ORIGINAL ARTICLE

The effects of genetic polymorphisms and diabetes mellitus on the development of peripheral artery disease

Periferik arter hastalığı oluşumunda genetik polimorfizmin ve diabetes mellitusun etkisi

Zafer Yalım, M.D.,[1] Serap Tutgun Onrat, M.D.,[2] Sümeyra Alan Yalım, M.D.,[3] Mustafa Aldemir, M.D.,[4] Alaettin Avşar, M.D.,[1] İsmet Doğan, M.D.,[5] Ersel Onrat, M.D.[1]

[1]Department of Cardiology, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey; [2]Department of Medical Genetics, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey; [3]Department of Internal Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey; [4]Department of Cardiovascular Surgery, Health Sciences University, Bursa Higher Specialization Training and Research Hospital, Bursa, Turkey; [5]Department of Bioistatistic, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

ABSTRACT

Objective: Peripheral artery disease (PAD) is a condition caused by the narrowing of limb arteries due to atherosclerosis. In recent years, polymorphisms in a number of genes have been shown to contribute to the risk of PAD development. However, whether the contribution of these inheritable factors is independent of traditional cardiovascular risk factors remains unclear. This study was an investigation of the effects of diabetes mellitus (DM) and genetic background, examined singly and together, on the pathogenesis of PAD.

Methods: The effects of the factor V Leiden (G1691A), factor V H1299R, prothrombin G20210A, factor XIII V34L, B-fibrinogen -455 G>A, PAI-1 4G/5G, HPA1, MTHFR C677T, MTHFR A1298C, ACE I/D, APO B R3500Q, and APOE polymorphisms were evaluated using a cardiovascular disease strip assay (CVD StripAssay). Two groups were created: 100 patients with PAD (50 with DM, 50 without DM) and 60 controls without PAD (30 with DM, 30 without DM).

Results: There was a significantly greater presence of the *MTHFR A1298C* and *PAI 4G/5G* homozygous polymorphisms in the PAD patients compared with the control group (p=0.035, p=0.004, respectively). There were no significant associations between the other genotypes and polymorphism frequencies. In the presence of DM, the *PAI-1 4G/5G* homozygous polymorphism was linked to the formation of PAD (p=0.021). Regression analysis indicated that the PAI-1 4G/5G gene homozygous polymorphism demonstrated a 17.1 times greater risk for DM with PAD [95% confidence interval (CI): 2.113-138.660; p=0.008] and the *MTHFR A1298C* homozygous polymorphism demonstrated a 316.6 times greater risk (95% CI: 10.763-9315.342; p<0.001) for the possibility of DM with PAD.

Conclusion: The *MTHFR A1298C* and *PAI 4G/5G* homozygous polymorphisms may be associated with the development of PAD. The presence of the *PAI 4G/5G* homozygous polymorphism with DM was a powerful predictor for the development of PAD.

ÖZET

Amaç: Periferik arter hastalığı (PAD), ateroskleroz nedeniyle ekstremite arterlerinin daralmasından kaynaklanan bir durumdur. Geçtiğimiz yıllarda, birkaç gende polimorfizmin PAD gelişme riskine katkıda bulunduğu gösterilmiştir, ancak geleneksel kardiyovasküler risk faktörlerinden bağımsız olan bu kalıtımsal faktörlerin katkısı hala belirsizliğini korumaktadır. Bu çalışmada, diabetes mellitus (DM) ve genetik arkaplanın PAD patogenezinde sadece ve birlikte etkilerini araştırdık.

Yöntemler: Çalışmamızda faktör V Leiden (G1691A), Faktör V H1299R, Protrombin G20210A, Faktör XIII V34L, B-Fibrinojen-455 G> A, PAI-1 4G/5G, HPA1, MTHFR C677T, MTHFR A1298C, ACE I/D, APO B R3500Q ve APOE polimorfizminin kardiyovasküler hastalık Strip panel kullanılarak etkilerini değerlendirmeyi planladık. Hastalar iki gruba ayrıldı: PAD'lı 100 hasta (50 DM+, 50 DM-), PAD olmayan 60 kontrol hasta (30 DM+, 30 DM-).

