

Association of TNF- α -857 Polymorphism with Inflammatory Bowel Disease in a Group of Iranian Azeri Individuals

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ABSTRACT

BACKGROUND

Inflammatory bowel disease (IBD) is a chronic, relapsing, inflammatory disorder of the gastrointestinal tract that includes two entities, Crohn's disease (CD) and ulcerative colitis (UC). As with other complex diseases, both genetic susceptibility and environmental factors play role in the pathogenesis of these diseases. The tumor necrosis factor α (TNF- α) gene is located in the IBD3 region on chromosome 6p21 which is a good functional candidate for involvement in susceptibility to IBD. In addition, the promoter region of TNF- α contains various polymorphisms that have shown a significant association with IBD.

METHODS

In this case control study we investigated the TNF- α -857 polymorphism in 109 patients (89 UC and 16 CD) who suffered from IBD and 100 healthy age, sex and ethnicity matched adults selected from the same population, as the control group. The polymorphism was checked by amplification refractory system (ARMS) and polymerase chain reaction (PCR).

RESULTS

Investigation of the association of TNF- α -857 gene promoter polymorphism with both types of IBD showed no significant difference in genotype and allele frequencies of this polymorphism between UC patients and controls. However, a possible association of TNF- α -857 polymorphism ($p=0.03$) was identified with CD.

CONCLUSION

TNF- α -857 polymorphism may have a role in the development of CD in the Iranian Azeri Turkish population.

KEYWORDS

IBD; TNF- α ; ARMS-PCR

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract which refers to two chronic diseases - ulcerative colitis (UC) and Crohn's disease (CD). Although UC and CD are two distinct forms of IBD that have a number of common clinical, epidemiological, and immunological features, they can be distinguished by anatomical and histological analysis as well as serologic tests.^{1,2} IBD is a complex multifactorial disease in which immune dysregulation caused by genetic or environmental factors plays an important role.³ Tumor necrosis factor alpha (TNF- α) is a key cytokine in the initiation and propagation of IBD. This is evidenced by an increased amount of TNF- α in intestinal tissues and peripheral phagocytes of patients with IBD.⁴

The TNF- α gene has been mapped to the chromosome 6P21.3 region. This gene consists of four exons with several polymorphisms. However, most of the reported polymorphisms are located in the promoter region of the gene.⁵ One of these polymorphisms is a single nucleotide substitution (C/T) at the -857 position. Several studies have shown an association between this C/T single nucleotide polymorphism (SNP) and autoimmune diseases such as Behcet's disease.⁶ Genetic associations have also been observed between promoter polymorphisms of TNF- α and IBD. However, these associations vary because they are not universally replicated. These conflicting results may be attributed to genetic variations of the different populations or systemic differences in the ancestry of cases and controls.⁴

In this study, we have analyzed the TNF- α gene promoter (-857 C/T) polymorphism in unrelated Iranian Azeri Turkish patients with CD and UC to evaluate the contribution of this SNP in genetic susceptibility to IBD.

MATERIALS AND METHODS

Subjects

We obtained blood samples from 209 subjects, 109 IBD patients and 100 healthy controls matched for age, sex and ethnicity. Subjects were all selected from the same population. None of the healthy

controls had any evidence of autoimmune diseases such as IBD, diabetes, or other autoimmune diseases. All patients and controls gave informed consent to participate in this study which was approved by the local Ethics Committee. The diagnosis and extent of IBD was made on the basis of clinical symptoms, endoscopic, radiological and histopathological findings, according to conventional criteria.²

DNA extraction and genotyping

Blood samples were collected from volunteers and placed in tubes that contained an EDTA anti-coagulant. Then, genomic DNA was extracted from peripheral blood cells by the "salting out" technique. DNA concentrations were determined by a UV spectrophotometer at 260 nm.⁷

DNA samples were genotyped for a promoter polymorphism in the -857C/T position of the TNF- α gene by amplification refractory system (ARMS) and polymerase chain reaction (PCR). For the amplification of polymorphism at the -857C/T, antisense 5'-TCA CAT GGC CCT GTC TTC G-3' or 5'-CTC ACA TGG CCC TGT CTT CT-3' and sense 5'-AAG ATA AGG GCT CAG AGA G-3' were used (289 bp PCR product). The internal control was a constant PCR band of 650 bp amplified with conx26 primer. PCR conditions were as follows: denaturation at 94°C for 5 min, 32 cycles at 94°C for 30 s, 58°C for 25 s, and 72°C for 30 s, followed by one cycle of final extension at 72°C for 7 min.

Distilled water was used as the negative control. The PCR products were analyzed by phototyping under ultraviolet light in 1.5% agarose gels stained with ethidium bromide.

