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Evaluation of genetic polymorphisms related to thrombophilia in patients with stroke in the Turkish population: A case-control study

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Keywords

Angiotensinogen; Endothelial Nitric Oxide Synthase; Methylenetetrahydrofolate Reductase; Stroke; Polymorphism

Abstract

Background: This study investigates the association between thrombophilia-related genetic variations and ischemic stroke risk in a Turkish population.

Methods: This study included 200 patients with ischemic stroke and 90 controls. Etiologies were classified using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) and Atherosclerosis, Small vessel disease, Cardiac source, Other cause (ASCO) criteria. Genetic analysis screened for methylenetetrahydrofolate reductase (MTHFR), factor V (FV), factor II, factor XIII, fibrinogen beta (FGB), integrin beta-3 (ITGB3), angiotensin-converting enzyme (ACE), angiotensinogen (AGT), angiotensin II receptor type 1 (AGTR1), endothelial nitric oxide synthase (NOS3), plasminogen activator inhibitor-1

(PAI-1), and protein C receptor (PROCR) variants using multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS).

Results: The MTHFR A1298C polymorphism was found to have a significant association with ischemic stroke in the recessive model [odds ratio (OR): 3.45, 95% confidence interval (CI): 1.157–10.332; P = 0.026]. According to the ASCO and TOAST classifications, significant associations were also identified between MTHFR polymorphisms and the cardioembolic and small vessel disease subtypes. The ACE polymorphism showed a significant association with cardioembolic stroke in both the TOAST and ASCO classifications (OR: 3.4, 95% CI: 1.0–10.8; P = 0.037 and OR: 2.7, 95% CI: 1.0–7.6; P = 0.047, respectively).

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Additionally, the NOS3 E298D polymorphism was associated with stroke risk in patients with hypertension (HTN), while the AGT M268T and NOS3 T-786C polymorphisms were linked to stroke risk in patients with coronary artery disease (CAD). No significant associations were observed for FV, factor II, factor XIII, PAI-1, PROCr, AGTR1, FGB, or ITGB3 variants.

Conclusion: MTHFR A1298C gene polymorphism was found to be significantly associated with increased stroke risk and stroke etiology.

Introduction

Stroke is the second leading cause of death worldwide, accounting for 5.5 million deaths annually.¹ It is also associated with high morbidity, as up to 50% of survivors suffer from varying degrees of disability.²⁻⁴ The cost of stroke care in European countries constitutes 3%-4% of total healthcare expenditures.⁵ Despite technological advances, the etiology of stroke remains largely unknown. For these reasons, clearly understanding the mechanism of stroke and providing appropriate preventive treatment are crucial.

Risk factors for stroke include non-modifiable factors such as gender, race, and age, and modifiable factors such as hypertension (HTN), diabetes, hyperlipidemia, and heart disease. Genetic factors can increase stroke risk through various mechanisms by influencing both modifiable and non-modifiable factors. First, specific single-gene disorders, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), can directly cause strokes. Second, complications of single-gene mutations, like sickle cell anemia, may lead to stroke. Third, common genetic polymorphisms, such as those at the 9p21 locus, have been implicated in stroke occurrence. Finally, genetic underpinnings of known stroke risk factors, including diabetes, HTN, and atrial fibrillation (AF), may indirectly augment stroke risk.^{6,7} The etiology of stroke is commonly classified into large-vessel atherosclerosis, cardioembolism, small vessel disease, strokes of unknown cause, and other stroke-associated conditions.^{8,9} Genetic factors influence all etiological categories with both direct and indirect effects. Whole-genome single nucleotide polymorphism (SNP) studies suggest that 37% of all ischemic strokes are heritable, with the prevalence being highest in large-artery atherosclerosis (40%), followed by cardioembolic stroke (33%), and lowest in small vessel disease

(16%).¹⁰ This underscores the significance of genetic factors in both known and unknown stroke etiologies.⁶

