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The effects of factor V leiden, prothrombin G20210A, MTHFR C677T, MTHFR A1298C, factor XIIIA Val34Leu, factor XIIIB His95Arg and apolipoprotein E genotypes on coronary artery disease

[Faktör V leiden, protrombin G20210A, MTHFR C677T, MTHFR A1298C, faktör XIIIA Val34Leu, faktör XIIIB His95Arg ve apolipoprotein E genotiplerinin koroner arter hastalığına etkileri]

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ABSTRACT

Objectives: The aim of our study was to evaluate the possible roles of factor V Leiden, prothrombin G20120A mutations, factor XIIIA and B, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C and apolipoprotein (Apo) E polymorphisms in the development of coronary artery disease (CAD).

Methods: A total of 442 subjects (234 CAD and 208 non-CAD) were included in the study according to their coronary angiography. Polymerase chain reaction (PCR), real-time PCR and restriction enzyme analysis (REA) were carried out in the study.

Results: Logistic regression analysis showed that when the biochemical variables and genetic risk factors were placed in a model, the most important variables were smoking, alcohol, Diabetes Mellitus (DM), factor V G1691A, MTHFR A1298C, factor XIIIA Val34Leu and XIIIB His95Arg. We showed that smoking, DM, factor V G1691A, MTHFR A1298C increased the risk of CAD 3.19, 2.27, 3.32, 1.97-fold, respectively. On the other hand, alcohol, factor XIIIA Val34Leu and XIIIB His95Arg decreased the risk of CAD 0.44, 0.46 and 0.17-fold, respectively. Apo E polymorphisms were not found important statistically in our study.

Conclusions: It is concluded that presence of Leu allele at factor XIIIA Val34Leu and Arg allele at factor XIIIB His95Arg is protective against CAD. In addition, Factor V G1691A mutation, MTHFR 1298 AC genotype could have an important role in the progression of CAD.

Key Words: Coronary artery disease, Factor V G1691A, prothrombin G20210A, MTHFR C677T, and A1298C, factor XIIIA and B.

Conflict of Interest: Authors declare no conflict of interest

ÖZET

Amaç: Bu çalışmadaki amaç faktör V leiden ve protrombin G20210A mutasyonları, faktör XIIIA ve B, metilentetrahidrofolat redüktaz (MTHFR) C677T ve A1298C ve apolipoprotein (Apo) E polimorfizimlerinin koroner arter hastalığı (KAH) gelişimindeki olası rollerini incelemektir.

Gereç ve Yöntemler: Koroner anjiyografi sonuçlarına göre 442 örnek (234 KAH ve 208 KAH olmayan) çalışmaya dahil edilmiştir. Çalışmada, polimeraz zincir reaksiyonu (PCR), real-time PCR ve restriksiyon enzim analizi (REA) kullanılmıştır.

Bulgular: Biyokimyasal değişkenler ve genetik risk faktörlerinin lojistik regresyon modelinde en önemli değişkenler sigara, alkol, Diabetes Mellitus (DM), faktör V G1691A, MTHFR A1298C, faktör XIIIA Val34Leu and XIIIB His95Arg olarak belirlenmiştir. Sigara, DM, faktör V G1691A ve MTHFR A1298C polimorfizminin KAH riskini sırasıyla, 3.19, 2.27, 3.32 ve 1.97 kat arttırdıkları gösterilmiştir. Bunun yanında alkol, faktör XIIIA Val34Leu ve XIIIB His95Arg polimorfizminin KAH riskini sırasıyla 0.44, 0.46 ve 0.17 kat azalttıkları bulunmuştur. Apo E polimorfizimlerinin CAD üzerine önemli bir etkisi saptanmamıştır.

Sonuçlar: Faktör XIIIA Val34Leu ve faktör XIIIB His95Arg polimorfizminde Leu alleli ve Arg allel varlığının KAH'de koruyucu rolü olduğu düşünülmektedir. Bunun yanında faktör V G1691A mutasyonu ve MTHFR 1298 AC genotipinin CAD gelişiminde önemli rolü olabilir.

Anahtar Kelimeler: Koroner arter hastalığı, Faktör V G1691A, protrombin G20210A, MTHFR C677T ve A1298C, faktör XIIIA ve B.

Çıkar Çatışması: Yazarlar arasında çıkar çatışması bulunmamaktadır.

