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Original Article

Association Study of Interleukin 23 Receptor (IL-23R) Polymorphisms with Inflammatory Bowel Disease (IBD) in Khuzestan Province of Iran

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Abstract

Background: Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a complex multifactorial disease for which the exact cause is not clear. In this study, the researcher aimed to evaluate interleukin 23 receptor (IL23R) gene polymorphisms in patients with inflammatory bowel disease.

Methods: In this case-control study, we evaluated 125 patients of the Iranian population (Khuzestan) with IBD, including 35 patients with CD, 90 patients with UC, and 125 healthy controls. The polymorphisms of C/A-97952 (rs10889677), and G/A-43045 (rs1004819) were genotyped, using ARMS-PCR and G/A-78790 (rs11209026) using RFLP-PCR methods in the studied population. The collected data were analyzed using SPSS software.

Results: In this study, no significant association was observed between IL23R polymorphisms and IBD in this population, however the association between C/A-97952 AA genotype and penetrate to the tissue (*P*=0.048 OR=2.812 (1.23-6.44)), G/A-43045, AA genotype, and Ileocolic (*P*=0.031 OR=5 (1.071-31)). and G/A-43045 AA genotype and Pan Colitis (*P*=0.028 OR=4 (1.082-17.00)) in IBD were strengthened and emphasized.

Conclusion: The present study showed that there were no associations between IL23R polymorphisms, CD, and UC in the Khuzestan population. In addition, we found that C/A-97952 and G/A-43045 gene polymorphisms in IL-23R are related to special phenotypes. Further functional analysis with a larger sample size and other known IL-23 receptor genotypes is necessary to confirm our population's association with UC and CD.

Keywords: IL-23R; Inflammatory Bowel Disease; Crohn's Disease; Ulcerative Colitis; Polymorphism.

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Introduction

IBD includes a group of chronic diseases of the digestive system CD and UC diseases are the most common types and are caused by the immunity system (1). CD can occur in any area of the gastrointestinal tract, but UC is limited in the large intestine. All thickness of the intestinal wall is often affected by Crohn whereas ulcerative colitis disease only involves the mucous layer. Inflammatory bowel disease is a common disorder worldwide with a prevalence rate of 0.4% to 0.1% of the general population (2).

The exact causes of IBD (CD, UC) diseases are unknown, however, risk factors such as family history, smoking, air pollution, pregnancy pills, and diet type can contribute to susceptibility to the disease. Likely, a combination of factors, including genetic defects, aberrant mucosal immune response, dysfunction of bowel aptly, mucus, and disorder of host interaction in bowel microbes, could have a significant role in susceptibility to CD and UC (3-5).

IL-23R in the membrane of T-cells memory and other immune cells such as NK cells of monocytes and dendritic cells are very much responsible for detecting external elements and defending the body against external problems. This receptor reacts with IL-23 and with activation of the TH17 lymphocytes and is achieved by producing IL-17 cells. TH17 cells produce many pro-inflammatory cytokines including IL-17, IL-6, and alpha tumor necrosis (6-9).

IL-23 receptor with protein coder gene is formed from IL-12p40 and IL-23- p19 and located on chromosome 1p31.1 and its ligand IL-23 is a key component of the immune regulation (10). IL-23R is a heterodimer cytokine that has the same performance as wasIL-12 in promoting cellular immunity. Unlike IL-23, IL-12 causes the polarity of CD4+ T cells, to Th17 cells producing IL-17 instead of TH1 cells (11).

Using various methods of genetic investigation including analysis of gene linkage, the study of selected genes, and evaluation of genetic relationships in all genomes approximately have been identified associated with disease in different chromosomes (12, 13). About a third of these genetic risk factors are common between CD and UC, which can explain both diseases in some families and the response to these two diseases toward treatment due to the common pathogenesis of autoimmune diseases and epidemiological creation (14, 15). Among these, many polymorphisms have been studied and, in some cases, there is a significant relation between the accompaniment of the polymorphisms and inflammatory bowel disease (16-20).

In G/A-78790 polymorphism placed in Exon 9 (non-synonymous Arg381Gln) at 3'UTR(3'untranslated region), guanine is replaced by adenine and as a result changed Arginine 381 to Glutamine (21) and G/A-43045 polymorphism is placed in intron 5 of Interleukin 23 receptor gene where guanine is replaced by adenine [22]. C/A-97952 polymorphism was also investigated which is placed in the Interleukin 23 receptor gene Exon 11-3'UTR (synonymous) and Adenine is replaced for Cytosine (22).