Bulgular: *MTHFR A1298C* ve *PAI 4G/5G* homozigot polimorfizmi, PAD hastalarında kontrol grubuna göre anlamlı derecede yüksek bulundu (sırasıyla, p=0.035, p=0.004). Diğer genotipler ve polimorfizm sıklıkları arasında anlamlı bir ilişki bulunamamıştır. Ayrıca DM varlığında *PAI 4G/5G* homozigot polimorfizmi PAD oluşumunu etkili olduğu belirlendi (p=0.021). Regresyon analizinde PAD oluşumunda DM+ hastalarda *PAI-1 4G/5G* gen homozigot polimorfizmi 17.1 kat daha riskli, (p=0.008) Güven Aralığı (GA) %95 (2.113–138.660), *MTHFR A1298C* homozigos polimorfizmi 316.6 kat daha riskli (p<0.001) GA %95 (10.763–9315.342) olduğu görüldü.

Sonuç: *MTHFR A1298C* ve *PAI 4G/5G* homozigoz polimorfizmi, PAD oluşumu ile ilişkili olabilir. *PAİ 4G/5G* homozigot polimorfizmi, DM varlığında PAD oluşumunda güçlü bir belirleyicidir.

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Correspondence: Dr. Zafer Yalım. Afyonkarahisar Sağlık Bilimleri Üniversitesi, Kardiyoloji Anabilim Dalı, Afyonkarahisar, Turkey.
Tel: +90 272 - 246 33 00 e-mail: zaferyalm@yahoo.com.tr

Peripheral arterial disease (PAD) is defined as the narrowing and obstruction or aneurysmal dilatation of the antegrade flow of major systemic arteries other than those of the cerebral and coronary arteries. PAD is also a marker for atherothrombotic disease in other vascular beds. There are many causes of PAD, including vasculitis, dysplastic syndromes, degenerative conditions, hypercoagulopathy, vascular dissection, vascular compression syndromes, and cardiac or vascular thromboembolism; however, the most common cause is atherosclerosis.[1,2]

PAD occurs most frequently in the lower limbs and causes a range of clinical conditions. Most cases are asymptomatic, but even so, it must be kept in mind that clinically silent PAD disease can increase vascular morbidity and mortality. The most common symptom of PAD is intermittent claudication, which is defined as pain in the legs and thighs that increases with walking exercise and is relieved with rest. More extreme presentations of PAD include rest pain, tissue loss, or gangrene. These limb-threatening manifestations of PAD are collectively termed critical limb ischemia.[3]

PAD is frequently concurrently observed in patients with advanced coronary and cerebrovascular diseases, leading to elevated rates of stroke, myocardial infarction, and death among individuals with PAD.[4] The major risk factors for PAD are smoking, hypertension, hyperlipidemia, diabetes mellitus (DM), obesity, and a family history of vascular disease, and smoking is the strongest factor.[5] Smoking and DM are the strongest predictors of morbidity and mortality, and each confers a more than 2.5 times greater risk of mortality and major morbidity from PAD.[6] The remaining risk is accounted for by other unmeasured environmental and genetic components.[7,8]

The presence of DM in patients with PAD is significant. In people with diabetes, the risk of PAD increases with age, duration of diabetes, and the presence of peripheral neuropathy. It is important to note that diabetes is most strongly associated with femorapopliteal and tibial (below the knee) PAD, whereas other risk factors (e.g., smoking and hypertension) are associated with more proximal disease in the aorto-iliofemoral vessels. Both symptomatic and asymptomatic PAD is associated with increased cardiovascular morbidity and mortality. Therefore, effective diagnosis and treatment of the disease could be expected to reduce cardiovascular morbidity and mortality.[9] Variation in genetic structure may be an important determinant of the interaction of PAD and DM. Previous studies have identified polymorphisms in genes that contribute to the extent of atherosclerosis, but we have much less information about which polymorphisms in genes influence PAD.

