are located outside of the coding sequence such as introns and 5'- or 3'- regulatory sequences. These non-coding SNPs may significantly affect the process of transcription by altering the structure of transcription factor binding sites within gene promoters or the structure of enhancers or silencers within introns or at other regulatory sites. These alterations result in changes of protein production thus may significantly contribute to the pathogenesis of various diseases (5).

Among the fore mentioned SNPs, -863 affects the binding site of the TNF- α protein to NF- κ B, a well characterized transcriptional regulator which increases the transcription of TNF- α ; while -857 mutation affects the binding site to OCT1, a transcription regulator which decreases the transcription of TNF- α . It has been suggested that variability in the promoter and coding regions of the TNF- α gene may modulate the magnitude of the secretory response of this cytokine (6).

In this study, we evaluated the frequency of five TNF- α gene promoter polymorphisms (-1031, -863, -857, -308 and -238) in Iranian CD and UC patients comparing with healthy controls. Such studies for clearing the association of important immune system related genes with IBD may be useful to determine the probability of the disease appearance familial members of the patients.

Materials and Methods

In this case-control study a total of 206 patients with IBD (156 patients with UC and 50 patients with CD) and 200 unrelated healthy sex and age matched individuals of Iranian origin were enrolled. The study was performed during a two year period (2008-2010) at Taleghani Hospital, Shahid Beheshti University of medical sciences, Tehran, Iran.

The diagnosis of CD and UC was based on the clinical, endoscopic, radiologic, and histopathologic findings (7) by an expert gastroenterologist. Controls were selected from healthy subjects without any GI symptoms or positive familial or history of IBD.

A written informed consent, confirmed in "Research Center of Gastroenterology and Liver diseases (RCGLD)", Shahid Beheshti University M.C. Ethics committee was obtained from all participants.

Genomic DNA was extracted from peripheral blood by the standard "Phenol-chloroform" method (8). PCR was performed for investigating TNF- α gene promoter polymorphisms (-1031, -863, -857, -308 and -238) by specific primers (9). The PCR reactions were done in a 25- μ l volume

containing 10 mM Tris-HCl, pH 8.8, 1.5 mM MgCl₂, 50 mM KCl, 250 μM dNTPs, a 0.50-mM concentration of each primer, 2 U of Taq DNA polymerase (Fermentas, Germany), and 200 ng of genomic DNA.

The PCR Protocol for all polymorphisms was as follows: initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 10 min. Appropriate synthesis of PCR products was confirmed by agarose gel electrophoresis (1.5%) and visualized by ethidium bromide staining (0.5 μg/ml). Amplification of the flanking regions was

followed by restriction fragment length polymorphism (RFLP) method to detect each polymorphism. The RFLP products were run on 2% agarose gel after digestion and stained with ethidium bromide (0.5 μg/ml) for visualization under UV light. Details of the materials assessing the studied SNPs are listed in Table 1.

Genotype and allele frequencies for the polymorphisms were calculated from observed genotype counts. These frequencies were assessed for association with CD or UC, Using the standard χ^2 test. Results were analyzed by the SPSS software (version 13, USA), and $P < 0.05$ was considered significant.

Table 1: Details of the materials assessing the TNF- α gene polymorphisms

SNP Name	Forward primer Reverse primer	PCR fragment Size(bp)	Restriction enzyme, incubation temperature (°C)	RFLP fragment Size(bp)
-238	5'-AAACAGACCACAGA CCTGGTC-3' 5'-CTCACACTCCCCATCCTCCCG-GATC-3'	155	Bam H1, 37	130+25
-308	5'-GAGGCAATAGGTT TTGAGGGCCAT-3' 5'-GGGACACACAAGC ATCAAG-3'	147	Nco I, 37	122+25
-857	5'-GGCTCTGAGGAAT GGGTTAC-3' 5'-CCTCTACATGGCC CTGTCTAC-3'	128	Tai I, 65	107+21
-863	5'-GGCTCTGAGGAAT GGGTTAC-3' 5'-CTACATGGCCCTG TCTTCGTTACG-3'	125	Tai I, 65	101+24
-1031	5'-TATGTGATGGACTC ACCAGGT-3' 5'-CCTCTACATGGCC CTGTCTT-3'	264	Bbs I, 37	68+183+13

