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IL-23 Receptor Gene rs7517847 and rs1004819 SNPs in Ulcerative Colitis

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ABSTRACT

Background: Crohn's disease (CD) and ulcerative colitis (UC) are two major clinical presentations of inflammatory bowel disease (IBD). Many novel candidate genes have been found to be associated with increased risk for IBD. Recently IL-23 receptor gene is identified as an IBD associated gene in genome-wide studies. **Objective:** To ascertain whether rs7517847 and rs1004819 SNPs in the IL-23 receptor gene are associated with UC in our population in Kerman, south east of Iran. **Methods:** A total of 85 patients with UC and 100 healthy controls enrolled in our study. Endoscopic procedure was performed for all patients to determine their disease severity. IL-23 receptor genotyping at positions rs7517847 and rs1004819 was done by PCR-RFLP technique. **Results:** The results of this study showed no association between the studied polymorphisms in the IL-23 receptor gene and UC in our population. However, we found a significant association between rs7517847 gene polymorphism in IL-23 receptor and two important clinical variables including blood in stool and bowel movements in UC patients. **Conclusion:** The rs7517847 gene polymorphism in IL-23R may be related to the presence of blood in stool and bowel movements in patients with UC. Further functional analysis with other known IL-23 receptor genotypes and/or other candidate genes is necessary to confirm any genetic association with UC in our population.

Keywords: IL-23R, rs7517847, rs1004819, Ulcerative Colitis

INTRODUCTION

Diagnosis of IBD predisposing genes could be important in understanding the mechanisms of pathogenicity of the disease. The results of epidemiological studies have introduced common predisposing genes for CD and UC. Extensive genetic studies in 2001

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led to the diagnosis of CD gene CARD15 as a major gene in which predisposing mutations influences the innate immune mechanisms (1). There are many other reports showing association between mutations in a number of other genes with CD and UC, although their precise role in susceptibility to IBD has been classified, in some cases, a review is necessary (2-6). Genetic studies on genes affecting IBD have not progressed rapidly due to several factors such as restrictions on studies of identical twins in the human population, inappropriate statistical tools and inadequate control for each single race (7). The very interesting findings in genetic studies on IL-23 receptor (IL-23R) gene polymorphisms suggest the influence of genotype on the risk of CD and UC (8,9). Results of German cohort study showing the relationship between both UC and CD with the IL23R gene polymorphisms (10) makes IL23R a predisposing gene in Caucasian IBD patients in Northern Europe, however such a relationship is not observed in the Japanese population (11). Although the exact mechanisms mediated by IL-23R in susceptibility to IBD is still not well understood, it seems that Th17 cells expressing IL-23R play an important role in this process (12). The latest results published in 2008 on a German population of patients with IBD showed a relationship between rs1004819 gene polymorphism in IL-23R and the increasing risk of CD, whereas rs11209026 gene polymorphism in the above mentioned gene presented a protecting effect against the disease (13). The same study on patients with CD and UC in England and Scotland, defines correlations of several mutations in IL-23R gene and UC among which rs1004819 polymorphism has the strongest relationship with the disease (14). Márquez and colleagues in a genetic analysis on two single nucleotide polymorphisms (SNPs) in IL-23R including rs7517847 and rs11209026 in 707 patients with IBD (344 patients with CD and 363 patients with UC) and 547 healthy controls, all from the same ethnic origin (Caucasian Spaniards), showed powerful associations of rs7517847 gene polymorphism in IL-23R with CD and UC diseases and this association was stronger with CD (15). In another study in England on IL-23R gene variants, eight polymorphisms were found to be correlated with CD and UC patients and a weak association were reported for rs7517847 and rs11209026 variants with UC disease (16). Additionally, a genetic study on eight IL-23R gene polymorphisms in German UC and CD patients showed significant associations of rs1004819 and rs7517847 with UC (17). Duerr and colleagues, in the study on the population of Jews and Gentiles, reported associations of some of IL-23R gene polymorphisms such as rs7514847and rs1004819 with CD (8). Based on the above studies, we chose two UC-associated IL-23R gene polymorphisms including rs7517847 and rs1004819 in order to detect the presence of any association between these variants and UC in our population in south east of Iran.

MATERIALS AND METHODS

Study Populations. The study population included 85 UC patients who were born and lived in Kerman in south east of Iran and 100 unrelated healthy blood donors from the Kerman blood transfusion centre who were originally from the same geographical area. Written, informed consent was obtained from all patients and healthy controls prior to participation in the study.

Table 1. Demographic and clinical characteristics of the study population.

