Eur J Pediatr
DOI 10.1007/s00431-012-1844-z

ORIGINAL ARTICLE

Plasminogen activator inhibitor-1 gene polymorphism in Iranian Azeri Turkish patients with FMF disease and its association with amyloidosis

M. Bonyadi · Z. Shaghaghi · M. Haghi · S. Dastgiri

Received: 24 April 2012 / Revised: 11 September 2012 / Accepted: 17 September 2012

Abstract Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by intermittent episodes of fever with serositis, arthritis, or eriseplemya. Plasminogen activator inhibitor 1 (*PAI-1*) is a key element in the inhibition of fibrinolysis by inactivating tissue-type and urokinase-type plasminogen activators. We evaluated the association of *PAI-1* -675 4G/5G polymorphism with the severity of FMF disease. For this purpose, 89 FMF patients with M694V homozygous mutation and 95 healthy controls from Iranian Azeri Turks were selected. Detection of this polymorphism was performed by polymerase chain reaction using allele-specific primers. No significant association was found between patients and control group. However, these data showed that FMF patients with M694V homozygous mutation carrying 4G/4G genotype have a reduced risk for development of pleuritis (odds ratios (OR) 0.36; 95 % confidence intervals (CI) 0.5–0.85; *P* value= 0.007) compared with 5G/5G homozygotes who have increased risk for development of amyloidosis (OR=2.46; 95 %CI=1.29–4.72; *P* value=0.001), pleuritis (OR=2.55; 95 %CI=1.31–4.99; *P* value=0.001), and fever (OR=4.68; 95 %CI=2.04–10.96; *P* value=0.000). Furthermore, the allelic frequency of the 4G among the patients with pleuritis was significantly low (OR=0.5, 95 % CI=0.27–0.92, *P* value= 0.008). *Conclusion* Our data suggest a protective role for the 4G allele against pleuritis in FMF patients with M694V homozygous mutation in this cohort. More evaluation of this polymorphism may be important and require further studies.

Keywords Familial Mediterranean fever · Plasminogen activator inhibitor 1 4G/5G Polymorphism · Amyloidosis · Iranian Azeri Turks

Introduction

Familial Mediterranean fever (FMF) (MIM 249100) is an autosomal recessive autoinflammatory disorder characterized by painful episodes of fever with serositis, arthritis, or erysipelas-like skin lesions and may be complicated by amyloidosis [32]. The disease mostly occurs in Armenians, Sephardic Jews, North African, Turks, and Arabs [2, 4]. Amyloidosis due to chronic inflammation leading to renal failure is one of the most severe complications of this disease [24, 32]. During FMF attacks, not only a developed acute-phase response occurs but also high levels of inflammatory mediators like tumor necrosis factor–alpha, interleukin-6, and serum amyloid-A can be found [22, 23, 29]. The gene responsible for FMF, *MEFV*, is located on chromosome 16p13.3 and composed of 10 exons which encode a protein, called pyrin or marenostrin [39, 42]. Pyrin is expressed mainly in neutrophils and myeloid cell lineage [2, 9] and suppresses inflammatory process by inactivating the immune response; without this control, an inflammatory reaction occurs [39, 42]. Different mutations have been identified in the *MEFV* gene and the most frequent causative mutations are V726A, M694V,

M. Bonyadi · Z. Shaghaghi · M. Haghi
Center of Excellence for Biodiversity, Faculty of Natural Sciences,
University of Tabriz,
Tabriz, Iran

M. Bonyadi (✉) · Z. Shaghaghi
Liver and Gastrointestinal Disease Research Center,
Tabriz University of Medical Sciences,
Tabriz, Iran
e-mail: jabbarpour@tabrizu.ac.ir

S. Dastgiri
Department of Community and Family Medicine,
School of Medicine, National Public Health Management Center,
Tabriz University of Medical Sciences,
Tabriz, Iran

Published online: 05 October 2012

M694I, M680I, and E148Q [7, 17]. Phenotype–genotype correlation of the disease shows that M694V mutation is a significant factor in predicting the development of amyloidosis [3, 4]. Therefore, patients who are homozygous for M694V mutation have severe FMF disease and a greater tendency to develop amyloidosis [8, 36].

Plasminogen activator inhibitor type-1 (*PAI-1*) is a glycoprotein with a molecular weight of approximately 50 kDa [18, 21]. *PAI-1* is member of a superfamily of serine protease inhibitors and is the major plasminogen activator inhibitor in humans [6]. It is synthesized by the vascular endothelium and also exists in platelets [37]. Hormones, cytokines, and growth factors can induce *PAI-1* production [6, 12, 28]. *PAI-1* is a key element in the inhibition of fibrinolysis by inactivating tissue-type and urokinase-type plasminogen activators [18, 27, 35, 40, 41]. The *PAI-1* not only inhibits the fibrinolytic system, but it is also involved in regulation of cell migration, invasion, and adhesion during the inflammatory process [1].

