Original Article

Association of Plasminogen Activator Inhibitor-1 Gene Polymorphism with Inflammatory Bowel Disease in Iranian Azeri Turkish Patients

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ABSTRACT

Background/Aim: Previous studies have shown the association of some genetic factors, such as Plasminogen activator inhibitor type-1 (PAI-1) 4G/5G polymorphism, with the development of inflammatory bowel disease (IBD). We aimed to study this polymorphism as a risk factor in IBD patients in this cohort. **Patients and Methods:** One hundred and fifteen IBD patients and 95 healthy controls were selected from Iranian Azeri Turks and -6754G/5G polymorphism of PAI-1 gene was tested by polymerase chain reaction using allele-specific primers confirmed by sequencing. **Results:** There was no significant difference of PAI-1 polymorphism between IBD patients and the control group ($P > 0.05$). Furthermore, these data showed no significant difference between Crohn's disease and ulcerative colitis patients. However, 4G/4G homozygotes have reduced probability to progression of loss of appetite, whereas 5G/5G genotypes have increased risk for development of chronic diarrhea without blood, nausea, and loss of appetite. **Conclusions:** Although our study showed no significant association of PAI-1 polymorphism between patients and control group, the carriers of 4G/4G genotype and 4G allele had reduced risk for the progression of IBD features in this cohort.

Key Words: Inflammatory bowel disease, Iranian Azeri Turks, plasminogen activator inhibitor 1, 4G/5G polymorphism

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Inflammatory bowel disease (IBD) is a chronic remittent immune disorder, which is divided into two major clinically defined forms, ulcerative colitis (UC) and Crohn's disease (CD).[1] CD may affect the entire gastrointestinal tract, whereas UC affects the colon.[2] IBD is characterized by diarrhea, fever, cramping, abdominal pain, rectal bleeding, weight loss, and nausea. IBD is a heterogeneous disorder of multifactorial etiology in which represents the clinical effect of the three interactive factors: genetic mutations, environmental elements, and immune dysregulation.[3] Immunomodulatory drugs such as infliximab can be used for treatment of this disease. Infliximab is an anti-TNF-α monoclonal antibody that has been studied in CD and UC.[4] Some factors have been associated with IBD, including IL-1, IL-6, IL-8, TNF-α, and PAI-1.[4]

Plasminogen activator inhibitor type-1 (PAI-1) is a glycoprotein of serine protease inhibitors (*SERPINs*) superfamily.[5] PAI-1 is a key inhibitor of fibrinolytic system by inactivating tissue-type and urokinase-type plasminogen activators, as well as it is involved in the regulation of cell migration, invasion, and adhesion.[6]

The human PAI-1 gene is located on the long arm of chromosome 7 and is composed of nine exons and eight introns.[7] A single nucleotide insertion/deletion (-6754G/5G) polymorphism has been detected within this gene.[8] The 4G/4G genotype is associated with an overexpression of PAI-1 compared with 5G/5G genotype because both 4G and 5G alleles can bind a transcriptional activator, whereas the 5G allele also binds a repressor protein at this site and eventuating in lower transcription of the PAI-1 gene.[6] Several studies showed that 4G/4G genotype is associated

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with a decreased fibrinolysis, therefore, the development of vascular complications in IBD patients.[9]

The aim of this study is to assess the role of PAI-1 gene 4G/5G polymorphism in patients with IBD and association of this polymorphism with severity of IBD complications in Azeri Turkish ethnic group from North West of Iran.