Abbreviations:

ABI	*Ankle-brachial pressure index*
CAD	*Coronary artery disease*
CRP	*C-reactive protein*
CVD	*Cardiovascular disease*
DM	*Diabetes mellitus*
LDL	*Low-density lipoprotein*
PAD	*Peripheral artery disease*
PCR	*Polymerase chain reaction*
PTCA	*Percutaneous transluminal coronary angioplasty*

PAD is a complex disorder from a genetic standpoint. Unlike monogenic vascular syndromes, such as Marfan and Loeys-Dietz syndromes, with a Mendelian inheritance pattern, atherosclerotic PAD likely results from dozens or hundreds of genes interacting with each other and the environment.[10,11] Genetic susceptibility to PAD is likely due to successive variants in multiple genes, each with modest effects. Although many of these variants probably alter susceptibility to both PAD and coronary artery disease (CAD), there is also a set of variants specific to PAD susceptibility.[12] A candidate gene approach allows for a search for an association between a specific variant in a specific gene (e.g., a single-nucleotide polymorphism) and a clinical phenotype [generally defined by the low ankle-brachial index (ABI) in PAD patients]. Such polymorphisms may alter a gene's expression by affecting the binding of the required transcription factors, impairing stability or intracellular trafficking of mRNA transcription, or limiting the ability to be translated into a functional protein. Often, the polymorphism may be linked to another gene responsible for the disease.[13,14] An activated clotting system contributes to the development of late luminal narrowing after percutaneous transluminal coronary angioplasty (PTCA). A number of genetic factors that influence hemostasis have been identified, including β-*fibrinogen–455 G/A, PAI-1 4G/5G,* and factor *V Leiden 1691 G/A* gene polymorphisms.[15] The β-*fibrinogen–455 G/A*, and *PAI-1 4G/5G* genotypes have been associated with CAD and myocardial infarction.[15,16]

PAD is a complex disorder with many genetic and environmental risk factors and as yet there is no definite disease-modifying treatment. Revealing genetic

modifiers in PAD may lead to potential treatment options for gene-specific personalized treatment in the future. In this study, a gene panel related to vascular pathology: *factor V Leiden (G1691A), factor V H1299, prothrombin G20210A, factor XIII V34L, B-fibrinogen -455 G>A, PAI-1 4G/5G, HPA1 1, MTHFR C677T, MTHFR A1298C, ACE 1, Apo B R3500Q,* and the *APOE* gene polymorphisms, was evaluated in 100 PAD patients and 60 controls without PAD. The aim of this study was to examine the effects of DM and genetic background, singly and together, on the pathogenesis of PAD.

METHODS

Determination of the patient group

This study was performed between March 2014 and December 2017 in the Medical Genetics, Cardiology and Cardiovascular Surgery Departments of Afyonkarahisar Health Sciences University Faculty of Medicine. The study was approved by the Afyon Kocatepe University Faculty of Medicine Ethics Committee (no: 2014-282) and all of the participating individuals signed an informed consent form. In all, 100 patients with PAD and 60 control patients without PAD were included in this study. Patients with DM made up half of the patient and control groups (50 patients with DM and PAD, 30 patients with DM and without PAD). The groups were designed to determine the effect of DM.

The patients were selected from among subjects admitted to the outpatient clinic in the Cardiology and Cardiovascular Surgery Department of Afyonkarahisar Health Sciences University. The patient group consisted of those who presented with complaints suggestive of PAD. A definitive diagnosis of the disease was made with conventional peripheral angiography. At least 30% stenosis was accepted as PAD. However, the majority of patients had severe, critical lesions. The location of the lesions was classified as iliofemoral and popliteotibial based on the results of angiographic evaluation.

