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Factor VII-401 and -402 polymorphisms and acute myocardial infarction in southern Turkey population

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ABSTRACT

Objectives: Factor VII has a crucial role in the extrinsic coagulation pathway and initiates the thrombus formation. Some studies showed that high plasma factor VII level was related to increased acute myocardial infarction (AMI) risk. But, some studies were reported opposite findings. Some polymorphisms can change the factor VII level. There is limited information about factor VII polymorphisms in southern Turkey population. Our aim was to determine the frequencies of Factor VII-401 and -402 polymorphisms and their relation to AMI in southern Turkey area.

Methods: We enrolled 83 patients with AMI and 71 healthy subjects. Routine laboratory tests and factor VII-401 and -402 polymorphisms were determined from blood samples. Factor VII -401 and -402 polymorphisms were analyzed by LightCycler device using Real-Time PCR technique.

Results: Family history of coronary artery disease and smoking frequencies were higher in patients group ($p < 0.001$ and $p = 0.013$, respectively). Patients had higher LDL cholesterol ($p = 0.011$) level, and lower HDL cholesterol ($p = 0.025$) level compared to healthy subjects. Factor VII-401 and -402 polymorphism genotypes were not significantly different in both groups. Also allele frequencies were similar in both groups.

Conclusion: Factor VII-401 and -402 polymorphisms do not seem to increase AMI risk in southern Turkey.

Keywords: Acute myocardial infarction, factor VII-401 and -402 polymorphisms

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Intracoronary thrombus is one of the reasons of acute myocardial infarction (AMI). It occurs via combination of circulating factor VII and tissue factor exposure after plaque rupture [1]. In clinical practice, it is observed that some patients with serious coronary artery disease (CAD) do not have any AMI. On the contrary, some patients without serious coronary artery lesions suffer from AMI. These facts have led the investigators to look for other abnormal thrombosis developing reasons. Some investigators focused mainly to the blood coagulation system. Increased plasma fibrinogen level was determined as a cardiovascular risk factor [2]. Factor VII is also a vitamin K dependent coagulation factor circulating as an inactive zymogen in blood. It has a crucial role in the extrinsic coagulation pathway and synthesized by liver [3]. There are some controversial reports in the literature about high plasma factor VII level's relation to increased AMI risk [4-7]. Factor VII level can be altered via genetic and environmental factors [8]. Guanine to

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thymine (G/T) base substitution at the -401st position of factor VII gene's promoter leads a decreased gene transcription and lower plasma factor VII level. Guanine to adenine (G/A) base substitution at the -402nd position of the same area increases the gene transcription and the factor VII level. Together both polymorphisms are responsible for 18% and 28% of the variation in the plasma concentrations of total factor VII and activated factor VII molecules, respectively. Both factor VII-401 and factor VII-402 polymorphism frequencies were reported as low [9]. There is limited information about factor VII polymorphisms in general Turkish population. The aim of this study was to determine the frequencies of these polymorphisms and their relation to AMI in southern Turkey population.

METHODS

We enrolled 83 patients who admitted with AMI to our coronary intensive care unit and 71 healthy subjects. Local Ethics Committee of Çukurova University was approved the study. Written informed consent was collected from all subjects. Diagnosis of AMI was based on typical chest pain, ST segment elevation in the admission electrocardiogram, and cardiac enzyme increase criteria. All patients were recruited consecutively, and all patients had their first myocardial infarction. Control subjects were selected randomly from healthy individualswho admitted for an examination to our polyclinic unit. None of these controls had any cardiovascular and valvular diseases. All the patients and control subjects were from southern Turkey. Diabetes mellitus (DM) was considered as a risk factor for atherosclerosis development [10]. Therefore, we excluded patients with DM. All patients and controls were questioned about age, gender, hypertension, smoking, hyperlipidemia, and family history. Body mass indexes were recorded. Complete blood count, glucose, lipid levels, and renal functions were recorded from routinely taken blood samples.

DNA isolation

2 cc of K3 EDTA anticoagulated venous blood sampleswere collected from all subjects for DNA analysis. DNA samples were isolated from whole blood with the aid of MagNa Pure LC DNA Isolation Kit I by MagNa Pure LC Automated DNA isolation instrument (Roche Applied Sciences). DNA samples were stored at –20 ºC until mutations were investigated.

Real-Time PCR

Primer and hybridization probes for the factor VII –401 G/T and -402 G/A polymorphisms were designed and synthesized by OlfertLandt (Tib-Molbiol, Germany). All polymorphism-related gene regions were amplified in 20 µl PCR capillary tubes. Amplification process was established using LightCycler FastStart DNA Master Hybridization Probes (Roche Applied Science). After preparation of primers, probes and kit mixtures, 18 µl of the reaction mixture and 2 µl (~40 ng) genomic DNA were added in each LightCycler capillary tube. Water was used as negative control. Capillary tubes were sealed and briefly centrifuged in a microcentrifuge and then placed into the LightCycler carousel. The PCR products were detected by using 3'- fluorescein (FLU) labelled probe and 5'- Red 640 labelled probe. When both probes hybridize in close proximity, fluorescence resonance energy transfer (FRET) occurs, producing a specific fluorescence emission of LC-Red as a result of FLU excitation. Fluorescence intensity depends on the amount of specific PCR products. Amplification was monitored on-line per cycle via LightCycler device. At the end of the amplification, LightCycler device increased the temperature and measured the fluorescence same time. Temperature / fluorescence curve (melting curve) was obtained this way and polymorphisms were determined with the analysis of this curve.

