Association of FXII 5'UTR 46C>T polymorphism with FXII activity and risk of thrombotic disease

FXII 5'UTR 46C>T polimorfizmi ile FXII aktivitesi ve trombotik hastalık riskinin ilişkisi

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Abstract

Objective: Thrombotic diseases are caused by genetic and environmental factors. There are a number of well-characterized genetic defects that lead to increased risk of thrombosis. Results from previous studies have indicated that FXII is involved in the pathogenesis of thrombophilic diseases. However, the results in this regard are highly controversial. One of the most important determinants of Plasma FXII level is 46CgT polymorphism in the FXII gene. In the present study, the risk of thrombophilic diseases related to this polymorphism was investigated in a case-control study.

Material and Methods: One hundred and sixty subjects were studied: 120 patients diagnosed with thrombophilia (96 venous thromboembolism, 24 arterial thrombosis), and 40 age-gender-matched controls. For each subject, FXII activity level was measured by a one-step clotting assay with FXII-deficient plasma, and 46CγT polymorphism was genotyped using a restriction fragment length polymorphism (RFLP) method.

Results: In this study, the previous observation that individuals with different genotypes for the 46 CγT polymorphism show significant differences in FXII activity levels was confirmed. Most importantly, FXII activity ≤68% was associated with an increased risk of venous thrombosis with an adjusted odds ratio (OR) of 4.7 (95% confidence interval [CI]: 1.03-21.1, p=0.04). However, it was not a risk factor for arterial thrombosis with adjusted OR of 5 (95% CI: 0.91-27.1, p=0.09). In CT and TT genotype, the adjusted ORs were 2 (95% CI: 0.9-4.4, p=0.11) and 2.3 (95% CI: 0.45-11, p=0.48), respectively, for patients with venous thrombosis compared with the controls. Similarly, the adjusted ORs in arterial thrombosis were 1.2 (95% CI: 0.4-3.6, p=0.76) for CT and 1.8 (95% CI: 0.2-14.9, p=0.59) for TT genotype. Thus, we did not find any association of the mutated T allele in the heterozygous or homozygous state with an increased risk of venous or arterial thrombosis.

Conclusion: Lower FXII activity is not a risk factor; rather, it simply represents a risk marker for thrombosis.

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Key words: Factor XII, factor XII polymorphism, venous thromboembolism, arterial thrombosis

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Özet

Amaç: Trombotik hastalıklar genetik ve çevresel faktörlerden kaynaklanmaktadır. Yüksek tromboz riskine yol açan çok sayıda iyi karakterize edilmiş genetik defekt mevcuttur. Önceki çalışmalardan elde edilen sonuçlarda, FXII'nin trombofilik hastalıkların patojenezinde yer aldığı gösterilmiştir. Bununla birlikte, sonuçlar bu bakımdan oldukça tartışmalıdır. FXII genindeki 46CγT polimorfizmi FXII aktivite düzeylerini etkileyen faktörlerden birisidir. Mevcut çalışma kapsamında, bu polimorfizm ile ilişkili trombofilik hastalıklar riski bir vaka kontrol çalışmasında araştırılmıştır.

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Yöntem ve Gereçler: Yüz altmış denek incelenmiştir: 120 hastaya trombofili (96'sı tromboembolizm, 24'ü arteriyel tromboz) tanısı konmuş olup 40 hasta yaş ve cinsiyet açısından eşleştirilmiştir. Her bir denek için, FXII aktivite düzeyi, FXII'den yoksun plazma ile tek adımlı pıhtılaşma testi kullanarak ölçülmüş ve 46CγT polimorfizmi, (RFLP) yöntemi ile genotiplenmiştir.
Bulgular: 46 CγT polimorfizmi için farklı genotipleri olan bireylerin FXII aktivite düzeylerinde anlamlı farklılıklar sergilediğine dair önceki gözlem, bu çalışmada doğrulanmıştır. Daha da önemlisi, ≤%68 olan FXII aktivitesi, 4.7 ayarlanmış risk oranı (OR) ile venöz tromboza yönelik yüksek risk ile ilişkilendirilmiştir (%95 güven aralığı [CI]: 1.03-21.1, p=0.04). Ancak bu, 5 ayarlanmış OR ile arteriyel tromboza yönelik bir risk faktörü değildir (%95 CI: 0.91-27.1, p=0.09). Ct ve TT genotipte, ayarlanmış OR değerleri, kontrollere kıyasla venöz trombozlu hastalar için sırasıyla 2 (%95 CI: 0.9-4.4, p=0.11) ve 2.3 (%95 CI: 0.45-11, p=0.48) idi. Benzer şekilde, arteriyel trombozda ayarlanmış OR değerleri CT genotip için 1.2 (%95 CI: 0.4 - 3.6, p=0.76) ve TT genotip için 1.8 (%95 CI: 0.2-14.9, p=0.59) idi. Böylelikle, heterozigot veya homozigot halde mutasyona uğramış T aleli ile venöz ya da arteriyel tromboza ilişkin yüksek risk arasında herhangi bir ilişki tespit edilmemiştir.
Sonuç: Düşük FXII aktivitesi bir risk faktörü olmamakla birlikte, yalnızca tromboza yönelik bir risk göstergesini temsil etmektedir. *(Turk J Hematol 2010; 27: 15-9)*
Anahtar kelimeler: Faktör XII, faktör XII polimorfizm, venöz tromboembolizm, arteriyel tromboz