PATIENTS AND METHODS

In this study 115 IBD patients and 95 healthy, unrelated, age- and gender-matched individuals as a control group were selected from Iranian Azeri Turks. All samples were sent by physicians from various medical specialties, including internal medicine and gastroenterologists. Selection and diagnosis of disease was made according to clinical criteria of IBD.[10] A standard questionnaire was designed, including demographics, family history of IBD, and the presence of IBD symptoms. Written informed consent was received from all of the participants and DNA was extracted from white blood cells by a salting-out method. 4G/5G polymorphism of PAI-1 gene was tested by the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique using an upstream control primer (5′-AAGCTTTTACCATGGTAACCCCTGGT-3′), a 4G or 5G allele-specific primer (5′-AGAGTCTGGACACGTGGGGA-3′ and 5′-AGAGTCTGGACACGTGGGGG-3′, respectively), and a common downstream primer (5′-TGCAGCCAGCCACGTGATTGTCTAG-3′). 138-or 139-bp fragments for, respectively, 4G and 5G alleles at an annealing temperature of 55°C and also 257-bp fragment for positive control were obtained from amplification by these primers. The conditions for the PCR reaction were denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 10 s, and extension at 72°C for 20 s, followed by a final extension at 72°C for 3 min. The PCR products were fractionated by 2% agarose-gel electrophoresis and visualized under UV light. A number of samples also were also confirmed by sequencing method using 5′-GATTGGCGCTCAGGCACAT-3′ (forward) and 5′-GGCTCCGTGGGCACAGTAAC-3′ (reverse) primers.

In this study the association of PAI-1 4G/5G polymorphism was performed between different groups using Chi-square and Fisher's exact tests. All statistical analyses were calculated with SPSS for Windows 16.0. The Fisher's exact test was used to test for departure from Hardy–Weinberg equilibrium of the genotype frequencies ($P > 0.05$). The odds ratios (OR) and confidence intervals (CI) at the 95% significance level were calculated for all data. P values less than 0.05 were regarded as significant. Furthermore, each clinical manifestation of IBD was tested with regard to PAI-1 4G/5G polymorphism and significance of the differences of the alleles and genotypes between patients with/without a specific clinical symptom were examined using Chi-square and Fisher's exact tests.

RESULTS

In this study the PAI-1 -675 4G/5G polymorphism was investigated in 115 IBD patients with a mean age onset of 27.27 years and in 95 healthy controls. The study included 59 males (51.3%) and 56 females (48.69%) for patient group and 53 males (55.78%) and 42 females (44.21%) for control group. The frequency of clinical symptoms of IBD in these patients was 44.34% (51/115) rectal bleeding, 35.96% (41/114) bloody diarrhea, 15.65% (18/115) chronic diarrhea without blood, 50.43% (58/115) abdominal cramps and pain, 18.26% (21/115) fever, 13.04% (15/115) nausea, 31.3% (36/115) loss of appetite, 36.52% (42/115) weight loss, 5.21% (6/115) severe urgency to have a bowel movement, and 20.37% (22/108) arthritis.

Twenty samples of both control and patient groups confirmed using sequencing method. There was no significant difference between IBD patients and control group [Table 1]. Also, no significant difference was seen in both genotypic and allelic distributions of PAI-1 polymorphism in comparison between IBD subgroups (CD and UC) [Table 2]. In addition, the clinical symptoms of

Table 1: Genotypic and allelic distribution of PAI-1 between patients and control group

PAI-1 genotypes and alleles	IBD patients (*n*=115)		Control group (*n*=95)		OR (95% CI)	*P* value
	n	*F*	*n*	*F*		
4G/4G	27	0.2347	23	0.2421	0.96 (0.47-1.93)	0.455
4G/5G	48	0.4173	46	0.4842	0.763 (0.42-1.38)	0.155
5G/5G	40	0.3478	26	0.2736	1.41 (0.74-2.7)	0.147
4G	102	0.4434	92	0.4842	0.84 (0.46-1.53)	0.297
5G	128	0.5565	98	0.5157	1.17 (0.65-2.13)	0.275

IBD: Inflammatory bowel disease, OR: Odds ratio, CI: Confidence interval, PAI: Plasminogen activator inhibitor

Table 2: Genotypic and allelic distribution of PAI-1 gene polymorphism between CD and UC groups

PAI-1 genotypes and alleles	CD group (*n*=19)		UC group (*n*=96)		OR (95% CI)	*P* value
	n	*F*	*n*	*F*		
4G/4G	5	0.2631	22	0.2291	1.2 (0.6-2.4)	0.267
4G/5G	7	0.3684	41	0.427	0.78 (0.42-1.43)	0.172
5G/5G	7	0.3684	33	0.3437	1.11 (0.59-2.07)	0.396
4G	17	0.4473	85	0.4427	1.01 (0.56-1.85)	0.5
5G	21	0.5526	107	0.5572	0.98 (0.54-1.78)	0.499