The present study aimed to investigate the associations of thrombophilia-associated genetic polymorphisms, including factor V Leiden (FVL), factor V (FV), FV Cambridge, prothrombin, methylenetetrahydrofolate reductase (MTHFR), plasminogen activator inhibitor-1 (PAI-1), coagulation factor XIII A chain (F13A1), fibrinogen beta chain (FGB), apolipoprotein B (APOB), apolipoprotein E (APOE), endothelial nitric oxide synthase (NOS3), angiotensin-converting enzyme (ACE), angiotensinogen (AGT), angiotensin II receptor type 1 (AGTR1), lymphotoxin alpha (LT α), integrin beta-3 (ITGB3), and protein C receptor (PROCcr), with ischemic stroke, its etiological subtypes, and established stroke risk factors. This study hypothesizes that genetic variations in these thrombophilia-related genes contribute significantly to ischemic stroke susceptibility and its etiological diversity.

Materials and Methods

This prospective case-control study was approved by the University's Pharmaceutical and Non-Medical Device Research Ethics Committee (Approval No. 2021/3366) and was supported by the Scientific Projects Management Unit (Project No. 211518016). All participants were informed about the study, and their written informed consent was obtained.

The study included 200 patients diagnosed with ischemic stroke, either in the acute or chronic phase, admitted to the Neurology Clinic of the University Hospital between 2019 and 2022. A control group of 90 individuals with no history of ischemic cerebrovascular events was also included. Exclusion criteria included: patients under 18 years of age, individuals with insufficient data for etiological subgroup classification, and patients who did not provide informed consent.

Demographic and clinical data for both the patient and control groups were obtained from the hospital registry system and supplemented with direct information provided by the patients or their relatives.

Brain imaging for patients with stroke was conducted using computed tomography (CT) or magnetic resonance imaging (MRI). Intracranial and extracranial vascular assessments were performed using CT angiography, magnetic resonance (MR) angiography, or doppler ultrasound.

Stroke etiology was classified using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria for causal classification and the Atherosclerosis, Small vessel disease, Cardiac source, and other cause (ASCO) criteria for phenotypic assessment. Statistical analyses were based on the ASCO classification, considering etiologies with high or moderate probability as the primary cause. Patients with multiple high-probability etiologies or those with undetermined causes were categorized into the 'unknown cause' group.^{8,9,11}

Genetic Analysis

Peripheral venous blood samples (3 ml) were collected from each participant into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Genomic deoxyribonucleic acid (DNA) was extracted using standard protocols. Targeted amplification of selected gene regions was performed using a multiplex polymerase chain reaction (PCR) approach.

Variant analysis was conducted by reverse in situ hybridization using the NextFlex Cardiovascular Diseases Amplicon Panel (NOVA-4255-02). Library preparation was subsequently completed, and sequencing was performed on the MiniSeq Sequencing System (Illumina, USA) based on next-generation sequencing (NGS) technology.

Sequencing data were analyzed using the SEQ

analysis platform (Genomize, Turkey). Genetic variation was assessed through the identification of sequence variants within predefined target regions rather than through population genetics-based diversity metrics, such as the average number of pairwise nucleotide differences or the number of segregating sites (Figure 1).

For statistical analysis, SPSS software (version 25.0, IBM Corporation, Armonk, NY, USA) was utilized. Descriptive statistics were reported using mean, median, quartiles, and standard deviation (SD). To assess the distribution normality of the variables, kurtosis, skewness values, and the Kolmogorov-Smirnov test were employed. The Student's t-test was used to compare normally distributed independent variables. Categorical data were analyzed using the chi-square test.

In addition to these tests, genetic models were applied to investigate the influence of specific alleles on the condition being studied. The dominant model assumed that a single copy of the allele was sufficient to affect the trait, combining heterozygotes with homozygotes for the dominant allele and comparing them to homozygotes for the recessive allele. Conversely, the recessive model required two copies of the allele to influence the trait, comparing homozygotes for the recessive allele against all others.