Determination of the control group

The control group comprised individuals without PAD symptoms or findings and an ABI measurement in the normal range (0.9–1.3). The patients in the control group had demonstrated normal vascularity via Doppler ultrasound.

Risk factors for atherosclerosis were identified by performing routine biochemical examinations of the patients and controls. Classic risk factors are age, gender, smoking, hypertension, family history, and hyperlipidemia. The following definitions were used: hypertension: blood pressure ≥140/90 mmHg and/or use of antihypertensive treatment, hypercholesterolemia: total plasma cholesterol level of >200 mg/dL, plasma low-density lipoprotein (LDL) cholesterol level of ≥130 mg/dL, triglyceride level of ≥150 mg/dL, HDL cholesterol level in males ≤40 mg/dL and ≥50 mg/dL in females and/or lipid lowering agents, DM: fasting plasma glucose 126 mg/dL (6.94 mmol/L) and/or glucose-lowering treatment. Smokers were defined as participants who reported smoking currently and regularly (at least 5 cigarettes per day). The ischemic or hemorrhagic nature of cerebrovascular events was investigated. Patients who had angiographically detected coronary artery stenosis, patients who underwent PTCA or stenting, and patients who had undergone coronary bypass surgery were defined as having atherosclerotic heart disease. The body mass index was calculated as weight in kilograms divided by the square of height in meters.

DNA isolation, PCR, and reverse hybridization

DNA samples were collected by the cardiology department and sent to the medical genetics department. The genomic DNA of 160 samples was extracted from anticoagulated fresh blood and ethylenediaminetetraacetic acid, using either a cardiovascular disease (CVD) strip assay lysis solution (CVD StripAssay; ViennaLab Diagnostics GmbH, Vienna, Austria) and GENTRACT resin (ViennaLab, Diagnostics GmbH, Vienna, Austria) or the QIAamp DNAblood Midi (Qiagen, Hilden, Germany) extraction kit, which uses a silica membrane-based DNA purification method that can yield up to 60 mg of DNA from an initial 2 mL of blood. The manufacturer's instructions were followed in all cases. Use of a CVD strip assay to screen for *PAI-1* gene polymorphisms with the reverse hybridization principle has already been reported. A number of target gene sequences were concurrently amplified and labeled with biotin in a single amplification reaction. The reaction was effected by adding 0.1 mg DNA to 15 mL of polymerase chain reaction (PCR) amplification mixture that was previously prepared. This mixture

included primers that flanked the target sequences and deoxyribonucleotide triphosphates in the presence of 1 unit of Taq polymerase. PCR cycles were optimized as follows: 2 minutes initial denaturation at 94°C followed by 35 cycles of amplification (15 seconds denaturation at 94°C, 30 seconds annealing at 58°C, and 30 seconds extension at 72°C), and a final extension for 3 minutes at 72°C. The amplification products were denaturated and selectively hybridized to a test strip containing allele-specific oligonucleotide probes (wild type and mutant) and immobilized as an array of parallel lines. Biotin-bound sequences were detected using streptavidin alkaline phosphatase and color substrates.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 22.0 software (IBM Corp., Armonk, NY, USA). Variables were investigated using visual and analytic methods to determine normal distribution. Descriptive analyses are presented using the mean±SD for normally distributed variables. A chi-square test was used to compare nominal and categorical variables (all genes, gender, hypertension, DM, smoking, hyperlipidemia, CAD, family history, congestive heart failure). The mean value of variables with normal distribution was tested with a t-test, and abnormally distributed variables were tested with the non-parametric Mann-Whitney U test. Hardy-Weinberg equilibrium was tested for each genotype within groups with a chi-square test. Univariate and multivariate analysis using a logistic regression model was performed to determine genotype relationships in PAD and odds ratios (OR) with a 95% confidence interval (CI) were calculated. A p value of <0.05 was considered statistically significant.