Many studies have shown significant associations between the G/A-78790, C/A-9,7952, and G/A-43045 polymorphisms and CD and UC, and in some, cases no significant association was observed (23-41). As far as we know, this is the first study for IL-23R gene polymorphism including G/A-78790, C/A-97952, and G/A-43045 in Iranian IBD patients living in Khuzestan province.

Materials and Methods

The study groups were comprised of 125 Iranian IBD patients from Khuzestan province, located in southwest Iran, Including 35 patients with CD, 90 patients with UC, and 125 healthy unrelated controls. A detailed questionnaire concerning the demographic and clinical features of the disease has been completed for each patient. This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (**ajums.REC.1393.134**). All detailed information of patients with CD and UC were summarized in **Table 1**.

DNA extraction and Genotyping of the IL23R

Genomic DNA was extracted from the peripheral blood containing EDTA using the conventional phenol-chloroform extraction method (42). Genotyping was performed using RFLP-PCR for G/A-78790 and ARMS-PCR for G/A-43045 and C/A-97952. All primers for each PCR in this

study were designed and are shown in **table 2**.

RFLP -PCR

Using 5-10 ng genomic DNA, PCR was performed using a T100™ Thermal Cycler (BIO-RAD, USA). The amplified PCR products were digested with Hpy188I restriction endonuclease (New England Biolabs, MA, USA) over three hours at 37°C, and the digestion products were run on a 2.5 % agarose gel and stained by DNA Safe Stain (SinaClone, Iran) for visualization on a UV transilluminator.

ARMS- PCR

ARMS- PCR was applied for genotyping two variants of IL-23R using 20 ng genomic with an annealing temperature of 62°C. The PCR products of the IL23R gene in the location of C/A-97952 and G/A-43045 SNPs were run on a 1.5 % agarose gel and stained with DNA Safe Stain for visualization on a UV transilluminator by Universal Hood II Gel doc (BIO-RAD, USA). (**Figure 1,2**)

Sequencing

For three polymorphisms, three controls, containing: homozygous wildtype, heterozygous and homozygous mutant were sequenced by the dideoxy chain termination method19 using the Big Dye Terminator cycle sequencing kit on an ABI 310 sequencer (Applied Biosystems, Darmstadt, Germany). All the following studies included these reference individuals

Statistical analysis

Statistical analysis was carried out using IBM®SPSS®Statistics ver22 for Windows. Data are given as mean ± SD. Allele and genotype frequencies in patients and controls were compared by Chi-square $\chi 2$ test or Fisher exact test when an expected value was < 0.5; P values were considered significant at a level of < 0.05. The odds ratio (OR) and P values were calculated using a standard package.

Results

Frequency distribution of IL23R genotypes and their role in IBD susceptibility

In all three subgroups (CD, UC, and controls), the genotype frequencies of the IL23R SNPs were by the Hardy-Weinberg equilibrium. IL-23R gene polymorphisms at positions G/A-78790, G/A-43045 and C/A-97952 were analyzed in healthy controls and UC & CD patients. Genotype and allele frequencies are shown in **Table 3-5.**

In this study, minor allele frequency was achieved as 0.071 for Crohn's patients, 0.066 for ulcerative colitis patients, and 0.056 for the control group, and there was no significant difference between the alternative genotype and the control group. We observed that the minor allele frequency for Crohn's patients and ulcerative colitis patients was 0.41 and for the control group was 0.36. No significant relationship was observed between the frequency of genotypes AA + GA, the frequency of AA group genotypes and A allele control group, and CD, UC, and IBD patients.

Minor allele frequency is 0.37 for group CD, 0.36 for group UC and 0.34 for the control group. Comparing the frequency of genotypes AA + CA, frequency of AA group genotypes, and allele A control group and CD, UC, and IBD patients, no significant difference was observed between Crohn's and ulcerative colitis patients as well as IBD patients.

Associations between IL23R genotype and IBD phenotype

By investigating the polymorphisms with age and gender variables using canonical correlation in all subjects it was observed that in IBD individuals the independent variables are not associated with any amount of variance of the dependent variable. Wilkes's statistic value is 0.97090 the degree of freedom is 6 and the *P*-value is 0.734. In UC individuals, the independent variables are not associated with any variance of the dependent variable. Wilkes's statistic value is 0.89375, the degree of freedom is 6 and the p-value is 0.140. In CD individuals, the independent variables are not associated with any amount of variance of the dependent variable. Wilkes's statistic value is 0.71550, the degree of freedom is 6 and the *P*-value is 0.110.