CD: Crohn's disease, UC: Ulcerative colitis, OR: Odds ratio, CI: Confidence interval, PAI: Plasminogen activator inhibitor

Table 3: Genotypic and allelic differences of the PAI-1 4G/5G polymorphism between IBD patients with/without symptoms

Symptoms	4G/4G	4G/5G	5G/5G	4G	5G
Rectal bleeding (*n*=115)					
With	13 (0.2549)	14 (0.2745)	24 (0.4705)	0.3921	0.4218
Without	14 (0.2187)	26 (0.4062)	24 (0.375)	0.6078	0.5781
OR	1.22	0.553	1.48	0.884	1.31
P value	0.547	0.049	0.172	0.669	0.669
Bloody diarrhea (*n*=114)					
With	9 (0.2195)	16 (0.3902)	16 (0.3902)	0.4146	0.5853
Without	13 (0.178)	29 (0.3972)	31 (0.4246)	0.3767	0.6232
OR	1.29	0.97	0.86	1.7	0.85
P value	0.462	0.919	0.621	0.584	0.584
Chronic diarrhea without blood (*n*=115)					
With	3 (0.1666)	2 (0.1111)	13 (0.7222)	0.2222	0.7777
Without	24 (0.2474)	38 (0.3917)	35 (0.3608)	0.4432	0.5567
OR	0.62	0.194	4.6	0.35	2.78
P value	0.08	0.000	0.000	0.001	0.001
Abdominal cramps and pain (*n*=115)					
With	18 (0.3103)	19 (0.3275)	21 (0.3620)	0.4741	0.5258
Without	11 (0.1929)	20 (0.3508)	26 (0.4561)	0.3684	0.6315
OR	1.88	0.9	0.67	1.54	0.64
P value	0.056	0.728	0.176	0.13	0.13
Fever (*n*=115)					
With	6 (0.2857)	8 (0.3809)	7 (0.3333)	0.4761	0.5238
Without	21 (0.2234)	32 (0.3404)	41 (0.4361)	0.3936	0.6063
OR	1.39	1.19	0.64	1.4	0.71
P value	0.312	0.551	0.135	0.239	0.239
Nausea (*n*=115)					
With	3 (0.2)	4 (0.2666)	8 (0.5333)	0.3333	0.6666
Without	24 (0.24)	36 (0.36)	40 (0.4)	0.42	0.58
OR	0.79	0.64	1.71	0.69	1.44
P value	0.304	0.07	0.026	0.206	0.206
Loss of appetite (*n*=115)					
With	6 (0.1666)	12 (0.3333)	18 (0.5)	0.3333	0.6666
Without	21 (0.2658)	29 (0.367)	29 (0.367)	0.4493	0.5506
OR	0.55	0.86	1.72	0.62	1.63
P value	0.039	0.618	0.039	0.111	0.093
Weight loss (*n*=115)					
With	8 (0.1904)	14 (0.3333)	20 (0.4761)	0.3571	0.6428
Without	19 (0.2602)	26 (0.3561)	28 (0.3835)	0.4383	0.5616
OR	0.66	0.904	1.46	0.71	1.4
P value	0.237	0.736	0.186	0.241	0.241
Severe urgency to have a bowel movement (*n*=115)					
With	2 (0.3333)	2 (0.3333)	2 (0.3333)	0.5	0.5
Without	25 (0.2293)	38 (0.3486)	46 (0.422)	0.4036	0.5963
OR	1.68	0.93	0.68	1.47	0.67
P value	0.083	0.43	0.107	0.171	0.171
Arthritis (*n*=108)					
With	7 (0.3181)	6 (0.2727)	9 (0.409)	0.4545	0.5454
Without	20 (0. 2325)	32 (0.372)	34 (0.3953)	0.4186	0.5813
OR	1.54	0.63	1.05	1.15	0.86
P value	0.175	0.133	0.843	0.609	0.609

IBD: Inflammatory bowel disease, OR: Odds ratio, CI: Confidence interval, PAI: Plasminogen activator inhibitor, : *P*<0.05 (statistically significant)

IBD with regard to PAI-1 4G/5G polymorphism were studied. These results showed a significant difference between patients with/without rectal bleeding (4G/5G; $P = 0.049$), chronic diarrhea without blood (4G/5G; $P = 0.0001$, 5G/5G; $P = 0.0001$, 4G $P = 0.001$ and 5G $P = 0.001$), nausea (5G/5G; $P = 0.026$), and loss of appetite (4G/4G; $P = 0.039$, 5G/5G; $P = 0.039$) [Table 3].