RESULTS

Demographic characteristics of the participants

The mean age of the PAD patients and the control group was 64.3±1.1 years and 61.1±1.8 years, respectively. There were significant differences between the groups with regard to the variables of gender, smoking, hyperlipidemia, amputation, and C-reactive protein (CRP). The demographic characteristics of the participants in the study are summarized in Table 1.

Comparison of the genetic study results

The assessment of gene polymorphism results for *factor VG1691A (Leiden)* (p=0.067) and prothrombin *G20210A* (p=0.078) between the 2 groups did not reveal a difference that reached statistical significance. However, there was a significant difference in the *PAI-1 4G-5G* polymorphism results (p=0.004), which was due to a higher homozygous polymorphism rate in the group with PAD (+). This result is presented in Figure 1. The *MTHFR A1298C* gene also yielded a significant difference (p=0.035). No significant differences were observed between the groups with respect to the other gene polymorphisms. The PAD (+) and PAD (-) group comparison results are summarized in Table 2.

Other analyses

The incidence of the *PAI-1 4G/5G* homozygous polymorphism was greater in the PAD (+) and the DM (+) groups than in the PAD (-) and the DM (+) groups (p=0.021). The genotypes and allele frequencies of all of the gene groups were analyzed using the Hardy-Weinberg calculation. Genes found to be significant are shown in Table 3.

Regression analysis was performed for the patient and control groups due to the presence of several variables that may affect the diagnosis of PAD. Risk factors and genetic polymorphisms were identified as categorical variables. The estimated proportion of patient relative risk (OR) and 95% CI were determined. The results of the regression analysis comparing the PAD (+) and PAD (-) groups are summarized in Table 4.

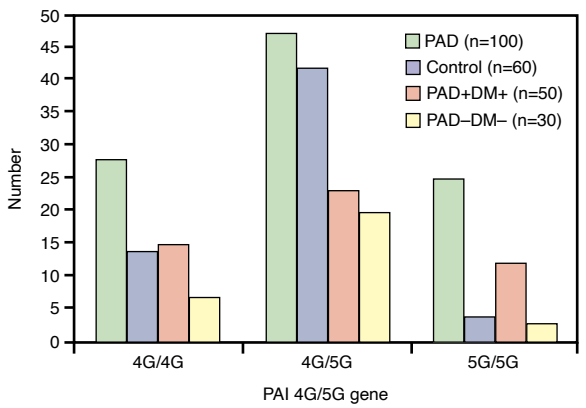

Figure 1. Results of the PAI gene comparison between study patients. DM: Diabetes mellitus; PAD: Peripheral artery disease.

Table 1. Demographic features of the study participants

Values	PAD (n=100)	Control (n=60)	*p* value
Gender			
Male	85	32	<0.001*
Female	15	28	
Age (years)	64.3±1.1	61.1±1.8	0.675
Body mass index	29.36±0.34	29.06±0.57	0.337
Diabetes mellitus	50	30	1.000
Hypertension	54	33	0.902
Smoking	67	16	<0.001*
Hyperlipidemia	47	17	0.024*
Coronary artery disease	68	33	0.099
Congestive heart failure	17	15	0.221
Chronic renal failure	15	6	0.365
Cerebrovascular disease	4	2	0.831
Lung disease	12	7	0.952
Amputation	20	0	<0.001*
Fasting glucose (mg/dL)**	131±4.8	135±5.2	0.497
C-reactive protein (mg/dL)**	3.35±0.42	1.06±1.4	<0.001
Hemoglobin (g/dL)	12.7±0.22	12.6±0.25	0.396
Creatinine (mg/dL)	1.22±0.1	1.15±0.14	0.685
Total cholesterol (mg/dL)	172±4.2	170±7	0.532
Triglyceride (mg/dL)	153±9.2	147±10.5	0.481
Low-density lipoprotein (mg/dL)	115±3.4	107±6.1	0.343
High-density lipoprotein (mg/dL)	37.7±1.05	39.3±1.6	0.217

* Chi-square test, p<0.05 statistical significance; **Mann-Whitney U test, mean±SD. PAD: Peripheral artery disease; SD: Standard deviation.