Discussion

In some populations, the IL-23R gene is reported to be associated with IBD. To regulate

Table 1. Demographic and clinical characteristics of the study population

Variables	CD N (%)	UC N (%)	IBD N (%)	Control N (%)
Gender				
Female (%)	22(62.9)	49(54.5)	71(56.8)	71(56.8)
Male (%)	13(37.1)	41(45.5)	54(43.2)	54(43.2)
Age mean±SD	33.63±14.87	37.23±10.35	36.22±11.84	41.96±14.89
Range	15-82	18-68	15-82	
Age at diagnosis (Year) mean±SD	26.09±10.69	30.80±9.28	29.48 ±9.88	
Range	14-60	13-60	13-60	
Below 20 (%)	14(40)	15(16.7)	29(23.2)	
Between 21- 30 (%)	15(42.9)	41(45.6)	56(44.8)	
Between 31 and 40 (%)	2(5.7)	24(26.7)	26(20.8)	
Between 41 and 50 (%)	2(5.7)	7(7.7)	9(7.2)	
Above 50 (%)	2(5.7)	3(3.3)	5(4)	
Crohn's disease behavior				
Inflammatory	27(77 .1)			
Penetrating	2(5.7)			
Stricturing	6(17.1)			
Crohn's disease location				
Colon (Granulomatous)	7(20)			
Ileal (ileitis)	5(14.3)			
Ileocolonic (ileocolitis or Enterocolitis)	8(22.9)			
only the small intestine (enteritis)	15(42.9)			
Disease extent UC				
Rectum		14(15.5)		
Left colon		35(38.9)		
Pancolitis		41(45.5)		

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

Table 2. Primers sequences used for the genotyping of IL23R SNPs

PCR-RFLP					
SNPS	**Sequence**	**PCR Product Size**	**Restriction Enzyme**	**PCR-RFLP Products Size**	**Genotypes**
	For 5' AAGTTGTTTCCTGGGGTAGTTGTG 3'	508 bp	Hpy188I	288+103+82+35	Homozygous Wild type
	Rev 5' CTTTTCTGGCAGGGTCATTTTG 3'			323+288+103+82+35	Heterozygous
				323+103+82	Homozygous Mutant
ARMS-PCR					
	Sequence				
C/A-97952	For 5' CTAGGTAGGGGATTGCTGGG 3'				
	Rev 5' CACGCCTGGCCTAATGATTC 3'				
	SSP* "a" 5' TTTAATTTTAGCCATTCTTCTGCCTA 3'				
	SSP "c" 5' TAATTTTAGCCATTCTTCTGCCTC 3'				
G/A-43045	For 5' TCAACATCTGAGTCTTGTGTAACA 3'				
	Rev 5' CCAAGAAGTAGAGGTTGCAGC 3'				
	SSP "a" 5' CTTTATGCTGTGATTCTTACTA 3'				
	SSP "g" 5' CTTTATGCTGTGATTCTTACTG 3'				

*** Sequence, specific PCR primers; SNP, single nucleotide polymorphism**

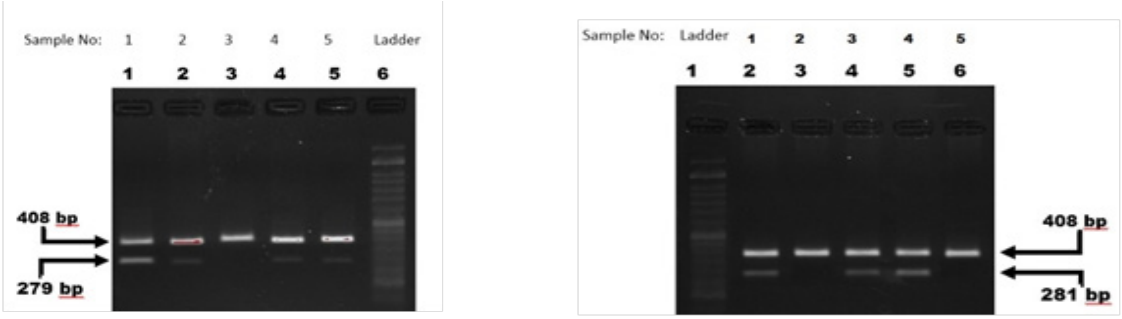

Figure 1. ARMS-PCR Assay for rs10889677 IL-23R. Left: Agarose gel electrophoresis showing the 408 bp & 279 bp ARMS-PCR amplicons of IL-23R gen rs10889677 with "C" SSP primer. Lane 1, 2, 3, 4, and 5 samples Lane 6, 50 bp DNA molecular weight marker; Right: Agarose gel electrophoresis showing the 408 bp & 281 bp ARMS- PCR amplicons of IL-23R gen rs10889677 with "A" SSP primer. Lane 1, 50 bp DNA molecular weight marker lane 2, 3, 4, 5 and 6 samples; Sample No. 1 and 4 Heterozygous (CA), 3 homozygous (AA) mutant, 2 and 5 homozygous (CC) wild-type.