Introduction

Coronary artery disease (CAD) is a major health problem and the leading cause of mortality in the industrialized countries [1,2]. Many risk factors are known to increase the likelihood of morbidity and mortality for cardiovascular disease [1]. Coronary artery disease occurs with the interaction between environmental influences and genetic factors [3]. Beside the conventional risk factors, it is known that coagulation processes play an important role among the causes of CAD [4,5]. Previous studies investigated the role of haemostatic markers as predisposing factors to thrombus formation and atherosclerosis. Several genetic mutations affecting coagulation proteins have been suggested as prothrombotic inherited risk factors [5]. These include the factor V G1691A (FV Leiden; R506Q), prothrombin G20210A gene mutations, methylenetetrahydrofolate reductase (MTHFR) and factor XIII gene polymorphisms [5,6]. Nucleotide variants in several genes for lipid metabolism influence the risk of atherosclerosis. One of the polymorphisms is apolipoprotein (Apo) E gene variants that are thought to be a candidate for atherosclerosis risk [7]. Many research efforts continue to identify inherited risk factors of this complex disease. But there are controversial results with regard to these gene mutations being candidate as risk factors for CAD.

In this study, we investigated the prevalence of FV Leiden, prothrombin G20120A mutations, factor XIIIA and B, MTHFR C677T and A1298C and Apo E gene polymorphisms and the possible roles of these factors in the development of CAD.

Materials and Methods

Subjects. Among the study population, 234 had CAD and 208 had normal coronary arteries (non-CAD). Four hundred and forty-two patients who underwent coronary angiography and admitted to the department of cardiology during 1 year period were included in the study. An informed consent from each patient was obtained and the study protocol was approved by Clinical Research local ethical committee of Çukurova University, Faculty of Medicine. Major risk factors for CAD like age, gender, family history, hypertension (HT), diabetes mellitus (DM) were determined. A positive family history was defined as CAD in a parent or sibling noted under the age of 55 for men and 65 for women. A sustained blood pressure ≥ 140 mmHg systolic and ≥90 mmHg diastolic or the use of antihypertensive drugs at the time of investigation was defined as HT. Diabetes mellitus was considered to be present if there was a history of diabetes, fasting blood glucose ≥126 mg/dL, or the use of an antidiabetic medication [8].

Coronary Angiography: Coronary angiography was performed by the Judkins technique. Angiographies were interpreted by two cardiologists. CAD was defined as ≥50% reduction of internal diameter of left anterior descending, right or circumflex coronary artery or their primary branches. Patients without angiographic lesions were considered as without CAD.

Genotyping Procedures. Venous blood samples (3 mL) were taken from patients into the tubes with EDTA for mutation analysis. DNA was extracted from leucocytes by using the phenol/chloroform protocol [9] and stored at -20ºC.

The mutation Factor V G1691A were detected by using the following forward and reverse primers for amplification; 5'- CAGGCAGGAACAACACCATG-3' and 5'-CTTGAAAATGCCCCATTA-3'. PCR conditions were applied as described by Russeva et al. [10].

To identify the G20210A mutation of coagulation factor II (prothrombin) the forward and reverse primers were; 5'-GCACAGACGGCTGTTCTCTT-3' and 5'-TAGCACTGGGAGCATTGAAGC-3'. PCR was carried out as described by Danneberg et al. [11].

Factor XIIIA Val34Leu and factor XIIIB His95Arg polymorphisms were determined by polymerase chain reaction. The forward and reverse PCR primers for FXIIIA were as fallows; 5'-CATGCCTTTTCTGTTGTCTTC-3' and 5'-TACCTTGCAGGTTGACGCCCCGGGCACTA-3'. When a mutation is present the underlined base change from the native sequence and produce a new restriction *Dde*I site. The forward and reverse PCR primers for FXIIIB were as fallows; 5'-AAAGACAAGCTTAGTTTCATCATT and 5'-TCTTCAGTTTAGGAAATGATTCTTAT. The R95-allele has a restriction *Nsi*I site when a base change is present [12].