The human *PAI-1* gene is located on the long arm of chromosome 7 and includes nine exons and eight introns [38]. Various polymorphisms have been studied within the gene, including a HindIII restriction fragment length polymorphism, a cytosine–adenine (CA) dinucleotide repeat and a single nucleotide insertion/deletion (4G/5G) polymorphism 675 bp upstream of the transcriptional start site in the promoter [10, 11]. Subjects with the 4G/4G genotype have significantly higher *PAI-1* concentrations than those with the 4G/5G or 5G/5G genotype [15] because both 4G and 5G alleles can bind a transcriptional activator, while the 5G allele also binds a repressor protein at this site and resulting in lower transcription of the *PAI-1* gene [27]. There are numerous *PAI-1* antagonists like angiotensin-converting enzyme inhibitors and insulin-sensitizing agents that decrease plasma levels of *PAI-1*. These drugs can use for some disorders such as amyloidosis [31]. The association of *PAI-1* 4G/5G polymorphism with susceptibility to several disorders like coronary artery disease [25], acute myocardial infarction [20], type 2 diabetes [30], and other diseases like Buerger's disease [28] has been shown.

We hypothesized that *PAI-1* 4G/5G polymorphism may have an effect on the severity of FMF and it may be an important risk factor for having amyloidosis in the presence of M694V homozygous mutation. Therefore, the aim of this study was to investigate the effect of this polymorphism on the severity of FMF disease and progression of amyloidosis in FMF patients with M694V homozygous mutation of Azeri Turkish ethnic group from North West of Iran.

Materials and methods

This case–control study included 89 FMF patients with M694V homozygous mutation and 95 healthy, unrelated, age- and sex-matched individuals as a control group. All samples were tested for the M694V mutation by using amplification refractory mutation system (ARMS) PCR [14, 16] and confirmed by sequencing method (Sanger method) on only 15 patients and 10 control subjects using 5′-GATT GGCGCTCAGGCACAT-3′ and 5′-GGCTCCGTGGGC ACAGTAAC-3′ primers (forward and reverse, respectively). All subjects were selected from Iranian Azeri Turkish population, and clinical diagnosis was made according to criteria of familial Mediterranean fever [26].

Informed written consent was obtained from all subjects, and genomic DNA was extracted by a salting-out method from peripheral white blood cells. Detection of −675 4G/5G polymorphism of *PAI-1* gene promoter was carried out by the ARMS-PCR assay using an upstream control primer (5′-AAGCTTTTACCATGGTAACCCCTGGT-3′), a 4G or 5G allele-specific primer (5′-AGAGTCTGGACAC GTGGGGA-3′ or 5′-AGAGTCTGGACACGTGGGGG-3, respectively), and a common downstream primer (5′-TGCAGCCAGCCACGTGATTGTCTAG-3′). These primers amplify 138-bp fragment for 5G or 4G alleles at an annealing temperature of 55 °C and also 256-bp fragment for positive control. The PCR amplification started with DNA denaturing for 3 min at 95 °C, followed by 30 cycles each consisting of 20 s at 95 °C, 10 s at 55 °C, and 20 s at 72 °C, and final extension was one cycle of 3 min at 72 °C. Amplified products were separated by electrophoresis in 1.5 % agarose gels and visualized using ethidium bromide. Subjects were classified into 4G/4G, 4G/5G, or 5G/5G according to the presence of the 138 bp fragment produced by the two allele-specific primers. Furthermore, the sequencing method was used for confirming the samples. For this purpose, 20 samples of each patient and control groups were selected and promoter region of the PAI-1 gene (675 4G/5G) was amplified using the following primers: 5′-AAGCTTTTACCATGGTAACCCCTGGT-3′ and 5′-TGCAGCCAGCCACGTGATTGTCTAG-3′ (forward and reverse primers, respectively) and sequencing of these PCR products were performed by the method of Sanger.

We analyzed the association of *PAI-1* 4G/5G polymorphism between FMF patients and healthy controls using chi square and Fisher exact test. The SPSS software (version 16.0), VassarStat, and JavaStat websites (statpages.org/ctab2x2.html) were used for all of the genetic data analyses. The distribution of PAI-1 *4G/5G* genotypes for deviation from Hardy–Weinberg equilibrium was tested by Fisher's exact test ($p>0.05$). The odds ratios (OR) and confidence intervals (CI) at the 95 % significance level were estimated for all data. *PAI-1* 4G/5G polymorphism frequency was calculated for each clinical manifestation discretely, and the significance of the differences of observed alleles and genotypes