Variables	UC (n=%)	Controls (n=%)
Gender		
Male	38 (44.7%)	59 (59.0%)
Female	47 (55.3%)	41 (41.0%)
Age (yr)	37.79 ± 15.79	38.19 ± 12.24
Range	(14-84)	(19-63)
Disease duration (yr)	3.07 ± 3.44	-
Bowel movements		
Mild	45 (52.9%)	-
Moderate	28 (32.9%)	
Severe	12 (14.1%)	
Immunosuppressive drugs		
Cytotoxic and steroidal	14 (16.5%)	-
ASA	40 (47.1%)	
Others	31 (36.4%)	
Anemia		
Mild	41 (48.2%)	-
Moderate	35 (41.2%)	
Severe	9 (10.6%)	
Blood in stool		
Mild	39 (45.9%)	-
Moderate	26 (30.6%)	
Severe	20 (23.5%)	
Tachycardia		
Mild	72(84.7%)	-
Severe	13(15.3%)	
ESR		
Mild	58 (68.2%)	-
Severe	27 (31.8%)	
Age at diagnosis (yr)	34.72 ± 15.49	-
Range	(11-82)	
Appendectomy	3 (3.5%)	-
Oral contraceptive consumption (female)	10 (21.27%)	-
Smoking habit	5 (5.9%)	-
Opium consumption	14 (16.5%)	-
Family history of disease	8 (9.4%)	-
Endoscopic criteria		-
Mild	44 (51.8%)	
Moderate	25 (29.4%)	
Severe	16 (18.8%)	
Total	85	100

Data are presented as mean ± SD or frequency as n (%)

UC was diagnosed according to the criteria of American Gastroenterology Association (18). All patients underwent endoscopic study to define the severity of their disease. Patients were then stratified into three groups of "Mild", "Moderate" and "Severe" according to endoscopic findings. Exclusion criteria were pregnancy, acute or chronic renal failure, congestive heart failure, thyroid disorders, acute infections, and stroke and hospital admission in recent months. Demographic data including age, sex, duration of disease, history of cigarette smoking, history of drug abuse and family history of disease was also taken from the patients. The study was approved by the local Ethics committee (Kerman University of Medical Sciences, Kerman, Iran). Demographic and clinical data are summarized in Table 1.

Genotyping. Genomic DNA was isolated from whole blood using a routine salting out method. PCR-RFLP methods were applied for genotyping the variants IL-23R (rs1004819, rs7517847) gene using the forward and reverse primers according to a previously published article (19) and are shown in Table 2.

The PCR amplifications were performed based on the following conditions: initial denaturation at 96°C for 2 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 45 s, extension at 72°C for 45 s and final extension at 72°C for 5 min. Ten microliters of PCR products were digested by 1 U of allele specific restriction endonucleases *Taa*I (rs1004819) or *BseMII* (rs7517847). The restriction fragments were separated by electrophoresis on 2% agarose gels containing ethidium bromide and visualized by UV light.

Table 2. Primer sequences for the analyzed variants.

Gene Location	Primers	The Size of PCR Products	Genotypes	The Size of PCR-RFLP Products
rs1004819	Forward 5'- GCA TTC TAG GAC CGT TTT GG-3' Reverse 5'- ATC TGG TGG AAA TAT GTG AAA CCT A-3'	270bp	WT	13+71+185
			Heterozygous	13+71+185+257
			Homozygous	13+257
rs7517847	Forward 5'- AAA CAT TGA CAT TCC CTT CAT AC-3 Reverse 5'-GAA ATG AGT CAC CAA TAA TCC AC-3'	530bp	WT	29+91+410
			Heterozygous	29+91+410+501
			Homozygous	29+501

Statistical Analysis. All data are presented as mean ± standard deviation (SD) for quantitative variables and summarized by absolute frequencies and percentages for categorical variables. Variables were compared using *chi*-square test or *Fisher's* exact test. Sub-group analysis was also performed in patient groups, to compare the severity of the disease (mild, moderate, and severe) with the genotypes (wild, heterozygote, and homozygote) using Kruskal-Wallis test. For the statistical analysis, SPSS software version 15.0 was used. P values less than 0.05 were considered statistically significant.

Ethical Considerations. The study protocol was approved by the ethics committee of Kerman University of Medical Sciences and a written informed consent was obtained from each patient before enrollment in the study.

RESULTS

IL-23R gene polymorphisms at positions rs7517847 and rs1004819 were analyzed in healthy controls and UC patients from Kerman, Iran. Genotype distributions were in Hardy-Weinberg equilibrium both in patients and controls. Genotype and allele frequencies are shown in Table 3.

Table3. Genotype and allele frequencies of IL23R used in the present study.