SNP ID	Gene Name	Chr	SNP Description
rs1801133	MTHFR	1	C677T, Methylenetetrahydrofolate reductase
rs1801131	MTHFR	1	A1298C, Methylenetetrahydrofolate reductase
rs6025	Factor V Leiden	1	Factor V
rs1800595	Factor V R2 (H1299R)	1	Factor V
rs118203906	Factor V Cambridge	1	Factor V
rs9574	PROCR, H1 haplotype/C	20	Protein C receptor
rs867186	PROCR, H3 haplotype/G	20	Protein C receptor
rs863223428	ACE	17	Angiotensin I converting enzyme
rs5186	AGTR1	3	Angiotensin II receptor type 1
rs699	AGT M268T	1	Angiotensin
rs1799889	PAI-1	7	Plasminogen activator inhibitor type 1
rs1799762	PAI-1	7	Plasminogen activator inhibitor type 1
rs1799983	NOS3 E298D	7	Nitric oxide synthase 3
rs2070744	NOS3 T-786C	7	Nitric oxide synthase 3
rs1799963	Factor II (G20210A)	11	Prothrombin F2
rs5985	Factor XIII V34L	6	Coagulation factor XIII
rs1800790	FGB -455 G>A	4	Fibrinogen
rs5918	ITGB3, L33P	17	Integrin beta-3
rs429358	APOE	19	Apolipoprotein E
rs1041981	LTA C804A(T60N)	6	Lymphotoxin alpha
rs5742904	Apolipoprotein B R3500Q	2	Apolipoprotein B

Figure 1. List of the genetic variants and single nucleotide polymorphisms (SNPs) analyzed in the study

Logistic regression analysis was then used to identify independent predictors, incorporating variables with a P-value < 0.10 in univariate analyses and those with clinical significance into the models. A 95% confidence interval (CI) was established for parameter estimation. A P-value of less than 0.05 was considered statistically significant for all group comparisons.

Results

The age and gender distributions were comparable between the patient and control groups ($P = 0.249$ and $P = 0.744$, respectively). The mean age of patients with stroke was 60.27 ± 13.30 years, and 43.5% were women, while controls had a mean age of 58.52 ± 11.20 years, with 45.6% being women. Regarding comorbidities, diabetes (36.5% vs. 23.3%; $P = 0.027$) and hyperlipidemia (51% vs. 30%; $P = 0.001$) were significantly more prevalent in patients with stroke compared to controls. Other comorbid conditions, including HTN (53.5% vs. 48.9%; $P = 0.467$) and coronary artery disease (CAD) (31.0% vs. 24.4%; $P = 0.255$), were also more frequent among patients with stroke, but the differences were not statistically significant. AF, heart valve disease, epilepsy, and migraine showed no significant differences between the groups ($P > 0.05$) (Table 1).

Stroke etiologies were classified using both the ASCO and TOAST systems. The phenotypic ASCO classification is shown in figure 2. Figure 3 illustrates the TOAST classification and the reclassification of the phenotypic ASCO categories into the causal ASCO groups. According to the TOAST classification, strokes of unknown etiology

were the most common subtype ($n = 65$, 32.5%), followed by cardioembolic strokes ($n = 52$, 26.0%) and small vessel disease ($n = 40$, 20.0%). The ASCO classification identified unknown etiology as the most frequent subtype ($n = 87$, 43.5%), followed by small vessel disease ($n = 49$, 24.5%) and cardioembolism ($n = 38$, 19%).

Among the genetic polymorphisms analyzed, the MTHFR A1298C polymorphism showed a significantly higher frequency in the stroke group under the recessive model ($P = 0.016$). Patients homozygous for this mutation were more prevalent in the stroke group (14%) compared to the controls (4.4%) (Table 2). Logistic regression analysis confirmed a strong association between the MTHFR A1298C homozygous mutation and ischemic stroke, with an odds ratio (OR) of 3.45 (95% CI: 1.157–10.332; $P = 0.026$) (Table 3).