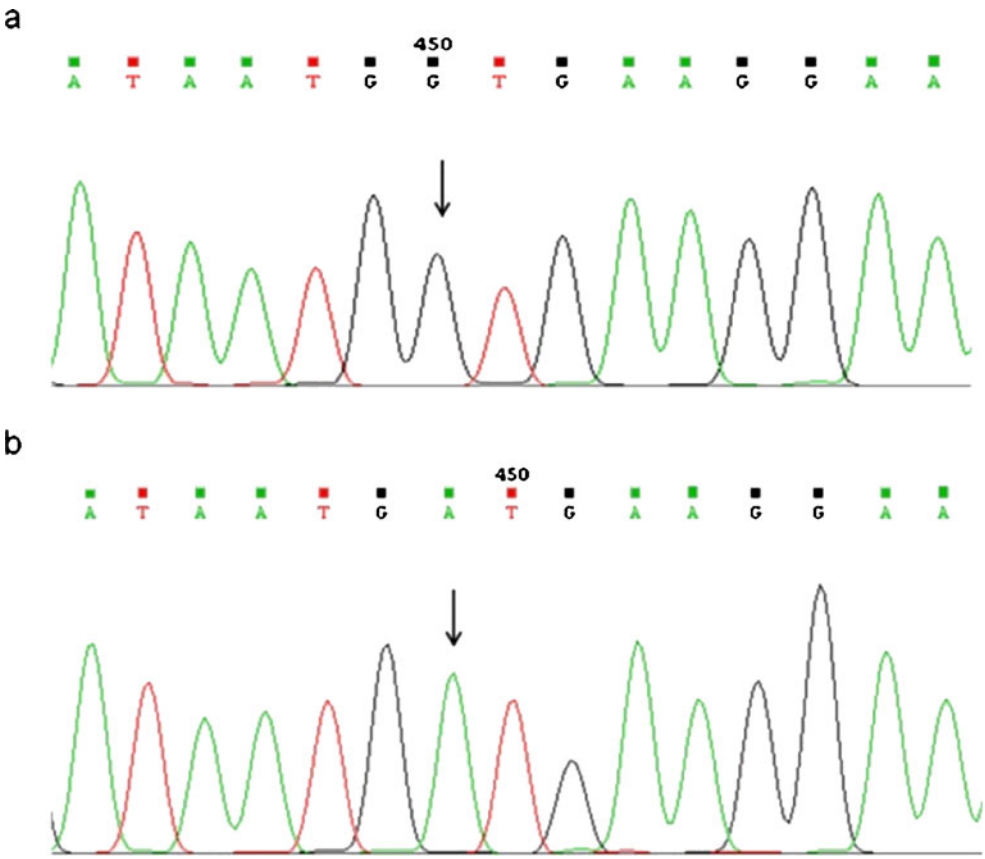

Fig. 1 Sequence results of *MEFV* gene in order to confirm genotype of FMF patients. **a** M694V homozygous genotype; **b** normal genotype

between patients with or without a specific clinical symptom was tested using chi square test and comparing confidence intervals. Probability values less than 0.05 were considered statistically significant.