DISCUSSION

Atherosclerosis is a result of injuries that lead to endothelial dysfunction followed by chronic inflammation of the arterial wall. Not surprisingly, there is substantial overlap between the pathogenesis of PAD and other forms of systemic atherosclerotic diseases, such as CAD. PAD and CAD share several risk factors, and approximately 70% of PAD cases (as defined by the ABI) can be attributed to these risk factors. Some of these risk factors have proportionately greater effects on the development of PAD than CAD. For instance, DM and smoking are particularly strong risk factors for PAD. However, even in the presence of traditional risk factors, the progression of PAD seems to be highly variable, which suggests the presence of other determinants of the disease. At least some are probably inherited, and thus genetic in nature.

Other research[17,18] has indicated that PAD appears to develop about 10 years earlier in smokers. In our study, smoking was one of the most important factors to influence the development of PAD; we found a significantly higher prevalence of PAD in smokers. In the presence of other major risk factors for atherosclerosis, hyperlipidemia plays a very important role in increasing the incidence of PAD.[19] In our study, we found a significant association between hyperlipidemia and PAD. However, this was not statistically significant with respect to serum triglycerides, total cholesterol, LDL, or HDL levels. The majority of the patients were receiving statin therapy, which decreases the LDL cholesterol level, and this therapy was not interrupted by the clinicians. Obesity, central obesity, hypercholesterolemia, high LDL cholesterol, and hypertriglyceridemia were not found to be associated with the incidence of PAD, as other authors have suggested.[20,21]

Table 2. Genotypes in the PAD (+) and PAD (-) groups

Genes	Genotype	PAD + (n=100)	Control (n=60)	*p* value
Factor V Leiden (G1691A)	Wild type (GG)	81	55	0.067
	Heterozygote (GA)	19	5	
	Polymorphic (AA)	0	0	
Factor V H1299R	Wild type (HH)	84	48	0.519
	Heterozygote (HR)	16	12	
	Polymorphic (RR)	0	0	
Prothrombin G20210A	Wild type (GG)	95	60	0.078
	Heterozygote (GA)	5	0	
	Polymorphic (AA)	0	0	
Factor XIII V34L	Wild type (VV)	67	37	0.472
	Heterozygote (VL)	29	22	
	Polymorphic (LL)	4	1	
B-fibrinogen -455 G>A	Wild type (GG)	48	36	0.207
	Heterozygote (GA)	46	23	
	Polymorphic (AA)	6	1	
PAI-1 4G/5G	Wild type (4G/4G)	28	14	0.004*
	Heterozygote (4G/5G)	47	42	
	Polymorphic (5G/5G)	25	4	
HPA1 a/b	Wild type (AA)	83	50	0.531
	Heterozygote (AB)	15	10	
	Polymorphic (BB)	2	0	
MTHFR C677T	Wild type (CC)	50	26	0.528
	Heterozygote (CT)	36	27	
	Polymorphic (TT)	14	7	
MTHFR A1298C	Wild type (AA)	39	27	0.035*
	Heterozygote (AC)	47	32	
	Polymorphic (CC)	14	1	
ACE I/D	Wild type (II)	20	15	0.732
	Heterozygote (ID)	50	27	
	Polymorphic (DD)	30	18	
APOB R3500Q	Wild type (RR)	100	60	.[a]
	Heterozygote (RQ)	0	0	
	Polymorphic (QQ)	0	0	
APOE genotype	2/2	1	0	0.864
	2/3	23	11	
	2/4	3	1	
	3/3	63	40	
	3/4	8	7	
	4/4	2	1	

*Chi-square test, p<0.05 statistical significance; [a]APO B is a constant. PAD: Peripheral artery disease.