To evaluate the relationship between genetic polymorphisms and stroke risk factors, ischemic stroke patients with specific risk factors were compared to a control group with the same risk factors. In the analysis comparing ischemic stroke patients with HTN to hypertensive controls, the NOS3 E298D polymorphism was found to be significantly associated with ischemic stroke, increasing the risk according to the dominant model (OR: 3.37, 95% CI: 1.02–11.01; $P = 0.045$). Similarly, in the comparison of ischemic stroke patients with CAD to the corresponding control group, the NOS3 T-786C and AGT M268T polymorphisms were shown to be associated with an increased risk of ischemic stroke by 4.38 (95% CI: 1.25–15.39; $P = 0.021$) and 4.99 times (95% CI: 1.14–21.72; $P = 0.032$), respectively.

Table 1. Comparison of demographic and comorbidity data between patients with stroke and controls

Variable	Control group (n = 90)	Patient group (n = 200)	P
Age (year)	58.52 ± 11.20	60.27 ± 13.30	0.249*
Gender			
Women	41 (45.6)	87 (43.5)	0.744**
Men	49 (54.4)	113 (56.5)	
Comorbidities			
Hypertension	44 (48.9)	107 (53.5)	0.467**
Diabetes	21 (23.3)	73 (36.5)	0.027**
Coronary artery disease	22 (24.4)	62 (31.0)	0.255**
Hyperlipidemia	27 (30.0)	102 (51.0)	0.001**
Atrial fibrillation	12 (13.3)	20 (10.0)	0.402**
Heart valve disease	3 (3.3)	18 (9.0)	0.093**
Epilepsy	1 (1.1)	6 (3.0)	0.303**
Migraine	1 (1.1)	4 (2.0)	0.591**

Data are presented as mean ± standard deviation (SD) or n (%).

*Student's t-test, **Chi-square test;

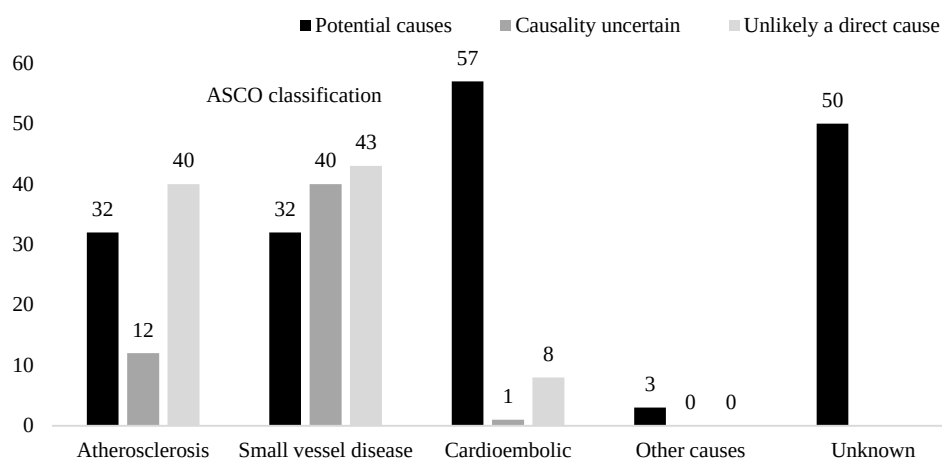


Figure 2. Phenotypic distribution of stroke etiologies and causality levels according to the Atherosclerosis, Small vessel disease, Cardiac source, other cause (ASCO) classification

However, no significant associations were identified between genetic polymorphisms and ischemic stroke in groups with diabetes, hyperlipidemia, or AF (Table 4).

The relationship between genetic polymorphisms and different stroke subtypes and etiologies was investigated based on ASCO and TOAST classifications. According to the ASCO classification, in the "Potential Cause" (PC) group for small vessel disease, the MTHFR C677T polymorphism was significantly associated with an increased risk of ischemic stroke (OR: 5.4, 95% CI: 1.2–24.2; $P = 0.024$).

In the cardioembolic stroke subgroup, which included both PC and "Causality Uncertain" (CU)

patients (PC + CU), MTHFR A1298C, MTHFR C677T, and ACE polymorphisms were associated with an increased risk of ischemic stroke by 7.7, 5.7, and 2.7 times, respectively ($P < 0.05$). Likewise, in the TOAST classification, the cardioembolic stroke group showed significant associations with MTHFR A1298C, MTHFR C677T, and ACE polymorphisms (Table 5).