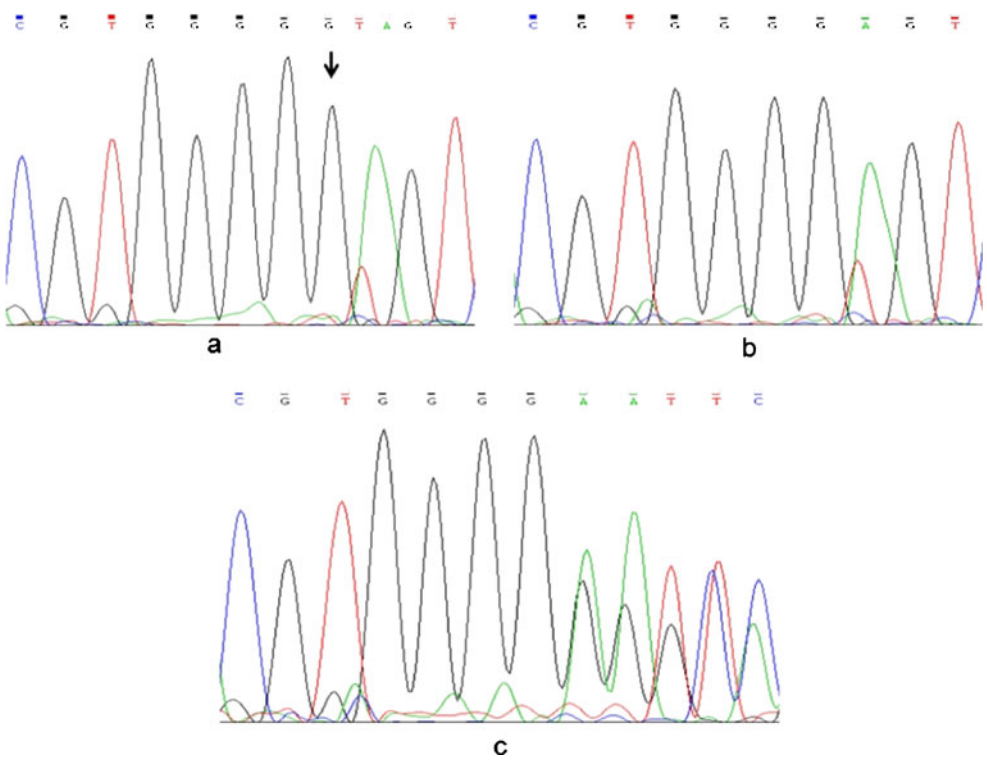

Fig. 2 Sequence result of *PAI-1* gene in order to confirm 4G/5G polymorphism. *a* 5G/5G which *arrow* displays an inserted guanine; *b* 4G/4G; *c* 4G/5G

Table 1 Genotype and allele distribution from wild type to risk groups

Genotypes and Alleles	FMF (*n* 89)		Control (*n*=95)		Odds ratio (95 %CI)	*P* value
	n	*F*	*n*	*F*		
4 G/4 G	15	0.1685	23	0.2421	0.63 (0.29–1.34)	0.539
4 G/5 G	45	0.5056	46	0.4842	1.08 (0.60–1.97)	0.388
5 G/5 G	29	0.3258	26	0.2736	1.343 (0.69–2.58)	0.177
4 G	75	0.4213	92	0.4842	0.77 (0.42–1.4)	0.215
5 G	103	0.5786	98	0.5157	1.28 (0.71–2.34)	0.181

Results

We analyzed allelic and genotypic frequencies of *PAI-1* 4G/5G polymorphism in 89 FMF patients with/without particular symptoms and 95 control individuals. Mean age of first symptoms in our study was 6.9, and the female-to-male ratio was estimated 0.79 (females 42, 44.21 %; males 53, 55.78 %) in controls and 0.85 (females 41, 46.06 %; males 48, 53.93 %) in patients. The frequency of each symptom in FMF patients with M694V homozygous mutation was: 89.4 % (76/85) peritonitis, 88 % (73/83) fever, 48 % (39/81) pleuritis, 49 % (41/83) arthritis, 27 % (20/74) myalgia, 35 % (27/76) amyloidosis, and 19 % (13/67) erysipelas-like erythema.

Twenty samples of each patient and control groups for 4G/5G genotype and also 15 patients and 10 control group genotypes for M964V mutation were confirmed using sequencing method (Figs. 1 and 2). Genotypic distribution and allelic frequencies of *PAI-1* 4G/5G polymorphism showed no significant difference between FMF patients and control individuals in this cohort (Table 1). A comparison between patients with/without specific features regarding *PAI-1* 4G/5G genotypes and alleles were performed. Results revealed a significant genotypic difference between patients with/without amyloidosis (4G/5G, *P* value=0.008; 5G/5G, *P* value=0.001), pleuritis (5G/5G, *P* value=0.001), fever (4G/5G, *P* value=0.000; 5G/5G, *P* value=0.000), and eriseplemya (4G/5G, *P* value=0.027) (Tables 2 and 3); in addition, a significant difference was seen in the allelic frequencies between patients with/without pleuritis (4G, *P* value=0.008; 5G, *P* value=0.007) (Table 3). Our data suggest a protective role for the 4G allele against pleuritis in FMF patients with M694V mutation from Iranian Azeri Turkish ethnic group.

Furthermore, the allelic and genotypic analysis of this polymorphism between controls and patients with/without symptoms were evaluated separately, that there was significant difference in genotype distribution and allelic frequency among some groups (Table 4).

Discussion

Familial Mediterranean fever is an autosomal recessive autoinflammatory disorder characterized by intermittent episodes of fever with serositis, arthritis, or erysipelas-like skin lesions. Genetic and non-genetic risk factors can be

Table 2 Genotype and allele distribution of *PAI-1* 4G/5G polymorphism between FMF patients with/without specific features

Features		4G/4G	4G/5G	5G/5G	4G	5G
Fever (*n*=83) *	With	13 (0.1780)	35 (0.7494)	25 (0.3424)	0.4178	0.5821
	Without	1 (0.1)	8(0.8)	1(0.1)	0.5	0.5
Pleuritis (*n*=81) **	With	4 (0.1025)	19 (0.4871)	16 (0.4102)	0.3461	0.6538
	Without	10 (0.2380)	23 (0.5476)	9 (0.2142)	0.5119	0.4880
Amyloidosis(*n*=83) *	With	4 (0.1481)	11 (0.4074)	12 (0.4444)	0.3518	0.6481
	Without	9 (0.1836)	28 (0.5714)	12 (0.2448)	0.4693	0.5306
Arthritis(*n*=83)	With	6 (0.1463)	24 (0.5853)	11 (0.2682)	0.4390	0.5609
	Without	8 (0.1904)	21 (0.5)	13 (0.3095)	0.4404	0.5595
Peritonitis(*n*=85)	With	13 (0.1710)	40 (0.5263)	23 (0.3026)	0.4342	0.5657
	Without	1 (0.1111)	5 (0.5555)	3 (0.3333)	0.3888	0.6111
Myalgia(*n*=74)	With	4 (0.2)	10 (0.5)	6 (0.3)	0.45	0.55
	Without	9 (0.1666)	30 (0.5555)	15 (0.2777)	0.4444	0.5555
Eriseplemya(*n*=67) *	With	3 (0.2307)	6 (0.4615)	4 (0.3076)	0.4615	0.5384
	Without	9 (0.1666)	32 (0.5925)	13 (0.2407)	0.4629	0.5370