The CRP level is a marker of active inflammation and has been found to be elevated in atherosclerosis.[22] Consistent with this finding, we a found significantly higher level of CRP in our PAD group. This

Table 3. Genotypes and allele frequencies analyzed with the Hardy-Weinberg Equation

Gene	Genotype	Observed	Expected	CHI2	Allele frequency		*p* value
					p	q	
DM+ PAD- patients							
β-fibrinogen -455G>A	GG	22	25.21	4.846	0.71	0.29	0.027*
	GA	27	20.59				
	AA	1	4.21				
DM+ PAD- patients							
HPA1 a/b	aa	43	41.41	7.585	0.91	0.09	0.0059*
	ab	5	8.19				
	bb	2	0.41				
DM+ PAD+ patients							
PAI-1 4G-5G	4G/4G	7	9.63	3.833	0.57	0.43	0.050
	4G/5G	20	14.73				
	5G/5G	3	5.63				

*P≤0.05. DM: Diabetes mellitus; PAD: Peripheral artery disease.

Table 4. Regression analysis results of groups with and without PAD

Data	*p* value	OR	Confidence interval (95%)
Smoking	<0.001	31.525	(6.052–164.217)
Family history	0.018	7.143	(1.395–36.583)
Factor V G1691A (Leiden) Heterozygote	0.005	21.521	(2.537–182.561)
Factor XIII V34L Heterozygote	0.047	21.27	(0.061–0.983)
β-fibrinogen -455G>A Homozygote	0.020	29.606	(1.719–509.824)
PAI-1 4G-5G Homozygote	0.008	17.118	(2.113–138.660)
MTHFR A1298C Homozygote	0.001	316.647	(10.763–9315.342)
APOE 2/3 genotype	0.026	7.015	(1.270–38.762)

*P<0.05.

significance was particularly dramatic in diabetics with PAD.

During platelet activation, *PAI-1* is released and stored by endothelial cells. In the event of vascular injury, *PAI-1* plays an important role in thrombus stabilization and the wound healing process.[17] *PAI-1* downregulation results in the inhibition of the conversion of plasminogen to plasmin by influencing the fibrinolysis process.[18] *PAI-1* is associated with vascular inflammation and atherosclerosis, especially in obesity, type 2 DM, and metabolic syndrome.[19] One study demonstrated that the *PAI-1 4G/5G* polymorphism was associated with CAD.[23] In our study, the incidence of the *PAI-1 5G/5G* genotype was significantly greater in the PAD group compared with the control group (p=0.004). In addition, we found that the *4G/5G* genotype was the most common polymorphism, with a rate similar to other studies in the literature.[24] In addition, the *PAI-1 5G/5G* polymorphism was significantly greater in patients with diabetic PAD (p=0.021). DM with the *PAI-1 5G/5G* genotype increases the risk of PAD development. Regression analysis revealed that the *PAI-1* gene polymorphism caused a 17-fold increased risk for PAD development. With these findings, it would be reasonable to suggest say that polymorphisms in the *PAI-1* gene have an effect on PAD development.