IL23R	Genotypes	UC n	UC %	Controls n	Controls %	Alleles	UC n	UC %	Controls n	Controls %
rs7517847	TT	41	48.2	52	52	T	118	69.4	145	72.5
	TG	36	42.4	41	41	G	52	30.6	55	27.5
	GG	8	9.4	7	7					
rs1004819	GG	26	30.6	29	29	G	91	53.5	107	53.5
	GA	39	45.9	49	49	A	79	46.5	93	46.5
	AA	20	23.5	22	22					

Table 4. *IL23R* gene variant, rs7517847, increases bowel movements and blood in stool in UC patients.

IL-23R SNP	Genotype	Blood in Stool n (%)			Bowel Movements n (%)		
		mild	moderate	severe	mild	moderate	severe
rs7517847	TT (n=41)	14(35.9)	12(46.2)	15(75)[a]	21(46.7)	14(50)	6(50)[b]
	TG (n=36)	20(51.3)	12(46.2)	4(20)	22(48.9)	12(42.9)	5(16.7)
	GG (n=8)	5(12.8)	2(7.7)	1(5)	2(4.4)	7(7.1)	3(33.4)
rs1004819	GG (n=26)	11(28.2)	10(38.5)	5(25)	15(33.3)	7(25)	4(33.3)
	GA (n=39)	21(53.8)	10(38.5)	8(40)	20(44.4)	14(50)	5(41.7)
	AA (n=20)	7(17.9)	6(23.1)	7(35)	10(22.2)	7(25)	3(25)

[a] severe blood in stool, wild versus homozygote, p value = 0.029
[b] mild bowel movements, heterozygote versus homozygote, p value= 0.038

The analysis of these SNPs showed no statistically significant associations with UC patients compared with the healthy controls. However, in contrast to rs1004819, the other IL-23R SNP's of rs7517847 had some influence on the disease pathologic symptoms such as blood in the stool and bowel movements in UC patients of our population (Table 4). To our knowledge, this is the first report showing that pathological symptoms such as bowel movements (p=0.038) and blood in stool (p=0.029) might be shifted from severe into the mild when patients show the homozygote GG genotype for IL23R rs7517847.

DISCUSSION

In the present study, two polymorphisms including rs1004819 and rs7517847 were selected for investigation of their associations with UC. It should be noted that the study on the relationship between these SNPs and the disease in a population of patients have already been conducted in other countries by several groups (8,16,17). Our data showed no association between the above mentioned polymorphisms and UC which is in concordance with the results of Duerr and colleagues (8) on the Jews and Gentiles population and also with a study on a Spanish population (16). The results of our research do not agree with study of Cummings and colleagues in England on the relationship between eight polymorphisms, including rs1004819 and rs7517847, with IBD. They had reported a positive association between the above mentioned polymorphisms with either UC or CD (17). Additionally, the results of a recent Meta analysis from all case-control studies showed that rs7517847 within the IL-23R gene might be a protective factor against developing CD (20). It should be noted that the geographic and genetic variations and also different ethnicities sometimes cause contradictory results in genetic studies. However, the majority of published data clearly show the importance of IL-23R gene in IBD. Studying the possible relationship between the severity of the disease, the endoscopic view, anemia, blood in stool and bowel movements, with UC, and using Kruskal-Wallis statistical test, a significant correlation was found between rs7517847 polymorphism and the presence of blood in the stool of our patients (p=0.029). The frequency of wild type genotype for rs7517847 polymorphisms was lowest in the mild type and highest in the severe type of blood in the stool of UC patients. In other words, the frequency of wild type allele (T) at position rs7517847 polymorphism was higher in the UC patients who had the greatest amount of blood in the stool and interestingly the homozygous genotype frequency of the same polymorphism in UC patients seems to be correlated with the lowest average of blood in the stool. Considering these results, we suggest that the allele at position rs7517847 may have an inhibitory effect on the severity of bleeding, thereby producing a protective role against UC. We also found a significant correlation between the frequency of rs7517847 polymorphism and bowel movements in UC patients (p=0.038). The frequency of the heterozygote genotype (rs7517847) was higher in patients with a mild type of bowel movements than the severe ones, while the frequency of homozygote polymorphism in patients with a severe type of bowel movements was more than the mild ones. It should be noted that the mild and severe form of bowel movements are described as less than 4 times a day and more than 6 times a day of bowel movements, respectively.

In conclusion, blood in the stool and bowel movements are qualitative variables which have been described by patients and the significant association between rs7517847 polymorphism and blood in the stool as well as the bowel movements might not be generalized to all patients. Therefore, further investigation is required for precise assessment of the association between the above mentioned factors and rs7517847 polymorphism in IL-23R gene in UC patients.

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