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Introduction

Thrombosis is a common cause of morbidity and mortality in industrialized nations. Both venous and arterial thrombosis can be life-threatening events, and both are of great public health importance [1]. Risk factors for thrombosis can be either genetic or acquired.

Current research on complex diseases, such as ischemic stroke (IS), now focuses on identifying genetic variants that increase the susceptibility to thrombotic disorders [2-4]. Among the clotting factors studied, factor XII (FXII) levels exhibited one of the highest heritabilities (67%) and a significant positive genetic correlation with thrombotic disease [5], indicating that some of the polymorphisms and mutations that influence variation in this physiological risk factor also influence liability to thrombosis.

Plasma coagulation FXII (Hageman factor) is an 80-KDa serine protease with an average plasma concentration of 30 μg/ml. Contact of FXII with negatively charged surfaces leads to proteolytic cleavage and activation of the FXII molecule. The generated FXIIa can initiate activation of factor XI and also seems to participate in the conversion of plasminogen to plasmin [6,7]. While *in vitro*, FXII plays a central role in the initiation of coagulation and fibrinolysis, the physiological function of FXII is still under discussion.

Factor XII gene spans a total of 12kb and is composed of 14 exons and 13 introns. It is reported to be located at 5q33-qter in the human chromosome [8]. The concentration of plasma FXII has been known to vary widely between individuals and races [9]. Recently, a frequent C→T promoter polymorphism at nucleotide 46 of the FXII gene has been identified in which the T allele creates a novel methionine-initiating codon, which results in a lower translation efficiency and decrease in FXII plasma levels [10].

Here, the relation of plasma FXII activity and a common C46T polymorphism of the FXII gene with the risk of thrombotic disease was explored in a case-control study. The identification of these relations may help to elucidate the mechanism underlying the risk of common thrombosis and therefore may suggest preventive strategies to reduce thrombosis-related morbidity and mortality.

Materials and Methods

The study group consisted of 120 patients (65 males, 54.2%; 55 females, 45.8%) diagnosed with a thrombophilic disorder based on clinicians' decision and 40 age-gender-matched control subjects. The patient group consisted of 96 venous thromboembolism (VTE) subjects (24 pulmonary embolism; 36 cerebral venous thrombosis; 36 deep vein thrombosis) and 24 subjects with arterial thrombosis [myocardial infarction [MI]). Patients were recruited from the hemophilia center in Iran. The patients were aged 21 to 64 years (41.9±10.8), with 73 (60.8%) individuals aged <45 and 47 (39.2%) aged >45 years. The patients who had no previous diagnosis of malignant disease or thrombophlebitis, with no history of diabetes mellitus or recent surgery, with normal renal and hepatic function, and who were not taking any drugs known to affect the coagulation system were included in the study protocol. Moreover, all of the patients were screened for FVL (factor V Leiden), prothrombin G20210A mutation, and protein C and S deficiency, and only patients with negative screening were included in the study. Control subjects were friends and spouses of patients who were recruited at the same time as the patients. They were included only if they had no personal history of thromboembolic disease, including venous and arterial thrombosis, cirrhosis, nephritic syndrome, or cancer. The control group was matched for age (20 to 60; mean 41.2±10.8; with 27 individuals (67.5%) aged <45 and 13 (32.5%) aged >45 years), and sex (22 males, 18 females). All individuals provided their informed consent to take part in the study, and the study was approved by the local ethics committee.

For FXII activity determination, a one-step clotting assay with FXII-deficient plasma (Technoclone, Austria) and the activated partial thromboplastin time (aPTT) reagent (Technoclone, Austria) were used on a Sysmex Ca-1500 coagulometer.