Discussion

In the present study, we found that the MTHFR A1298C CC genotype was significantly more prevalent in patients with ischemic stroke than in the control group when using the recessive genetic model (multivariable OR: 3.45; $P < 0.05$).

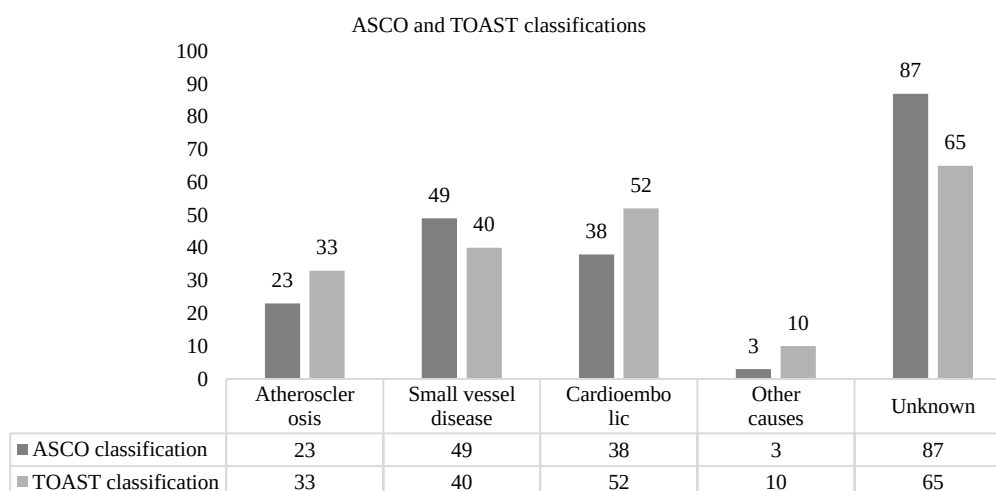


Figure 3. Distribution of stroke etiologies by Atherosclerosis, Small vessel disease, Cardiac source, other cause (ASCO) and Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classifications. For the ASCO, potential causes and uncertain causalities were considered primary causes. Patients presenting with two primary causes were classified as unknown.

Table 2. Distribution and comparison of thrombophilia gene polymorphisms and genetic variants in patients with ischemic stroke and controls

Gene/SNP	Dominant model [†]		P*	Recessive model [†]		P*
	Control [‡]	Patient [‡]		Control [‡]	Patient [‡]	
Folate metabolism-related genes						
MTHFR C677T	55.6	54.0	0.806	5.6	11.0	0.140
MTHFR A1298C	67.8	58.5	0.133	4.4	14.0	0.016
Coagulation-related genes						
Factor V Leiden	28.9	18.0	0.053	2.2	1.5	0.662
Factor V R2 (H1299R)	8.9	9.5	0.868	0.0	0.5	0.502
Factor V Cambridge	2.2	0.0	0.096	1.1	0.0	0.135
Factor II (G20210A)	4.4	5.5	0.707	0.0	0.5	0.502
Factor XIII V34L	22.2	21.0	0.814	3.3	1.5	0.310
FGB -455 G>A	35.6	37.0	0.813	3.3	4.5	0.644
ITGB3 L33P	27.8	24.0	0.493	2.2	3.5	0.562
Endothelial function-related genes						
PROCR H1 haplotype (C)	78.9	78.0	0.865	22.2	30.0	0.170
PROCR H3 haplotype (G)	25.6	27.0	0.797	3.3	1.0	0.158
NOS3 E298D	85.6	92.0	0.091	46.7	56.5	0.120
NOS3 T-786C	85.6	87.5	0.650	36.7	44.0	0.241
Renin-angiotensin system-related genes						
ACE (I/D)	63.3	62.5	0.892	13.3	14.5	0.792
AGTR1	42.2	44.5	0.718	3.3	8.5	0.108
AGT M268T	72.2	79.5	0.171	32.2	32.0	0.970
Fibrinolysis-related gene						
PAI-1 rs1799762	71.1	72.5	0.807	25.6	20.5	0.337

*Group comparisons were performed using the chi-square (χ^2) test; [†]Dominant models compare wild-type genotypes versus combined heterozygous and homozygous variant genotypes, whereas recessive models compare combined wild-type and heterozygous genotypes versus homozygous variant genotypes; [‡]Values are expressed as percentages (200 patients in the stroke group and 90 in the control group); Note: The following genes were not included in the table due to lack of allelic variation or insufficient genotype distribution for statistical comparison: Apolipoprotein E (APOE), apolipoprotein B (APOB), lymphotoxin alpha (LT α), and PAI-1 rs1799889.