This study suggests a protective role for the 4G allele, 4G/4G and 4G/5G genotypes. 5G/5G genotype and 5G allele probably are risk factors in IBD patients from Iranian Azeri Turks in this cohort.

DISCUSSION

PAI-1 is the key inhibitor of fibrinolysis. 4G/5G polymorphism of PAI-1 gene promoter related to altered plasma levels of PAI-1 protein by affecting on binding of the transcription-regulating proteins. The 4G/4G genotype of this polymorphism is related to a decreased fibrinolysis and, therefore, a progression of vascular complications in IBD patients by an overexpression of PAI-1 gene. It is reasonable to assume that PAI-1 gene may be as a modifier gene and 4G/5G polymorphism possibly will associate with the progression of IBD and its complications.

In this study no significant association was observed between IBD patients and controls from Iranian Azeri Turk ethnic group. In comparison between two subgroups of IBD patients (CD and UC) with respect to allelic and genotypic distribution of PAI-1 gene polymorphism, no significant difference was observed between these two subgroups. In addition, all declared symptoms of IBD patients were studied regarding PAI-1 4G/5G polymorphism for investigation of the PAI-1 effect on the severity of the disease. Therefore, we compared allelic and genotypic frequencies of PAI-1 gene polymorphism between patients with/without IBD complications. Rectal bleeding (4G/5G; $P = 0.049$), chronic diarrhea without blood (4G/5G; $P = 0.0001$, 5G/5G; $P = 0.0001$, 4G; $P = 0.001$, and 5G; $P = 0.001$), nausea (5G/5G; $P = 0.026$) and loss of appetite (4G/4G; $P = 0.039$, 5G/5G; $P = 0.039$) differed significantly between patients with/without these features.

Consequently, our data suggest that IBD patients with 4G/4G genotype have reduced sensitivity to loss of appetite and 4G/5G heterozygote individuals show lower sensitivity to rectal bleeding and chronic diarrhea without blood, whereas 5G/5G homozygotes are more susceptible to chronic diarrhea without blood, nausea, and loss of appetite. Therefore, 5G/5G versus 4G/4G homozygotes are at a greater risk for IBD phenotypes. Furthermore, 5G versus 4G allele probably is a risk factor against IBD complications in Iranian Azeri Turkish population. This is the first study showing an association of PAI-1 gene polymorphism with IBD in Azeri Turk population from North West of Iran.

The association of PAI-1 gene polymorphism with several diseases such as Familial Mediterranean Fever,[11] Type 2 Diabetes,[12] Acute Myocardial Infarction,[13] and Coronary Artery Disease[14] has been investigated. As indicated in some previous studies, 4G/4G genotype or 4G allele may be a risk factor for development of disease,[12-14] whereas others proposed a protective role for 4G/4G genotype or 4G allele against symptoms.[15-17] Also, in our previous study we showed that FMF patients carrying 5G/5G genotype are more susceptible to the progression of disease symptoms.[11]

Several studies have investigated the role of PAI-1 in the development of IBD. Some of these evaluations indicated that subjects with 4G/4G genotype are at a higher risk for development of Crohn's disease,[18,19] and have high concentrations of PAI-1 protein,[20] whereas Souto *et al.* showed that PAI-1 levels in IBD patients were clearly lower than in the controls.[21]

The genotypic frequency of PAI-1 varies among races and ethnic groups. Based on the published data, the prevalence of the 4G/4G genotypes is approximately 26.7% in Spanish population[22] and 26.3% in white population.[23] The prevalence of this polymorphism in our cohort (4G/4G was 23.47%) is in agreement with the literature data. The data in our present study confirm the findings of our previous reports that PAI-1 5G/5G versus 4G/4G homozygotes are at an increased risk for IBD symptoms.[11] In conclusion, these data suggest a protective role for 4G versus 5G allele and 4G/4G versus 5G/5G genotype against IBD complications in the studied cohort. Also, subjects who carry one or more 5G alleles are more susceptible to IBD symptoms.

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