For genetic analysis, DNA was extracted by the phenol-chloroform method from blood cells. Then, polymerase chain reaction (PCR) amplification of exon 1 of the FXII gene was performed using the following primer pair to produce a fragment of 142 bp. The forward primer was 5′-GAT AGG CAG CTG GAC CAA CG-3′; the reverse primer was 5′-TGA TAG CGA CCC CCC AGA AC-3′. The sequence surrounding the

C46T polymorphism contains a naturally occurring *Bsa*HI restriction enzyme (Fermentas, Russia) site. The C46 wild type sequence (GACGCC) contains this *Bsa*HI site, which is abolished in the T46 rare variant (GATGCC). In the presence of the C allele, *Bsa*HI digestion yields fragments of 116 and 26 bp, whereas in the presence of the T allele, digestion does not occur.

After overnight incubation of the PCR product with BsaHI restriction enzyme in 37°C, electrophoresis on 8% TBE-PAG (tris borate EDTA-polyacrylamide gel) for 90 min at 150V, and staining with ethidium-bromide for 10 min, bands were visualized on an UV transilluminator and photographed using a Polaroid land camera.

Statistical analyses were performed using SPSS software version 14.0, and values of $p<0.05$ were considered significant. Odds ratio (OR) was also calculated as relative risk for thrombosis.

Results

Of 40 tested healthy volunteers, 26 (65%) carried FXII 46C genotype, 12 (30%) were heterozygous, and 2 (5%) were homozygous for FXII 46T genotype. The allele frequencies of 46C and 46T were estimated as 0.8 and 0.2, respectively.

The FXII activity levels, analyzed as a function of the 46 C→T polymorphism, showed statistically significant differences between the different genotypes (p=0.002). Genotype *T/T* showed the lowest levels of FXII activity (58±11.3) compared with the levels of the other genotypes (*C/C* genotype: 123±30.3 and *C/T* genotype: 97.3±23).

In both the patient and control groups, the CC genotype had the highest frequency, and TT the lowest. CC frequency was higher in controls (65%) than patients with VTE and MI (48% and 58.3%, respectively), but CT and TT frequencies were higher in patients with VTE (44% and 8%, respectively) and MI (33.3% and 8.3%, respectively) than controls (30% and 5%, respectively). The C/T allele frequency in patients with VTE and MI were 70/30 and 75/25 compared with 80/20 in controls. Therefore, the risk of thrombosis associated with the genotype CT and TT was studied. CC genotype was considered as a reference group, and the OR of thrombosis associated with the 46 C→T polymorphism for genotypes CT and TT was calculated in VTE patients (Table 1) and MI patients (Table 2) compared with the controls. The results suggest that neither genotype CT nor TT is a risk factor for arterial or venous thrombosis.

In both the patient and control groups, CC demonstrated the highest FXII activity level and TT the lowest FXII activity levels. The mean values of plasma FXII activity levels were 112±32.2 in controls compared with 87±23 in VTE patients and 84±21 in MI patients. There were statistically significant differences in mean values of FXII activity levels between patients and controls (p=0.001). Thus, it was speculated that decreased FXII activity is a risk factor for thrombosis. Consequently, OR was calculated as a risk for thrombosis. Levels lower than the 10th percentile (lower than 68%) were used as a cut-off, and FXII activity >68% was considered as a reference group. The risk of thrombosis associated with FXII activity levels in VTE and MI patients is shown in Table 1 and Table 2, respectively. The results suggest that FXII activity ≤68% is an independent risk factor for venous thrombosis. However, it is not a risk factor for arterial thrombosis.

Table 1. Risk of VTE associated with CT, TT genotype and FXII activity

	Patients	Controls	OR	95% CI	P
CC	46 (48%)	26 (65%)	1 *	----	----
CT	42 (44%)	12 (30%)	2	0.9-4.4	0.11
TT	8 (8%)	2 (5%)	2.3	0.45-11	0.48
FXII activity >68%	77 (80.2%)	38 (95%)	1#	----	----
FXII activity ≤68%	19 (19.8%)	2 (5%)	4.7	1.03-21.1	0.04

* Reference group: subjects with CC genotype, # Reference group: subjects with FXII activity >68, Reference range for FXII activity: 50% - 150%

Table 2. Risk of MI associated with CT, TT genotype and FXII activity

	Patients (N=96)	Controls (N=40)	OR	95% CI	P
CC	14 (58.3%)	26 (65%)	1*	----	----
CT	8 (33.3%)	12 (30%)	1.2	0.4-3.6	0.76
TT	2 (8.3%)	2 (5%)	1.8	0.2-14.9	0.59
FXII activity >68%	19 (79.2%)	38 (95%)	1#	----	----
FXII activity ≤68%	5 (20.8%)	2 (5%)	5	0.91-27.1	0.09

* Reference group: subjects with CC genotype, #Reference group: subjects with FXII activity >68, Reference range for FXII activity: 50%-150%

Discussion

It is well-known that plasma level of FXII is variable among different individuals and races due to genetic and environmental factors. Various factors such as estrogen, interleukin 6 [11], specific lipoprotein particles [12] and a dysfunctional endothelium [13] were shown to have a significant influence on FXII levels. This study confirms and extends the previous observation that the 46C→T polymorphism in the FXII promoter region correlates with lower FXII plasma activity.