Results

The frequency of the mutant allele -1031 was significantly higher in patients with CD compared to healthy controls (26% in CD patients vs. 15.5% in controls, $P=0.02$, OR=1.91; 95% CI, 1.14-3.23). The frequency of this polymorphism was also higher in CD patients compared to UC patients (26% in CD vs. 12.2% in UC, $P = 0.001$, OR = 0.40, 95% CI: 0. 23-0.69). This allele frequency in

UC patients was similar to healthy controls (12.2% vs. 15.5%).

No association was observed between other evaluated SNPs frequency and Crohn's disease. None of the mentioned SNPs were more frequent in UC patients compared to healthy controls. All genotype and allele frequencies were in Hardy-Weinberg equilibrium for all polymorphisms.

The details of the mutant allele frequencies and their comparisons are demonstrated in Table 2 and 3.

Table 2: The frequency of TNF- α gene genotypes for 5 evaluated SNPs in Iranian CD and UC patients compared to healthy controls

Location and nucleotide change	dbSNP accession number	Genotypes	No. (%) of variant genotypes		
			UC (n=156)	CD (n=50)	Controls (n=200)
-238(G→A)	rs361525	GG	131 (84)	39 (78)	163 (81.5)
		GA	25 (16)	10 (20)	35 (17.5)
		AA	0 (0)	1 (2)	2 (1)
-308(G→A)	rs1800629	GG	122 (78.2)	42 (84)	158 (79)
		GA	33 (21.2)	6 (12)	39 (19.5)
		AA	1 (0.6)	2 (4)	3 (1.5)
-857(C→T)	rs1799724	CC	109 (69.9)	32 (64)	143 (71.5)
		CT	46 (29.5)	18 (36)	52 (26)
		TT	1 (0.6)	0 (0)	5 (2.5)
-863(C→A)	rs1800630	CC	117 (75)	33 (66)	157 (78.5)
		CA	33 (21.2)	14 (28)	33 (16.5)
		AA	6 (3.8)	3 (6)	10 (5)
-1031(T→C)	rs1799964	TT	122 (78.2)	28 (56)	149 (74.5)
		TC	30 (19.2)	18 (36)	40 (20)
		CC	4 (2.6)	4 (8)	11 (5.5)

Table 3: The allele frequencies of TNF- α gene polymorphisms in Iranian CD and UC patients compared to healthy controls

Location and nucleotide Change	allele	No. (%) of variant alleles		Pvalue	No. (%) of variant alleles		Pvalue
		CD (n=50)	Control (n=200)		UC (n=156)	Control (n=200)	
-238(G→A)	G	88 (88.0)	361 (90.2)	0.58	287 (92.0)	361 (90.2)	0.43
	A	12 (12.0)	39 (9.8)		25 (8.0)	39 (9.8)	
-308(G→A)	G	90 (90.0)	355 (88.7)	0.86	277 (88.8)	355 (88.7)	1.00
	A	10 (10.0)	45 (11.3)		35 (11.2)	45 (11.3)	
-857(C→T)	C	82 (82.0)	338 (84.5)	0.54	264 (84.6)	338 (84.5)	1.00
	T	18 (18.0)	62 (15.5)		48 (15.4)	62 (15.5)	
-863(C→A)	C	80 (80.0)	347 (86.7)	0.11	267 (85.6)	347 (86.7)	0.66
	A	20 (20.0)	53 (13.3)		45 (14.4)	53 (13.3)	
-1031(T→C)	T	74 (74.0)	338 (84.5)	0.02*	274 (87.8)	338 (84.5)	0.23
	C	26 (26.0)	62 (15.5)		38 (12.2)	62 (15.5)	

*: OR=1.91; 95% CI, 1.14-3.23.