Statistical analysis

Frequency of genotypes was assessed for Hardy-Weinberg equilibrium by the chi-square test or Fisher's exact probability if frequency in the cells of 2 by 2 tables was too small. The same test method was used to evaluate the correlation of the TNF- α -857 genotypes or alleles between patients and healthy controls.

Odds ratio (OR) with 95% confidence interval

Table 1: Genotypic and allelic frequencies of *TNF-α* -857 promoter polymorphism in inflammatory bowel disease (IBD) patients and healthy controls.

Genotype/allele		Control		IBD		Odds ratio (95%CI)	p-value
		N	%	N	%		
TNFα -857	CC	54	54	64	58.71	1.21 (0.7-2.2)	0.298
	CT	44	44	40	36.7	0.74 (0.4-1.4)	0.134
	TT	2	2	5	4.59	2.4 (0.4-18.4)	0.247
	C	152	76	168	77.06	1.06 (0.5-2.1)	0.495
	T	48	24	50	22.94	0.94 (0.5-1.9)	0.373

(95% CI) was calculated to show strength of correlation. All data were analyzed using SPSS version 15.0 software. A p value of <0.05 was considered significant.

RESULTS

In total, 109 patients with IBD and 100 healthy controls were enrolled in this study. Of these 109 patients, there were 89 UC cases and the remainder consisted of CD patients. The median age of onset of disease in this group was 24 years (range: 10- 69 years). Female to male ratios were 49:52 for the patients and 11:14 for the control group.

The distribution of the *TNF-α* promoter region -857C/T alleles and genotypes in the IBD and control groups is shown in Table 1. According to this table there was no difference in the *TNF-α* -857C/T genotypes and alleles detected between both groups.

Regarding the possible effects of *TNF-α* on different types of IBD, we compared *TNF-α* genotypes with both groups of patients (Table 2). Results showed no significant difference of *TNF-α* -857C/T polymorphism genotypes and alleles in UC patients. However, a comparison of this polymorphism between CD patients and controls revealed a possible association.

DISCUSSION

The most common type of IBD in our samples was UC which is in agreement with the previous reports based on the rarity of CD in Iran.¹

An active inflammatory response is an important feature of IBD. High serum levels of *TNF-α* as well as the increased expression of *TNF-α* have been documented in IBD.⁸ *TNF-α* is critically involved

in the pathogenesis of several chronic inflammatory diseases and therefore it is considered to be an appropriate target for interfering with the inflammatory responses. Blocking of *TNF-α* action by biological agents has been established as an effective treatment in various inflammatory diseases. Recent reports suggest that monoclonal antibodies against *TNF-α* can be effective for decreasing inflammation in IBD.¹ Alterations in *TNF* expression related to polymorphic alleles of the *TNF* genes may implicate a pathogenetic role in the increased activity of this cytokine in IBD. A comparison of the *TNF-α* promoter region -857C/T allelic and genotypic distribution in IBD patients and control groups has shown no difference between these groups. The results also showed no significant difference in *TNF-α* -857C/T polymorphism genotypes and alleles among UC patients. However, a comparison of this polymorphism between CD patients and controls revealed a possible association ($p=0.03$).

The *TNF-α* gene -857C/T single nucleotide polymorphism screened in this study showed differences between patients and controls. The main finding of this study was the association of *TNF-α* C-857T SNP with CD, which was similar to that seen in Japanese,⁹ Korean³ and Australian,¹⁰ Caucasian¹¹ and Chinese Han¹² ethnic populations. However, studies on New Zealand¹³ and Mexican¹⁴ populations did not confirm our findings.

In conclusion, the findings of this study have revealed an association between *TNF-α* -857 C/T polymorphism and CD. Other regulatory mechanisms effective on *TNF-α* expression need to be studied to clarify the role of the *TNF-α* related genetic contribution to the pathogenesis of IBD.

Table 2: Genotypic and allelic frequencies of TNF- α -857 promoter polymorphism in Crohn's disease (CD), ulcerative colitis (UC) patients and healthy controls.

Genotype/allele		CD				UC			
		N	%	Odds ratio (95% CI)	<i>p</i> -value	N	%	Odds ratio (95% CI)	<i>p</i> -value
TNFα -857	CC	11	68.75	1.9 (1.01-3.5)	0.023*	51	57.3	1.1 (0.6-2.08)	0.372
	CT	5	31.25	0.6 (0.3-1.07)	0.037*	33	37.08	0.75 (0.4-1.4)	0.191
	TT	0	0	-	-	5	5.62	2.9 (0.5-21.8)	0.156
	C	27	84.38	1.7 (0.8-3.7)	0.085	135	75.84	0.99 (0.5-2.0)	0.555
	T	5	15.62	0.6 (0.27-1.26)	0.063	43	24.16	1.01 (0.5-2.02)	0.444

*P value <0.05

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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