Figure 2. ARMS-PCR Assay for rs1004819 IL-23R. Left: Agarose gel electrophoresis showing the 487 bp & 363 bp ARMS-PCR amplicons of IL-23R gen rs1004819 with "G" SSP primer. Lane 1, 2, 3, 4, 5,6, and 7 samples Lane 8, 50 bp DNA molecular weight marker; Right: Agarose gel electrophoresis showing the 487 bp & 363 bp ARMS- PCR amplicons of IL-23R gen rs1004819 with "A" SSP primer. Lane 1, 50 bp DNA molecular weight marker lane 2, 3, 4, 5 ,6, 7, and 8 samples; Sample No. 1,3,4,6 and 7 Heterozygous (GA), 5 homozygous (AA) mutant, 2 homozygous (GG) wild-type.

Table 3. Genotype frequencies of G/A-78790 Polymorphism in the present study

IL23R	CD (N=35)	df,X^2 (*P*-value)	UC (N=90)	df,X^2 (*P*-value)	IBD (N=125)	df,X^2 (*P*-value)	Controls (N=125)
GG	30 (85.7%)	X2=0.25 *P*=0.6 df=1	78 (86.7%)	X2=0.22 *P*= 0.63 df=1	108 (86.4%)	X2=0.33 *P*= 0.56 df=1	111 (88.8%)
GA	5 (14.3%)		12 (13.3%)		17 (13.6%)		14 (11.2%)
GA+AA	35 (100%)		90 (100%)		125 (100%)		125 (100%)
AA	0		0		0		0
MAF_A	0.071		0.066		0.068		0.056
G	65 (92.85%)		168 (93.33%)		233 (93.2%)		229 (91.6%)
A	5(7.14%)	X2=0.18 *P*= 0.67 df=1	12 (6.66%)	X2=0.14 *P*= 0.7 df=1	17 (6.8%)	X2=0.31 *P*= 0.58 df=1	14 (5.6%)

X2, chi square statistic; df, degrees of freedom; or, odds ratio; MAF, minor allele frequency

Table 4. Genotype frequencies of G/A-43045 Polymorphism in the present study

IL23R	CD (N=35)	df,X^2 (*P*-value) OR (95%CI)	UC (N=90)	df,X^2 (*P*-value) OR (95%CI)	IBD (N=125)	df,X^2 (*P*-value) OR (95%CI)	Controls (N=125)
GG	11 (31.42 %)	*P*=0.328, 0.484 (0.11-2.098) X^2=0.95 df=1	28 (31.11 %)		39 (31.2 %)		47 (37.6 %)
GA	19 (54.28%)		50 (55.55%)		69 (55.2%)		65(52%)
GA+AA	24 (68.57%)		62 (68.88%)	X^2=1.6 *P*=0.2,0.528 (0.195-1.43)	8 6(76%)	X^2=1.12 *P*=0.28,0.648 (0.290-1.144) df=1	78 (62.4%)
AA	5 (14.28%)	X^2=0.68 *P*=0.7 df=2	12 (13.33%)	X^2=1.1 *P*=0.56	17 (13.6%)	X^2=1.4 *P*=0.5 df=2	13 (10.4 %)
MAF_A	0.41		0.41		0.41		0.36
G	41 (58.57%)		106 (58.88%)		147 (58.8%)		159 (63.6%)
A	29 (41.42%)	X^2=0.59 *P*=0.44 df=1	74 (41.11%)	X^2=0.98 *P*=0.32	103 (41.2%)	X^2=1.2 *P*=0.27df=1	91 (36.4%)

X2, Chi Square Statistic; df, Degrees of Freedom; CI, Confidence Interval; OR, Odds Ratio; MAF, Minor Allele Frequency