MTHFR 677 C→T and MTHFR 1298A→C mutations were detected by real-time flourescence PCR (Roche Diagnostics, Mannheim, Germany) by the manufacturer's instructions. 25 ng of genomic DNA in a total volume of 10 μL reaction mixture provided by the manufacturer (LightCycler DNA Master Hybridization Probes) with 0.5 μM each PCR primer, and 0.15 μM each florescent probe. PCR amplification included a first denaturation step of 10 min at 95 °C, followed by 35 cycles of 95 °C for 3 min, 55 °C for 10 s, and 72 °C for 15 s.

ApoE gene polymorphisms for ε2 (Cys-112; Cys-158), ε3 (Cys-112; Arg-158),ε4 (Arg-112; Arg-158); the template DNA was amplified by the following forward and reverse primers: 5'-TCCAAGGAGCTGCAGCGGCGCA and 5'-ACAGAATCGCCCCGGC. PCR conditions were optimized according to the method of Wenham et al. [13].

Statistical analysis: SPSS 11.5 package was used for all computations. The results of descriptive analysis in the samples were defined as mean ± standard deviation and range. Student's t-test was used to compare means of the biochemical parameters in CAD and the non-CAD groups. Chi-square test was used to analyze other risk factors. Multiple logistic regression analysis was used to determine the relationship between the risk factors, biochemical parameters and CAD. To assess the associa-

tion between genotype and CAD, odds ratios (ORs) with 95% CIs were calculated. P value <0.05 was considered as significant.

Results

Clinical Features of the Subjects: Among the study population, 234 subjects had CAD and 208 had normal coronary arteries (non-CAD). The mean age of patients were significantly higher than the controls (56.1 ± 9.6 vs. 52.2 ± 10.1, p< 0.001) (Table 2). The other risk factors such as smoking, family history and DM were also higher in the CAD group compared to the non-CAD (Table 1).

Laboratory findings of the subjects: Genotype distributions of both groups are shown in Table 3. The multivariate logistic regression analysis is shown in Table 4. Alcohol intake was found protective for CAD (Table 4). Factor V Leiden and prothrombin G20210A were not found important statistically among groups. MTHFR C677T was not found important in logistic regression model. MTHFR A1298C, factor XIIIA and B were important factors for CAD and the OR values were shown in Table 4. The genotype distributions of ApoE alleles are shown in Table 3.

Discussion

This study demonstrated that presence of Leu allele at factor XIIIA Val34Leu and Arg allele at factor XIIIB His95Arg is protective against CAD. In addition, FV Leiden mutation, MTHFR 1298 AC genotype could have an important role in the progression of CAD.

Factor V Leiden is known to be a common risk factor for venous thrombosis but this mutation is also associated with arterial thromboembolism [14]. The increased frequency of this mutation has been reported in patients surviving acute myocardial infarction (AMI) [15]. The wide variations of the frequency of FV Leiden were reported in different surveys, countries, geographical locations and also in the ethnic populations. In Caucasians, the carrier frequency of FV Leiden was reported to be 2–15% [14,16]. In a different study group the prevalence was found to be 10% in the middle part of Turkey [17]. In a study in the South of Turkey, the prevalence of FV Leiden mutation was obtained as 6.3% and 5.2% in patients and the controls, respectively [18]. Dunn et al. [19] suggested that FV Leiden heterozygosity alone may not be an independent predictor of CAD and pointed out that the homozygous state could play a more substantial role in the development of the disease. In our study, the multivariate logistic regression model revealed that the GA genotype had an 3.3-fold increased risk for CAD than the GG genotype. On the other hand if there were homozygote individuals for this mutation that could be more effective to estimate the CAD risk.

The prothrombin G20210A mutation is associated with the increased thrombosis risk [16,20]. Similarly, prothrombin G20210A gene mutation is also shown to be

Table 1. Clinical features of the CAD and non-CAD groups.

	CAD (n= 234)	Non-CAD (n= 208)	
Characteristic	n (%)	n (%)	p
Sex Male /Female	154 (65.8)/ 80 (34.2)	124 (59.6)/ 84 (40.4)	0.178
Current Smokers	66 (28.2)	48 (23.1)	0.219
Hypertension	78 (33.3)	84 (39.4)	0.184
Diabetes Mellitus	48 (20.5)	24 (11.5)	0.011
Alcohol	14 (6.0)	22 (10.6)	0.078
Family History	92 (39.3)	46 (22.1)	<0.001

Table 2. Age and Body Mass Index (BMI) of the CAD and non-CAD groups.