ACE: Angiotensin-converting enzyme; AGTR1: Angiotensin II receptor type 1; AGT: Angiotensinogen; FGB: Fibrinogen; ITGB3: Integrin subunit beta 3; MTHFR: Methylene tetrahydrofolate reductase; NOS3: Nitric oxide synthase 3; PAI-1: Plasminogen activator inhibitor-1; PROCR: Protein C receptor; SNP: Single nucleotide polymorphism

The C677T and A1298C gene polymorphisms have a significant impact on MTHFR enzyme activity. These polymorphisms are linked to reduced enzymatic activity, lower folate concentrations in serum, plasma, and red blood cells, and increased plasma total homocysteine concentrations. It has been suggested that the

increase in plasma homocysteine concentration is less pronounced in individuals with the MTHFR A1298C gene polymorphism compared to those with the MTHFR C677T gene polymorphism.¹² Elevated levels of homocysteine may increase the risk of atherosclerosis by damaging the endothelial layer, causing inflammation and oxidative stress.¹³

Table 3. Associations between genetic polymorphisms and stroke: univariate and multivariable analyses comparing patients and controls

Gene/SNP	Univariate analysis		Multivariable analysis		R ²
	OR (95% CI)	P	OR (95% CI)	P	
MTHFR A1298C (recessive model [†])	3.500 (1.190–10.298)	0.023	3.450 (1.157–10.332)	0.026*	0.107
NOS3 E298D (dominant model [†])	1.942 (0.891–4.230)	0.095	1.937 (0.847–4.431)	0.117*	

*Genetic variants with P < 0.10 in the univariate analysis and variables considered clinically significant were included in the multivariable binary logistic regression model. Specifically, the model was adjusted for hyperlipidemia and diabetes due to their clinical relevance. [†]Dominant models compare wild-type genotypes versus combined heterozygous and homozygous variant genotypes, whereas recessive models compare combined wild-type and heterozygous genotypes versus homozygous variant genotypes.

CI: Confidence interval; MTHFR: Methylene tetrahydrofolate reductase; NOS3: Nitric oxide synthase 3; OR: Odds ratio

Table 4. The association between genetic polymorphisms and stroke risk factors

	SNP	Model	P	OR (95% CI)	R ²
Hypertension	NOS3 E298D	Dominant	0.045*	3.37 (1.02–11.01)	0.220
Diabetes	-	-	-	-	-
Coronary artery disease	NOS3 T-786C	Dominant	0.021*	4.38 (1.25–15.39)	0.287
	AGT M268T	Dominant	0.032*	4.99 (1.14–21.72)	
Hyperlipidemia	-	-	-	-	-
Atrial fibrillation	-	-	-	-	-

*Binary logistic regression test was used. Variables with $P < 0.10$ in univariate analysis, together with clinically significant variables, were selected for models.

AGT: Angiotensinogen; CI: Confidence interval; NOS3: Nitric oxide synthase 3; OR: Odds ratio; SNP: Single nucleotide polymorphism

Numerous studies have explored the relationship between MTHFR gene polymorphisms and stroke etiology in different populations. Jin et al. investigated the association between the MTHFR C677T polymorphism and homocysteine levels in

the Chinese population, finding that high homocysteine levels were linked to the TT, CT, and CC genotypes. They further suggested that the TT genotype was associated with large-vessel atherosclerosis in stroke etiology.¹⁴