Statistical Analysis

The variables were divided into two groups as categorical and continuous. Categorical data were expressed as numbers and percentages, and compared with the chi-square test. Kolmogorov-Smirnov test was used to determine whether continuous variables had normal distribution or not. Normal distributed continuous variables were compared with the independent samples t-test. Not normal distributed variables were compared with Mann-Whitney U Test. Binominal logistic regression analysis was performed with significant variables. Independent predictors were found for AMI. Statisticalanalyses were

Table 1. Comparison of demographic findings

	Patients (n = 83)	Controls (n = 71)	*p* value
Age (years)	47.5 ± 7.5	45.6 ± 5.7	0.068
Male gender, n (%)	46 (55.4)	33 (46.5)	0.268
Systolic blood pressure (mmHg)	119.6 ± 17.0	122.7 ± 16.5	0.284
Diastolic blood pressure (mmHg)	70.2 ± 11.5	71.4 ± 11.2	0.508
Pulse (beat/minute)	81.7 ± 8.7	80.7 ± 8.1	0.489
BMI (kg/m^2)	24.5± 2.1	24.2± 2.1	0.394
Smoking, n (%)	56 (67.5)	34 (47.9)	**0.013**
Family history of CAD, n (%)	37 (44.6)	11 (15.5)	**< 0.001**
Hypertension, n (%)	65 (78.3)	56 (78.9)	0.933
Hyperlipidemia, n (%)	38 (45.8)	21 (29.6)	**0.039**

Data are shown mean ± standard deviation or number (%). BMI = Body mass index, CAD = Coronary artery disease

calculated with SPSS 20.0 (SPSS Inc., Chicago, IL, United States). A *P* value < 0.05 was considered to be statistically significant.

RESULTS

Demographic comparison was presented in the Table 1. Family history of CAD ($p < 0.001$), smoking ($p = 0.013$), and hyperlipidemia ($p = 0.039$) were significantly higher in patient group ($p < 0.05$). All other variables were similar between two groups. Low density lipoprotein cholesterol (LDL-C) levels were higher ($p = 0.011$) and high density lipoprotein cholesterol (HDL-C) levels were lower ($p = 0.025$) in patient group ($p < 0.05$). Other laboratory parameters were similar (Table 2). Factor VII-401 and -402 polymorphism genotype frequencies were similar between two groups (Table 3). It was determined that both polymorphisms were suitable to the Hardy-Weinberg equation. Factor VII-401 G/T allele frequencies were 0.59/0.41 and 0.51/0.49 in patients

Table 2. Comparison of laboratory findings

	Patients (n = 83)	Controls (n = 71)	*p* value
Glucose (mg/dl)	123.2 ± 82.5	100.7 ± 7.0	0.723
WBC (uL)	7.1 ± 1.5	6.8 ± 1.8	0.261
Hb (mg/dl)	12.1 ± 1.3	12.1 ± 1.5	0.979
BUN (mg/dL)	41.8 ± 4.6	41.1 ± 4.5	0.492
Cr (mg/dL)	0.9 ± 0.2	0.9 ± 0.1	0.95
Na (mmol/L)	137.7 ± 2.6	137.8± 3.6	0.94
K (mmol/L)	4.1 ± 0.3	4.1 ± 0.1	0.992
LDL-C (mg/dl)	128.7 ± 39.2	113.5 ± 31.3	**0.011**
HDL-C (mg/dl)	41.5 ± 8.7	44.9 ± 9.1	**0.025**
Triglyceride (mg/dl)	191.9 ± 102.8	183.5 ± 137.7	0.675
Total cholesterol (mg/dl)	202.0 ± 47.0	194.6 ± 35.4	0.284

Data are shown mean ± standard deviation. WBC = White blood cells, Hb = Hemoglobin, BUN = Blood urea nitrogen, Cr = Creatinine, LDL-C = Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol

Table 3. Genotype distributions of patients and control subjects

	Patients (n = 83)	Controls (n = 71)	*p* value
Factor VII -401 G/T			
Homozygous G, n (%)	33 (39.8)	24 (33.8)	
Heterozygous, n (%)	32 (38.6)	24 (33.8)	0.324
Homozygous T, n (%)	18 (21.7)	23 (32.4)	
Factor VII -402 G/A			
Homozygous G, n (%)	38 (45.8)	28 (39.4)	
Heterozygous, n (%)	25 (30.1)	18 (25.4)	0.318
Homozygous A, n (%)	20 (24.1)	25 (35.2)	
Allele distributions			
Factor VII -401 G/T, n	0.59/0.41	0.51/0.49	0.3
Factor VII -402 G/A, n	0.61/0.39	0.52/0.48	0.31

and controls, respectively. Factor VII-402 G/A allele frequencies were 0.61/0.39 in patients and 0.52/0.48 in control subjects. Both groups had similar allele frequencies (Table 3). Binominal logistic regression analysis was performed with statistically significant variables. Family history of CAD (OR: 5.101, 95% CI: 2.200 - 11.825, $p < 0.001$), LDL-C (OR: 1.018, 95% CI: 1.006 -1.031, $p = 0.003$), and HDL-C (OR: 0.938, 95% CI: 0.896 - 0.982, $p = 0.006$) were determined as independent predictors for AMI (Table 4).