The human *MTHFR* gene is localized at chromosome 1p36.3 and comprises 11 exons.[22,25] The second most common genotype is A1298C, while the most common polymorphism seen is the *MTHFR C677T* genotype.[26] The *MTHFR C677T* is a well-described polymorphism of the *MTHFR* enzyme, and elevated homocysteine levels have been described as a characteristic of carriers of the *677 C>T* allele. An elevated plasma homocysteine level may promote vascular disease through endothelial injury, predisposing vessels to atherosclerosis. Several studies have found a significant positive association between the *MTHFR C677T* polymorphism and PAD.[27–29] However, studies showing the effect of the *MTHFR A1298C* allele on PAD formation remain insufficient. In our study, which compared groups with and without PAD, there were significant differences in the *MTHFR A1298C* homozygous polymorphism results (p=0.035). When we adjusted this gene group with regression analysis for cardiovascular risk factors with respect to the presence of homozygous polymorphisms, we found that there was a 316.6 times greater probability than that seen before the adjustment (p=0.001). We did not find a significant difference between the 2 groups with respect to the *MTHFR C677T* polymorphism. We think this may be due to the likelihood that the control group was not entirely composed of healthy individuals and the relatively small number of patients.

The *prothrombin G20210A* polymorphism of plasma prothrombin increases the tendency to venous thrombosis.[30] The incidence of the *G20210A* polymorphism in the general population and venous thromboembolism cases have been reported as 2% and 9%, respectively. A study of the *prothrombin G20210A* allele indicated that the myocardial infarction risk was 40 times greater in smokers.[31,32] Particularly in young patients, myocardial and cerebral infarction have been found to be associated with the *factor V Leiden* and the *prothrombin G20210A* polymorphisms.[33,34] In a study with 433 patients with PAD and a control group, it was reported that there were no differences in terms of the gene polymorphisms of factor V Leiden, *prothrombin G20210A* and *MTHFR C677T*.[35] In our study, we found that there was a difference between groups with respect to the *factor V Leiden* and *prothrombin G20210A* heterozygous polymorphisms, but this was not statistically significant (respectively, p=0.067 and p=0.078). A larger number of cases may have led to statistical significance.

In some studies, an elevated fibrinogen level has been found to be consistently associated with arterial thrombotic disorders. Though the β-*fibrinogen 455G/A* polymorphism has been the most common polymorphism examined clinically, the relationship between arterial thrombotic disease and β-*fibrinogen 455G/A* remains undecided.[36] When our study group was adjusted for cardiovascular risk factors using regression analysis for β-f*ibrinogen 455G>A* for homozygous carriers of PAD, it was found to have a 29.6-fold greater probability (p=0.02).

There are 6 different *APOE* genotypes: 3 homozygous (*e2e2, e3e3, e4e4*) and 3 heterozygous (*e2e3, e3e4, e2e4*). The e3e3 genotype is considered to be the most common, with a prevalence of 62% in the general population. This result is similar to that observed in our study (64.4%).[37] *APOE* has many functions, including the transport of cholesterol and other lipids as a combination of various lipoproteins. An autosomal recessive disorder of *APOE* is associated with familial dysbetalipoproteinemia. This may be related to various diseases and conditions, and a link has been demonstrated to several alleles.[38,39] In our study, the *e3/3* genotype (64.4%) was the most frequent genotype. The second and third most frequent were the *e2/3* (21.2%) and the *e3/4* (9.4%), respectively. When cardiovascular risk factors were adjusted for using regression analysis, the *APOE e2/3* genotype was revealed to be a risk factor for PAD (OR: 7.015, 95% CI: 1.270–38.762; p=0.026).

Conclusion

Over the past decade, polymorphism in a number of genes has been shown to contribute to the risk of development of PAD. Our results indicated that the *MTHFR A1298C* and *PAI 4G/5G* homozygous polymorphisms could be considered significant risk factors for PAD. Also, in the presence of DM, the *PAI 4G/5G* homozygous polymorphism was associated with the development of PAD. We think that the results of our study shed some light on this area, which is still unclear.

Study limitations

The small number of cases is a significant limitation to this study. Research with a larger number of patients, as well as other contributions, is needed to support our findings.

Ethical statement: The study was approved by the Afyon Kocatepe University Faculty of Medicine Ethics Committee (no: 2014-282).

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Keywords: Diabetes mellitus; factor V G1691A; MTHFR A1298C; PAI-1 4G/5G; peripheral artery disease; polymorphism.

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