*$P<0.05$ (in genotypes); **$P<0.05$ (in genotypes and alleles)

Table 3 Genotype and allele distribution of *PAI-1* 4G/5G polymorphism between patients with/without amyloidosis, pleuritis, fever and Eriseplemya

Symptoms															
Genotypes and Alleles	Fever (n=83)				Pleuritis (n=81)				Amyloidosis (n=83)				Eriseplemya (n=67)			
	With	Without	OR	*p*	With	Without	OR	*p*	With	Without	OR	*p*	With	Without	OR	*p*
4G/4G	13	1	1.94	0.06	4	10	0.36	0.007*	4	9	0.77	0.214	3	9	1.5	0.09
4G/5G	35	8	0.74	0.246	19	23	0.78	0.217	11	28	0.51	0.008*	6	32	0.58	0.027*
5G/5G	25	1	4.68	0.000*	16	9	2.55	0.001*	12	12	2.46	0.001*	4	13	1.4	0.164
4G	0.4178	0.5	0.71	0.106	0.3461	0.5119	0.5	0.008*	0.3518	0.4693	0.61	0.055	0.4615	0.4629	1.06	0.369
5G	0.5821	0.5	1.39	0.153	0.6538	0.4880	1.98	0.007*	0.6481	0.5306	1.62	0.059	0.5384	0.5370	0.99	0.517

*P value < 0.05

associated with FMF disease. In other words, many modifier genes and environmental factors can affect the FMF phenotypes.

PAI-1 is the main inhibitor of fibrinolysis by inactivating tissue-type and urokinase-type plasminogen activators. Elevated plasma levels of this protein related to a deletion/insertion polymorphism (4G/5G) within the *PAI-1* locus. This polymorphism affects the binding of transcription regulating proteins of *PAI-1* gene. We hypothesized that *PAI-1* may be a modifier gene and possibly affect the development of FMF disease. However, our study revealed no genotypic and allelic association between cases and controls for 4G/5G polymorphism in this cohort. Moreover, we tested all declared symptoms of FMF disease regarding *PAI-1* -675 4G/5G polymorphism to study its function in the severity of disease. For this purpose, we compared the allelic and genotypic frequencies of patients with/without particular symptoms. Among the features, amyloidosis (4G/5G, *P* value=0.008; 5G/5G, *P* value=0.001), pleuritis (4G/4G, *P* value=0.007; 5G/5G, *P* value=0.001), fever (4G/5G, *P* value=0.000; 5G/5G, *P* value=0.000), and eriseplemya (4G/5G, *P* value=0.027) had statistically significant difference between patients with/without mentioned symptoms. These data showed that *PAI-1* 4G/4G homozygotes have a markedly reduced risk for development of pleuritis (OR=0.36; 95 %CI=0.5–0.85; *P* value=0.007) and also 4G/5G genotypes have low risk for development of eriseplemya (*P* value=0.027) and amyloidosis (*P* value=0.000) compared with *PAI-1* 5G/5G homozygotes who have increased risk for development of amyloidosis (OR=2.46; 95 %CI=1.29–4.72; *P* value=0.001), pleuritis (OR=2.55; 95 %CI=1.31–4.99; *P* value=0.001), and fever (OR=4.68; 95 %CI=2.04–10.96; *P* value=0.000). Furthermore, the allelic frequency of the 4G among the patients with pleuritis was low (*P* value=0.008, OR=0.5, 95 %CI=0.27–0.92). Therefore, these data showed that patients with 5G/5G genotype are more susceptible to amyloidosis, pleuritis, and fever, while 4G/4G genotypes show noticeably lower sensitivity to pleuritis, and also 4G/5G genotypes have reduced sensitivity to eriseplemya and amyloidosis. Therefore, 4G allele likely has a protective role against pleuritis in FMF patients with M694V homozygous mutation from Iranian Azeri Turkish population. This is the first study of the *PAI-1* gene polymorphism (−675 4G/5G) in Azeri Turkish FMF patients from North West of Iran.