	CAD (n= 234)		Non-CAD (n= 208)		
	Mean ± SD	Median (Min-Max)	Mean ± SD	Median (Min-Max)	p
Age (y)	56.1 ± 9.6	55 (36-75)	52.2 ± 10.1	52 (34-75)	<0.001
BMI(Kg/m^2)	27.0 ± 3.8	26.6 (16.5-36.7)	27.4 ± 4.5	27.1 (18-41.6)	<0.001

Table 3. Genotype distributions for CAD and non-CAD groups.

	CAD n=234 n (%)	non-CAD n=208 n (%)	p
FactorV G1691A			
GG	220 (94.0)	200 (96.2)	0.383
GA	14 (6.0)	8 (3.8)	
AA	0 (0.0)	0 (0.0)	
ProthrombinG20210A			
GG	222 (94.9)	204 (98.1)	0.080
GA	12 (5.1)	4 (1.9)	
AA	0 (0.0)	0 (0.0)	
MTHFR C677T			
CC	128 (54.7)	84 (40.4)	0.009
CT	84 (35.9)	94 (45.2)	
TT	22 (9.4)	30 (14.4)	
MTHFR A1298C			
AA	88 (37.6)	110 (52.9)	0.005
AC	126 (53.8)	82 (39.4)	
CC	20 (8.5)	16 (7.7)	
FactorXIIIAVal34Leu			
Val/Val	210 (89.7)	158 (76.0)	<0.001
Val/Leu	24 (10.3)	46 (22.1)	
Leu/Leu	0 (0.0)	4 (1.9)	
FactorXIIIBHis95Arg			
His/His	214 (91.5)	162 (77.9)	<0.001
His/Arg	18 (7.7)	38 (18.3)	
Arg/Arg	2 (0.9)	8 (3.8)	
Apolipoprotein E			
ε2ε2	2 (0.9)	0 (0.0)	0.024
ε2□3	58 (24.8)	74 (35.6)	
ε2ε4	2 (0.9)	6 (2.9)	
ε3ε3	122 (52.1)	100 (48.1)	
ε3ε4	44 (18.8)	24 (11.5)	
ε4ε4	6 (2.6)	4 (1.9)	

originated from a single mutational event and has the same geographical distribution [14]. The prevalence of the mutation in healthy control subjects in the Leiden Thrombophilia Study (LETS) was 2.3% [20]. Gerdes et al. [4] showed that the prothrombin G20210A mutation may be a relevant factor in patients with established atherosclerosis and found a higher prevalence in patients with premature CAD. In another study it was demonstrated that there is no significant difference for the heterozygote G20210A mutation among patients and the control group [21]. We found no significant difference in our study for the heterozygote mutation among groups according to the multivariate logistic regression analysis. On the other hand, there was no homozygote mutation and the number of the samples was not sufficient. This may be the reason that this mutation has not entered in multivariate regression model.

The mutation of C677T of the MTHFR gene produces a variant thermolabile enzyme with decreased activity. The other mutation of this gene, A1298C is a point mutation that forms at the 7th exon of the MTHFR coding gene. The frequency of the C677T mutation has been

Table 4. Results of multivariate logistic regression for CAD and non-CAD groups.

	CAD (n=234) Median (min-max)	non-CAD (n=208) Median (min-max)	p	OR*	95% CI
Age	56.1± 9.6 55 (36-75)	52.2± 10.1 52 (34-75)	<0.001	1.074	1.048-1.101
Smoking					
Non-Smoker	106 (45.7)	116 (55.8)		1	
Smoker	66 (28.4)	48 (23.1)	<0.001	3.191	1.812-5.622
Quit Smoking	60 (25.9)	44 (21.2)		2.295	1.324-3.977
Alcohol					
No	220 (94)	186 (89.4)	0.050	1	
Yes	14 (6)	22 (10.6)		0.446	0.199-0.999
Diabetes Mellitus					
No	186 (79.5)	184 (88.5)	0.007	1	
Yes	48 (20.5)	24 (11.5)		2.276	1.255-4.126
FactorV G1691A					
GG	220 (94.0)	200 (96.2)		1	
GA	14 (6.0)	8 (3.8)	0.042	3.322	1.043-10.582
AA	0 (0.0)	0 (0.0)		NA**	
MTHFR A1298C					
AA	88 (37.6)	110 (52.9)		1	
AC	126 (53.8)	82 (39.4)	0.011	1.973	1.265-3.076
CC	20 (8.5)	16 (7.7)		1.467	0.652-3.302
FactorXIIIAVal34Leu					
Val/Val	210 (89.7)	158 (76.0)		1	
Val/Leu	24 (10.3)	46 (22.1)	0.050	0.467	0.253-0.861
Leu/Leu	0 (0.0)	4 (1.9)		NA	
FactorXIIIBHis95Arg					
His/His	214 (91.5)	162 (77.9)		1	
His/Arg	18 (7.7)	38 (18.3)	<0.001	0.177	0.087-0.360
Arg/Arg	2 (0.9)	8 (3.8)		0.052	0.008-0.322