Table 5. Genotype frequencies of C/A-97952 Polymorphism in the present study

IL23R	CD (N=35)	df,X^2 (*P*-value) OR (95%CI)	UC (N=90)	df,X^2 (*P*-value) OR (95%CI)	IBD (N=125)	df,X^2 (*P*-value) OR (95%CI)	Controls (N=125)
CC	13 (37.14 %)		34 (37.77 %)		47 (37.6 %)		51 (40.8 %)
CA	18 (51.42%)		47 (52.22%)		65(52%)		63(50.4%)
CA+AA	22 (62.85%)	P=0.7* 0.64 (0.134-3.095)	56 (62.22%)	df = 1 X2=1.54 P=0.215 ,0.556 (219-1.413)	78 (62.4%)	df = 1 P=0.493, 1.29 (0.620-2.692)	74 (59.2%)
AA	4 (11.42%)	df = 2 X2=0.2 P=0.86	9 (10%)	df = 2 X2=0.24 P=0.89	13 (10.4%)	df = 2 X2=0.4 P=0.80	11 (8.8 %)
MAF_A	0.37		0.36		0.36		0.34
C	44 (62.85%)		115 (63.88%)		159 (63.6%)		165 (66%)
A	26 (37.14%)	df = 1 X2=0.24 P=0.62	65 (36.11%)	df = 1 X2=0.2 P=0.65	91 (36.4%)	df = 1 X2=0.31 P=0.57	85 (34%)

X2, Chi Square Statistic; df, Degrees of Freedom; CI, Confidence Interval; OR, Odds Ratio; MAF, Minor Allele Frequency

the IL-23R gene function; several possible mechanisms can be offered. First, the standard form of the IL-23R gene is coded by 12 exons; it has been shown that there are at least six isoforms of IL-23R premises that can be produced through alternative splicing. Different variants embellished with premature end led to the creation of the ectodomain receptors or through frameshift create different lengths of endo domain IL-23R. In addition, intronic polymorphisms such as G/A-43045 may apply their influence through differential splicing regulation. Also, polymorphism C/A-97952 may lead to overexpression of the receptors by increasing mRNA stability and stimulating T cell differentiation into Th17 cells and thus leads to inflammation by increased expression of cytokines(7, 43).

In the present study three polymorphisms, namely G/A-43045, G/A-78790, and C/A-97952 were chosen. Among various studies conducted, some researchers reported the association of these polymorphisms with IBD but others did not confirm it.

In Tremelling's study in G/A-78790, no significant association was observed on age at onset of CD, disease location, and disease behavior. In patients with CD affecting the colon only without small bowel disease appeared to be as strongly associated as those with exclusively ileal A allele frequencies. The age at disease onset ranged from 12 to 67 years in patients with CD who carried the A allele and from 0 to 80 years in wild-type GG cases. For UC, subgroup analysis by disease extent, smoking history, and sex also revealed no significant subgroup association. Age at onset of UC ranged from 14 to 79 years in cases that carried the A allele and from 2 to 81 years in wild-type GG cases (36).

Glas et al, observed that there is a significant relationship between polymorphism G/A-43045 and Crohn's disease. Also, SNP G/A-78790 was shown as a protection for Crohn's and weak susceptibility association for UC (28). Buning et al studied the G allele frequency in polymorphisms IL23R G/A-78790 (p.Arg381Gln) on (318 CD and 178 UC in German individuals) and (G/A-43045 CD and 118 UC Hungarian) (157 CD Dutch) 845 patients as controls. It was observed that G allele frequency was significantly low in all three patients' nations (was similar For CD and UC) (as ga roup, P<0.000001 and individually for Hungary P = 0.02 and for Germany P=0.001 and for Netherlands P=0.0002). Baptista et al (2008) investigated three variants (C/A-97952, G/A-43045, and G/A-78790) IL-23R was obtained for G/A-43045 (P=0.013) and C/A-97952 (P=0.036) which showed C/A-97952 and G/A-43045 can be associated with Crohn as risk alleles in IL-23R gene, and for G/A-78790 (P=0.009) which indicates that G/A-78790 can be associated with Crohn as G alleles protect in IL-23R gene (44).

Okazaki et al. investigated 315 CD patients and 118 UC patients and 315 healthy individuals for the IL23R G/A-78790 variant. In this study, G/A-78790 variants inversely were associated with this CD and UC, and this allele was reported as a protective factor. Also, research was carried out for IL23R C/A-97952 variants. In this study, a variant of C/A-97952 was strongly associated with CD (33).