The association of *PAI-1* 4G/5G polymorphism with susceptibility to several disorders like coronary artery disease, acute myocardial infarction, type 2 diabetes, and other diseases like Buerger's disease has been shown. Some of these studies have revealed that increased *PAI-1* levels are associated with the 4G allele of this polymorphism, and carrying 4G/4G genotype have increased susceptibility to several disorders. Lima et al. reported that patients with coronary

Table 4 Genotype and allele distribution of *PAI-1* 4G/5G polymorphism between controls and patients with/without features

Features		4G/4G	4G/5G	5G/5G	4G	5G
Fever (n=83)	With	13 (0.1780)	35 (0.7494)	25 (0.3424)	0.4178	0.5821
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.67	3.18	1.38	0.76	1.3
	P value	0.112	0.000*	0.128	0.153	0.168
	Without	1 (0.1)	8(0.8)	1 (0.1)	0.5	0.5
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.34	4.26	0.29	1.06	0.93
	P value	0.006*	0.000*	0.001*	0.467	0.42
Pleuritis (n=81)	with	4 (0.1025)	19 (0.4871)	16 (0.4102)	0.3461	0.6538
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.35	1.01	1.84	0.56	1.77
	P value	0.005*	0.507	0.014*	0.022*	0.023*
	without	10 (0.2380)	23 (0.5476)	9 (0.2142)	0.5119	0.4880
	Controls	23(0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.97	1.28	0.72	1.11	0.89
	P value	0.434	0.191	0.171	0.316	0.355
Amyloidosis(n=83)	with	4 (0.1481)	11 (0.4074)	12 (0.4444)	0.3518	0.6481
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.54	0.73	2.12	0.57	1.73
	P value	0.036*	0.123	0.005*	0.035*	0.027*
	without	9 (0.1836)	28 (0.5714)	12 (0.2448)	0.4693	0.5306
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.7	1.42	0.86	0.94	1.06
	P value	0.168	0.113	0.324	0.37	0.4
Arthritis(n=83)	with	6 (0.1463)	24 (0.5853)	11 (0.2682)	0.4390	0.5609
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.53	1.5	0.97	0.9	1.2
	P value	0.039*	0.079	0.426	0.324	0.254
	without	8 (0.1904)	21(0.5)	13 (0.3095)	0.4404	0.5595
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.73	1.06	1.19	0.83	1.19
	P value	0.233	0.46	0.338	0.312	0.261
Peritonitis(n=85)	with	13 (0.1710)	40 (0.5263)	23 (0.3026)	0.4342	0.5657
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.64	1.18	1.15	0.81	1.22
	P value	0.135	0.283	0.299	0.246	0.233
	without	1 (0.1111)	5 (0.5555)	3 (0.3333)	0.3888	0.6111
	Controls	23 (0.2421)	46 (0.4842)	26 (0.2736)	92 (0.4842)	98 (0.5157)
	OR	0.39	1.33	1.32	0.67	1.47
	P value	0.011*	0.162	0.166	0.07	0.084

*$P<0.05$

artery disease carrying 4G/4G genotype had considerably higher plasma *PAI-1* levels compared with 5G/5G genotypes [25]. Isordia-Salas et al. showed that subjects with 4G allele have higher concentrations of PAl-1, which possibly complicated in events cause ST elevation myocardial infarction [20]. Meigs et al. reported that 4G/4G homozygotes having high *PAI-1* levels which increase risk for type 2 diabetes [30]. Demirel et al. measured *PAI-1* levels in FMF patients and showed that there are statistically higher levels of *PAI-1* during the severe attack period compared with the attack-free period [13]. Some studies reported converse association between *PAI-1* 4G/5G polymorphism and severity of complications. Roest et al. reported that *PAI-1* 4G/4G homozygotes had a reduced risk of cerebrovascular mortality

compared with *PAI-1* 5G/5G homozygotes [34]. Bentley et al. showed that 5G/5G genotype relative to 4G/4G is associated with increased stroke risk [5]. Reiner et al. showed that women with *PAI-1* 5G/5G genotype had increased risk of hemorrhagic stroke [33], and Hoekstra et al. also reported a protective effect of the 4G allele against stroke [19].

Therefore, previous studies revealed that 4G/4G genotype is a risk factor in the pathogenesis of some diseases or their clinical manifestations, whereas the results of our study do not support this hypothesis. However, we found a significantly reduced risk for FMF symptoms in *PAI-1* 4G/4G homozygotes compared with *PAI-1* 5G/5G homozygotes. In conclusion, our results suggest a protective role for the 4G allele and 4G/4G genotype against pleuritis and 4G/5G genotype against eriseplemya and amyloidosis. Furthermore, the 5G allele probably is a risk factor for development of pleuritis and 5G/5G genotype for having fever, pleuritis, and amyloidosis in FMF patients with M694V homozygous mutation from Iranian Azeri Turkish ethnic group. More evaluation of this polymorphism may be important and require further studies.

Acknowledgments This study was funded by Center of Excellence for Biodiversity (University of Tabriz) and Center of Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences.