Age-adjusted ORs are given.
* OR = Odd Ratios
**NA = Not Applicable

shown to vary among the different ethnic groups in the world [22,23]. Madgy et al. [23] found the prevalence of CC variant 45%, CT and TT variants 35% and 20%, respectively. The prevalence of C677T variant has also been studied in a healthy population in Turkey and the frequencies of C677T heterozygosity and homozygosity were reported as 47.4% and 9.6%, respectively [24]. Our results were compatible with the prevalence rates observed in this study. However, our results do not confirm studies which suggested that this variant is a risk factor for CAD. There might be another underlying cause and homozygote samples were not sufficient. In another study the prevalence of A1298C variant for heterozygote and homozygote CAD patients was found 51.5% and 9.1%, respectively. They found no association between this MTHFR variant and CAD [25]. The multivariate logistic regression analysis for A1298C variant in our study showed that the AC genotype had 1.9-fold risk and the CC genotype had 1.4-fold risk for CAD than the AA genotype. The odds ratios were important statistically.

The point mutation in codon 34 of the factor XIIIA subunit has been reported to protect against AMI. Some

cross-sectional studies showed that Val34Leu genotype was associated with reduced risk of CAD [26,27]. Doggen et al. [28] indicated that the heterozygote Val34Leu in factor XIIIA gene was not associated with a decreased risk of AMI in heterozygote carriers. They found the risk of AMI was decreased 0.3-fold in homozygote carriers. In another study, the main finding was the identification of an independent molecular prognostic factor (factor XIIIA L34-allele) against the occurrence of future adverse cardiac events in patients after AMI with no effects on major bleeding complications [10]. Aleksic et al. [27] found no decrease in the risk of heterozygote or homozygote for the factor XIIIA ValLeu34 allele in their study. In our study the multivariate logistic regression showed that for factor XIIIA the Leu allele reduced the risk of CAD 0.4-fold. The risk could not be calculated for homozygote Leu genotype because of the insufficient number of samples.

Gemmati et al. [12] investigated the effects of factor XIIIB in patients after AMI. They did not demonstrate a relationship between factor XIIIB and CAD in their study. When put into multivariate logistic regression model, the risk for His/Arg was reduced 0.2-fold and for homozygote Arg genotype the risk reduced 0.05-fold for CAD. According to these results factor XIII A and B polymorphisms might have a protective role in CAD.

Many studies showed that Apo E mutation was an independent risk factor for CAD [29]. The three common alleles (e2, e3, e4) produce three homozygote and three heterozygote genotypes [30]. It is found that the frequencies of e2, e3 and e4 alleles are relatively constant in adult Caucasians as 8%, 78% and 14%, respectively [30]. These allele frequencies were compatible with our results. Eichner et al. [29] found an association between e4 allele and the risk of CAD. Another study pointed out that e4 allele increased 4-fold the risk of CAD in the Southern Turkey [30]. We could not find an association between this allele and CAD. A case control study reported that the e2/e3 genotype was a protective factor for CAD [31]. In our study the e2/e3 genotype was found in 35.6% of non-CAD group and 24.8% of CAD group. But these results were not significant statistically because of the insufficient sample number.

In conclusion, factor XIIIA Val34Leu and factor XIIIB His95Arg polymorphisms were found protective against CAD and FV Leiden mutation, MTHFR 1298 AC genotype could have an important role in the progression of CAD. However, these results require confirmation in a larger study group.

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Conflict of Interest: Authors declare no conflict of interest

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