Latiano studied polymorphism in IL23R G/A-78790 (p.Arg381Gln). After genotyping and statistical analysis of both groups, it was observed that allele A (Arg381Gln) related to IL23R is significantly lower in healthy subjects compared to patients. Allele A was reduced by 6% in patients and 38% in healthy subjects and the G allele significantly was reduced in UC patients. Gazouli showed that IL-23R (G/A-78790) (R381Q) in children and adults is less than in the control subjects (P=0.04, P = 0.0018)(31).

Lacher et al. (2010) investigated polymorphism IL23R G/A-78790 (Arg381Gln) in 221 CD patients, 132 UC patients, and 253 control groups using the TaqMan method. In this study, A allele variant was not found in UC patients And CD patients had a low incidence compared to the control group: 1.8 % vs. 7.1%, P<0.01 (41).

Hayatbakhsh et al. (2012) investigated polymorphism IL-23R (G/A-43045) in the Kerman province of Iran. In this study, it was indicated that there is no meaningful relationship between this polymorphism and UC (40).

Safrany studied polymorphisms IL23R C/A-97952 gene. The results stated that homozygous variant C/A-97952 can be significantly associated with Crohn's (45).

In Ferguson's study, by comparing polymorphisms G/A-78790 and C/A-97952 with inflammatory bowel disease phenotype it was observed that There is a significant relationship between polymorphisms G/A 78790 and influence and Ileocolic but no significant relationship was observed between polymorphism C/A-97952 and any of the disease phenotypes (46).

None of the SNPs (G/A-78790, C/A-97952, and G/A-43045) tested in Okazaki's study showed differences in genotype or carrier frequencies for comparisons between CD patients with a history of complications of internal fistulas

and/or strictures and CD patients with only inflammation (33).

In our study by comparing inflammatory bowel disease phenotype and studied polymorphisms it was observed that there is a significant association between the G/A-78790 GG genotype and Ileocolic and inflammatory behavior in CD patients. And it was observed, there is a significant relationship between polymorphism C/A-97952 AA genotype and the disease's behavior, in terms of penetrating the tissue. Likewise, by comparing the disease phenotype and polymorphism G/A-43045, the AA genotype and Ileocolic are statistically significant.

And finally, by Comparing the Allele frequency and behavior, and phenotype of ulcerative colitis disease it was observed that there is a significant relationship between Polymorphism G/A-43045 AA genotype and Pan Colitis

This study is the first study in Iran's Khuzestan population on three receptor polymorphisms on interleukin-23 and investigates the relationship between genotypes and phenotypes and different clinical manifestations. Judgment in this study needs collecting more samples of patients which can be performed in other provinces of Iran.

The Significant difference in different parts of the world can be a confirmation of the assumption that the epidemiology of IBD differs in different regions, and pathogenesis behavior may also differ. Likely the Genetic differences around the world can be considered as an important factor in the interpretation of information in our study compared to other studies. It is also possible to achieve more accurate results by increasing the number of data. In this study, we do not exclude IL-23R gene associations reported formerly with CD and UC. Because Iran country is composed of several ethnicities, further studies are recommended in larger sample sizes from different ethnicities are required to evaluate the role of the IL-23 polymorphisms in the Iranian population. According to different studies around the world and by observing the significant relationship of interleukin 23 receptor polymorphisms and inflammatory bowel disease, and by studying other polymorphisms in this gene signaling pathway such as JAK2 and STAT3, finding a stronger association to explain the disease in the future, whose results can be used to confirm the association of polymorphisms with inflammatory bowel disease, as an important factor in making a diagnosis of the disease and thus treatment.

Conclusion

Briefly, the current study suggests that investigated polymorphisms do not play a significant role in inflammatory bowel disease in the Khuzestan Province population in Iran. Consequently, it is essential to conduct studies with larger sample sizes and evaluate other polymorphisms associated with immunopathological pathways in inflammatory bowel disease.

Nevertheless, in this investigation of the relationship between patients' phenotypes and polymorphisms, some results proved that there are some significant association between phenotypes and genotypes. Polymorphism G/A-43045 was more frequently observed in ileocolic Crohn's disease and pancolitis ulcerative colitis which can play a role in the inflammation process in patients with inflammatory bowel disease. Moreover, a significant relationship was observed between polymorphism G/A-78790 of the GG genotype and ileocolic and inflammatory Crohn's disease. Note that Crohn's disease patients with polymorphism C/A-97952 may experience more tissue penetration of the disease.

Conflict of interest

The authors declare no conflict of interest.

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