References

1. Ariue Oh CKB, Alban RF, Shaw B, Cho SH (2002) PAI-1 promotes extracellular matrix deposition in the airways of a murine asthma model. Biochem Biophys Res Commun 294:1155–1160
2. Bakkaloglu A (2003) Familial Mediterranean fever. Pediatr Nephrol 18:853–859
3. Balci B, Tinaztepe K, Yilmaz E, Gucer S, Ozen S, Topaloglu R, Besbas N, Ozguc M, Bakkaloglu A (2002) *MEFV* gene mutations in familial Mediterranean fever phenotype II patients with renal amyloidosis in childhood: a retrospective clinicopathological and molecular study. Nephrol Dial Transplant 17:1921–1923
4. Ben-Chetrit E, Backenroth R (2001) Amyloidosis induced, end stage renal disease in patients with familial Mediterranean fever is highly associated with point mutations in the MEFV gene. Ann Rheum Dis 60:146–149
5. Bentley P, Peck G, Smeeth L, Whittaker J, Sharma P (2010) Causal relationship of susceptibility genes to ischemic stroke: comparison to ischemic heart disease and biochemical determinants. PLoS One 5:e9136
6. Binder BR, Christ G, Gruber F, Grubic N, Hufnagl P, Krebs M, Mihaly J, Prager GW (2002) Plasminogen activator inhibitor 1: physiological and pathophysiological roles. News Physiol Sci 17:56–61
7. Bonyadi M, Esmaeili M, Karimi A, Dastgiri S (2010) Common Mediterranean fever gene mutations in the Azeri Turkish population of Iran. Genet Test Mol Biomarkers 14:149–51
8. Cazeneuve C, Sarkisian T, Pecheux C, Dervichianet M, Nedelec B, Reinert P, Ayvazyan A, Kouyoumdjian JC, Ajrapetyan H, Delpech M, Goossens M, Dodé C, Grateau G, Amselem S (1999) MEFV-gene analysis in Armenians patients with FMF: diagnostic value, unfavorable renal prognosis of the M694V homozygous genotype, genetic and therapeutic implications. Am J Hum Genet 65:88–97
9. Centola M, Wood G, Frucht DM, Galon J, Aringer M, Farrell C, Kingma DW, Horwitz ME, Mansfield E, Holland SM, O'Shea JJ, Rosenberg HF, Malech HL, Kastner DL (2000) The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. Blood 95:3223–3231
10. Dawson S, Hamsten A, Wiman B, Henney A, Humphries S (1991) Genetic variation at the plasminogen activator-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb 11:183–90
11. Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney A (1993) The two allele sequences of a common polymorphism in the promoter of the plasminogen activator-1 (PAI-1) gene respond differently to interleukin- 1 in HepG2 cells. J Biol Chem 268:10739–10745
12. Dellas C, Loskutof DJ (2005) Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. Thromb Haemost 93:631–40
13. Demirel A, Celkan T, Kasapcopur O, Bilgen H, Ozkan A, Apak H, Arisoy N, Yildiz I (2008) Is familial Mediterranean fever a thrombotic disease or not? Eur J Pediatr 167:279–285
14. Eisenberg S, Aksentijevich I, Deng Z, Kastner D, Matzner Y (1998) Diagnosis of familial Mediterranean fever by a molecular genetics method. Ann Intern Med 129:539–42
15. Eriksson P, Kallin B, van t' Hooft FM, Bavenholm P, Hamsten A (1995) Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A 92:1851–1855
16. Esmaeili M, Bonyadi M, Rafeey M, Sakha K, Somi MH (2008) Common *MEFV* mutation analysis in Iranian Azeri Turkish patients with familial Mediterranean fever. Semin Arthritis Rheum 37:334–338
17. Gershoni-Baruch R, Shinawi M, Leah K, Badarnah K, Brik R (2001) Familial Mediterranean fever: prevalence, penetrance and genetic drift. Eur J Hum Genet 9:634–637
18. Hermans PWM, Hazelzet JA (2005) Plasminogen activator inhibitor type 1 gene polymorphism and sepsis. Clin Infect Dis 41:S453–8
19. Hoekstra T, Geleijinse JM, Kluft C, Giltay EJ, Kok FJ, Schouten EG (2003) 4G-4G genotype of PAI-1 gene is associated with reduced risk of stroke in elderly. Stroke 34:2822–2828
20. Isordia-Salas I, Leaños-Miranda A, Sainz IM, Reyes-Maldonado E, Borrayo-Sanchez G (2009) Association of the plasminogen activator inhibitor-1 gene 4G/5G polymorphism with ST elevation acute myocardial in-farction in young patients. Rev Esp Cardiol 62 (4):365–372
21. Kohler HP, Grant PJ (2000) Plasminogen-activator inhibitor type 1 and coronary artery disease. N Engl J Med 342:1792–801
22. Korkmaz C, Ozdogan H, Kasapcopur O, Yazici H (2002) Acute phase response in familial Mediterranean fever. Ann Rheum Dis 61:79–81
23. Lachmann HJ, Sengul B, Yavuzsen TU, Booth DR, Booth SE, Bybee A, Gallimore JR, Soyturk M, Akar S, Tunca M, Hawkins PN (2006) Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. Rheumatology (Oxford) 45:746–750
24. Lidar M, Livneh A (2007) Familial Mediterranean fever: clinical, molecular and management advancements. Neth J Med 65:318–324
25. Lima LM, Carvalho MDS, Neto CPF, Garcia JCF, Sousa MO (2011) PAI-1 4G/5G Polymorphism and plasma levels association in patients with coronary artery disease. Arq Bras Cardiol 97 (6):462–467