Table 4. Independent predictors for acute myocardial infarction

	Odds ratio	CI (95%)	*p*
Family history of CAD	5.101	2.200-11.825	**< 0.001**
Hyperlipidemia	1.182	0.472-2.958	0.722
Smoking	0.553	0.248-1.229	0.146
LDL-C	1.018	1.006-1.031	**0.003**
HDL-C	0.938	0.896-0.982	**0.006**

CAD = Coronary artery disease, LDL-C = Low density lipoprotein cholesterol, HDL-C = High density lipoprotein cholesterol

DISCUSSION

The main finding of our study was that this was the first study which investigated the relation between factor VII-401 and -402 polymorphisms and AMI in factor VII southern Turkey population. Classic CAD risk factors were significantly higher in patients. There was no association between these polymorphisms and AMI in this population.

It was thought that increased plasma factor VII levelcould affect the formation speed and growth of thrombus. These facts can play an important role in acute coronary syndromes. The studies whichaimed to investigate this hypothesis had controversial results in factor VII the literature [11].

Seven factor VII gene related polymorphisms have been identified. These are A/G base substitution at the 353rd codon in exon 8, hyper variable region 4 (HVR4) polymorphism of intron 7, decanucleotide insertion at positions -323, -401G/T, -402G/A, -59T/G, and -32A/C[12].

There were conflicting results in studies that

investigated the relation between CAD and first two polymorphisms [13-16]. It was reported that -323rd nucleotide polymorphism in promoter has no biological activity [9]. Effects of the -401 G/T and -402 G/A polymorphisms over CAD and cerebrovascular diseasewere investigated in limited number of studies [17-20].

Kang *et al.* [17] reported that activated factor VII (FVIIa) level and factor VII coagulant activity (FVIIc) were higher in the AMI patients. But, CAD patients without AMI had similar FVIIa, FVIIc, and factor VII antigen (FVIIag) levels compared to control subjects. Their study population consisted of 60 CAD patients (33 of these patients had AMI) and 149 control subjects. Factor VII-401 and -402 polymorphism genotype and allele distributions were not different between groups. Same investigators showed that FVIIa, FVIIc, and FVIIag levels were higher in 62 cerebral infarction patients compared to the 149 healthy subjects [18]. They also reported that polymorphism genotype and allele distributions were similar in both groups.

Evangelista *et al.* [19] investigated the effect of factor VII-401 and -402 polymorphisms in arterial and venous thrombotic events. Their patient group consisted of 104 participants and control group had 106 healthy subjects. They showed that there was no significant difference about these polymorphisms between patients and controls. Likewise, Ramzi *et al.* [20] reported a similar result in their study. They tried to identify the role of factor VII-401 G/T and HVR4 polymorphisms in CAD. They enrolled 110 patients and 110 control subjects. They found no association between these polymorphisms and CAD. There were no genotype distribution and allele frequency difference between our groups. Our results were compatible with these studies.

Allele distributions can vary significantly in different populations. Kang *et al.* [17] found allele frequencies 0.03/0.97 for factor VII-401 G/T and 0.48/0.52 for -402 G/A in patients. We found -401 G/T allele frequency 0.59/0.41 and -402 G/A allele frequency 0.61/0.39 in our patient group. Van't Hooft *et al.* [9] found -401 G/T and -402 G/A allele frequency 0.91/0.09 and 0.71/0.29 respectively in healthy European subjects. Kang *et al.* [17] found allele frequencies for -401 G/T and -402 G/A polymorphisms in healthy Chinese subjects 0.97/0.03 and 0.52/0.48, respectively. In our control group, -401 G/T allele frequency was 0.51/0.49 and -402 G/A allele frequency was 0.52/0.48. There was a noticeable difference in the factor VII-401 G/T allele frequency between our population and their populations.

Limitations

Factor VII-401 and -402 polymorphism's effects on the factor VII level have been identified before. Some of the studies did not include measurements of factor VII [21]. We also did not measure factor VII level for this reason. But, this is an important limitation for us. Also, our small sample size is another limitation.

Age, lipid levels, obesity, andsmoking can alter factor VII level [22]. When all the environmental and genetic risk factors taken into account, we still have limited information about which factor how much strongly affects factor VII level. Further studies are needed to clarify role of factor VII gene polymorphism over arterial thrombosis development.

CONCLUSION

There is an association between classic CAD risk factors and AMI, butfactor VII-401 and -402 polymorphisms do not seem to increase risk of AMI in southern Turkey population.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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