In this study, the allele frequency of 46 C/T was 0.8/0.2 in a small Iranian population (allele number=80), which is exactly the same as the allele frequency found by Kanaji [10] in Caucasians (allele number=40). Further, genotype frequency in that study corresponded to the genotype frequency in Austrians (who are representative of the Middle European population) reported by Endler et al. [14] in 100 healthy Austrian newborns and those in the United Kingdom found by Kohler et al. [15]. Conversely, the allele frequency of 46 C/T in Orientals has been shown to be 0.27/0.73 [10], which contradicts our results. This can be explained by racial and geographical differences.

It has been reported that the plasma levels of FXII in Orientals (125.4±56.3) are lower than in Caucasians (150±30.3) because of the differences in allele frequency of 46C/T in the FXII gene between the two races [10]. Although in the present study the allele frequency of 46C/T was exactly the same as in Caucasians, the level of plasma FXII activity in this study (112±32.2) was lower than in Caucasians. It may be because of the low patient number in both studies. Furthermore, although the C46T polymorphism in the FXII gene is an important determiner of plasma FXII level, it is not the only effective factor. As previously mentioned, there are other factors that have a significant influence on FXII levels. Thus, further large scale studies considering the other effective factors on FXII levels may be needed to elucidate these differences.

There is controversy over the clinical significance of FXII deficiency because most of subjects with complete deficiency of FXII have no clinical manifestations. Some authors considered low FXII levels to be associated with an increased risk for venous [1,16,17] as well as arterial thromboembolism [18-20], while other studies have reported increased FXII activity in people with acute coronary syndromes [21,22]. In some studies, no effect of FXII levels on thrombosis was observed [13,23,24]. Furthermore, there are conflicting results about the effect of polymorphism 46C→T in the FXII gene on thrombotic diseases. Previous genetic association studies showed that genotype TT increases significantly the risk for ischemic stroke [25], acute coronary artery disease [26] and cerebral venous thrombosis [27]. Conversely, there are also studies that either do not detect a genetic association between this genotype and cardiovascular risk [28] or alternatively report an association between the C/C genotype and thrombosis risk [29].

Although in this study, FXII activity ≤68% was associated with an increased risk of VTE, there was no association between FXII activity ≤68% and MI risk. Moreover, no association of the mutated T allele in the heterozygous or homozygous state with an increased risk of VTE and MI was found. Therefore, it was speculated that reduced FXII activity is not the cause of thrombosis, but the result of it [30],for the following reason: Previous studies [10,14] and this study showed that the substitution of T allele at nucleotide 46 in the FXII gene reduces FXII activity because the 46T allele creates a novel methionine-initiating codon that reduces the translation efficiency of FXII. As a result, the FXII 46C→T polymorphism is not merely a marker, but rather a strong determinant of plasma FXII levels. Notably, despite the importance of the FXII genotype on FXII levels, the studies by Bach et al. [20], Athanasiadis et al. [28] these results study did not observe an association between the FXII 46T/T genotype and thrombosis. This would suggest that lower FXII activity is not a risk factor; rather, it simply represents a risk marker [30]. Endler et al. [31] demonstrated that with decreasing FXII activity, the hazard ratio for all-cause mortality and death as a result of ischemic heart disease gradually increased in an almost linear manner. However, mortality was not increased in individuals who had FXII activity that was lower than 10% of normal. While this appears paradoxical, the suggested reason is that the linear association between low FXII activity and mortality may simply reflect the progression of atherosclerosis and inflammation, and that individuals with very low FXII activity may have mutations that cause FXII deficiency but do not alter their overall survival [30].

Furthermore, FXII is a plasma protein that activates both coagulation and fibrinolysis. The level of plasma FXII is suggested to be reduced as a consequence of activation of both coagulation and fibrinolysis upon thrombus formation [30].

According to our results, FXII activity is decreased as a result of thrombosis in both arterial and venous thrombosis.

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Conflict of interest

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

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