26. Livneh A, Langevitz P, Zemer D (1997) Criteria for the diagnosis of familial Mediterranean fever. Arthritis Rheum 40:1879–1885
27. Madách K, Aladzsity I, Szilágyi A, Fust G, Gal J, Penzes I, Prohaszka Z (2010) 4G/5G polymorphism of PAI-1 gene is associated with multiple organ dysfunction and septic shock in pneumonia induced severe sepsis: prospective, observational, genetic study. Critical Care 14:R79
28. Manduz S, Katrancioglu N, Karahan O, Ozdemir O (2010) Association of the plasminogen activator inhibitor-1(PAI-1) gene 4G/5G promoter polymorphism in Buerger's disease. (Tromboangiitis obliterans). Health 2:454–457
29. Medlej-Hashim M, Delague V, Chouery E, Salem N, Rawashdeh M, Lefranc G, Loiselet J, Megarbane A (2004) Amyloidosis in familial Mediterranean fever patients: correlation with MEFV genotype and SAA1 and MICA polymorphisms effects. BMC Med Genet 5:4
30. Meigs JB, Dupuis J, Liu C, Donnell CJ, Fox CS, Kathiresan S, Gabrie SB, Larson MG, Yang O, Herber AG, Wilson PWF, Feng D, Tofler GH, Cupples LA (2006) PAI-1 Gene 4G/5G Polymorphism and risk of type 2 diabetes in a population-based sample. Obesity 14:753–758
31. Ozel Demiralp DF, Ekim M, Akar N (2009) The effect of plasminogen activator inhibitor-1–675 4G/5G polymorphism on familial Mediterranean fever (FMF) disease. Clin Appl Thromb Hemost 15(4):443–7
32. Ozen S (2004) Renal amyloidosis in familial Mediterranean fever. Kidney International 65:1118–1127
33. Reiner AP, Schwartz SM, Frank MB, Longstreth WT, Hindorff LA, Teramura G, Rosendaal FR, Gaur LK, Psaty BM, Siscovick DS (2012) Polymorphisms of coagulation factor XIII subunit A and risk of nonfatal hemorrhagic stroke in young white women. American Heart Association 32:2580–2587
34. Roest M, van der Schouw YT, Banga JD, Tempelman MJ, Groot PG, Sixma JJ, Grobbee DE (2000) Plasminogen activator inhibitor 4G polymorphism is associated with decreased risk of cerebrovascular mortality in older women. Circulation 101:67–70
35. Schneiderman J, Sawdey MS, Keeton MR, Bordin GM, Bernstein EF, Dilley RB, Loskutoff DJ (1992) Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. Proc Natl Acad Sci USA 89:6998–7002
36. Shohat M, Magal N, Shohat T, Chen X, Dagan T, Mimouni A, Danon Y, Lotan R, Ogur G, Sirin A, Schlezinger M, Halpern GJ, Schwabe A, Kastner D, Rotter JI, Fischel-Ghodsian N (1999) Phenotype–genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis. Eur J Hum Genet 7:287–292
37. Simpson AJ, Booth NA, Moore NR, Bennett B (1991) Distribution of plasminogen activator inhibitor (PAI-1) in tissues. J Clin Pathol 44:139–143
38. Strandberg L, Lawrence D, Ny T (1988) The organization of the human-plasminogen-activator-inhibitor-1 gene: implications on the evolution of the serine-protease inhibitor family. Eur J Biochem 176:609–16
39. The International FMF Consortium (1997) Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. Cell 90:797–807
40. van Mourik JA, Lawrence DA, Loskutoff DJ (1984) Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. J Biol Chem 259:14914–21
41. Wong TY, Poon P, Szeto CC, Chan JC, Li PK (2000) Association of plasminogen activator inhibitor-1 4G/4G genotype and type 2 diabetic nephropathy in Chinese patients. Kidney Int 57:632–638
42. Yepiskoposyan L, Harutyunyan A (2007) Population genetics of familial Mediterranean fever: a review. Eur J Hum Genet 15:911–916