Discussion

Since IBD is characterized by a failure to confine the usual self-limited gut inflammatory response, genes involved in determining the level of the immune response in the inflammatory pathway

might be risk factors in the disease pathogenesis. TNF- α gene codifies one of the most potent pro-inflammatory cytokine and immune modulator of inflammation, thus it constitutes an important candidate for determining genetic susceptibility in inflammatory responses. Recent studies suggest

TNF- α gene as a strong positional and functional candidate for IBD (4). Our results showed a significant higher -1031 mutant allele frequency in Iranian CD patients compared to healthy controls. This polymorphism demonstrated a similar pattern in a study in Canadian population ($P=0.008$, $OR=9.9$, 95% CI: 1.3-78) (11) as well as in a Japanese study ($P=0.004$, $OR=1.68$, 95% CI: 1.18-2.39) (14).

Other studies in UK (12) (25) and a Canadian study (10) did not report any higher frequency of this polymorphism.

In our study, the -863 mutant allele frequencies were higher in UC and CD patients comparing to healthy controls but these frequencies were not statistically significant. Considering other studies assessing the same polymorphism, a wide range of results was reported. The similar results to this paper, were reported in two studies (10) (11) from Canada, a study on a European population in UK (12), and two separate studies in Asia including Korean (13) and Chinese (14) populations. On the other hand, in a Japanese study the -863 mutant allele demonstrated an association with Crohn's disease ($P=0.004$, $OR=1.72$, 95% CI: 1.19-2.48) (15).

In our study, the -238 mutant allele frequencies were higher in CD patients comparing to control group, but this difference was not statistically significant. Similar to our study, in other studies such as a Hungarian study (16), two Canadian studies (10) (11) also a Japanese study (15) and a Chinese study (14) for the SNP -238 the differences between patients and controls were not significant.

The allele frequency of SNP -308 was lower than both in UC and CD patients comparing to control group without any significance in our study.

Similar to our study, some studies (10, 12, 15, 17-19) did not find any significant difference in the frequency of the -308 mutant alleles in patients compared with healthy controls.

But on the other side, -308 mutant allele frequency between patients and controls in some studies (14, 16, 20-24,) a significant difference was found between the UC and controls but this difference was not significant between CD patients and controls.

The allele frequency of SNP -857 was also lower in UC patients and higher in CD patients comparing to control group with no statistical significance in this study.

Similar to our study in Canadian study (10), an Italian study (20) and a Chinese study (14) for the SNP -863 the difference between patients and controls were not significant. While in some studies such as two studies in UK (12, 25) and in a study in Korea (13) frequency of SNP -863 was significant in patients and controls. Furthermore, in a Japanese study (15) allele frequency in SNP -857 between CD and HC was significant and that wasn't significant between UC and HC.

In our study allele frequency of SNP -1031 was higher in UC and CD patients comparing to control group and this differences between CD patients and control group was significant. Similar to our study a Canadian study (11) and a Japanese study (15) showed allele frequency in polymorphism -1031 between CD and HC was significant and this mutant allele frequency was not significant between UC and HC similar to our study. This mutant allele frequency in some studies for example in UK (12) (25) and a Canadian study (10) was not significantly different.

In an overall meta-analysis in New Zealand on three common SNP in 2008 which was performed on previous studies shows that carrying the TNF-alpha receptor SNPs -238,-308 or -857 were not significantly associated with the risk of IBD, both in the New Zealand population as well as in the international studies (26).

Conclusion

As it was discussed there were some studies similar to and some different from our study throughout various parts of the world, but despite some conflicting results many of these studies have provided important evidences for the influence of cytokine gene polymorphisms on disease susceptibility, onset and course as well as response to drug therapy. The variability of the results may be due to genetic/ethnic variations among different populations or the presence of different pheno-

types among the patients in different studies. Besides, in Iran, the studies with high sample size and use of more advanced laboratory techniques seem to be necessary.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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