Table 5. Comparison of genetic polymorphisms between a control group and stroke patient subgroups classified by Atherosclerosis, Small vessel disease, Cardiac source, and other cause (ASCO) and Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria

	SNP	Model	P	OR (95% CI)	R ²
Atherosclerosis (PC + CU) n = 44 (22.0%)	-	-	-	-	
Atherosclerosis (PC) n = 32 (16.0%)	-	-	-	-	
Small vessel disease (PC + CU) n = 72 (36.0%)	-	-	-	-	
Small vessel disease (PC) n = 32 (16.0%)	MTHFR C677T	Recessive	0.024*	5.4 (1.2–24.2)	0.341
Cardioembolic (PC + CU) n = 58 (29.0%)	MTHFR A1298C	Recessive	0.034*	7.7 (1.1–51.4)	0.557
	MTHFR C677T	Recessive	0.035*	5.7 (1.1–28.8)	
	ACE	Dominant	0.047*	2.7 (1.0–7.6)	
Cardioembolic (PC) n = 57 (28.5%)	MTHFR A1298C	Recessive	0.027*	9.8 (1.3–75.8)	0.602
	MTHFR C677T	Recessive	0.028*	6.5 (1.2–35.1)	
Others n = 3 (1.5%)	-	-	-	-	
Unknown cause n = 50 (25.0%)	MTHFR A1298C	Recessive	0.025*	5.2 (1.2–22.5)	0.367
TOAST					
Large vessel atherosclerosis n = 33 (16.5%)	-	-	-	-	
Small vessel disease n = 40 (20.0%)	-	-	-	-	
Cardioembolic n = 52 (26.0%)	MTHFR A1298C	Recessive	0.019*	12.6 (1.5–105.3)	0.628
	MTHFR C677T	Recessive	0.012*	10.3 (1.6–60.6)	
	ACE	Dominant	0.037*	3.4 (1.0–10.8)	
Others n = 10 (5.0%)	MTHFR A1298C	Recessive	0.002*	36.7 (3.8–354.2)	0.400
Unknown cause n = 65 (32.5%)	-	-	-	-	

*Binary logistic regression test was used. Variables with $P < 0.10$ in univariate analysis, together with clinically significant variables, were selected for models.

ACE: Angiotensin-converting enzyme; ASCO: Atherosclerosis, Small vessel disease, Cardiac source, and other cause; CI: Confidence interval; CU: Causality uncertain; MTHFR: Methylene tetrahydrofolate reductase; PC: Potential cause; OR: Odds ratio; SNP: Single nucleotide polymorphism; TOAST: Trial of ORG 10172 in Acute Stroke Treatment

Similarly, Chita et al. Reported a high prevalence of MTHFR gene polymorphisms in patients with cardioembolic stroke related to non-valvular AF.¹⁵ Rutten-Jacobs et al. found an association between the MTHFR C677T polymorphism and lacunar infarcts but noted no significant link to large artery disease.¹⁶

Salem-Berrabah et al. identified a significant relationship between high homocysteine levels and ischemic stroke in the Tunisian population, although they found no significant association between the T allele and stroke.¹⁷ Conversely, Mialovytska and Nebor demonstrated significant associations between both the T allele and TT genotype and ischemic stroke.¹⁸ Huang et al. similarly found a significant association between the T allele and ischemic stroke in a Chinese population, though no link to stroke severity was reported.¹⁹ Dong et al.'s meta-analysis highlighted a significant association between MTHFR A1298C polymorphisms and stroke risk in both dominant (OR = 1.19; P = 0.004) and recessive (OR = 1.43; P = 0.001) genetic models.²⁰ In contrast, our study found no significant association between MTHFR C677T and ischemic stroke. However, the MTHFR A1298C polymorphism was significantly higher in the recessive genetic model (OR: 3.45; P < 0.05). Additionally, the MTHFR C677T polymorphism was significant in cardioembolic etiology according to the TOAST classification and in both cardioembolic and small vessel disease etiologies according to the ASCO classification. Likewise, the MTHFR A1298C polymorphism was significant in cardioembolic and other etiologies in the TOAST classification and in cardioembolic and cryptogenic etiologies in the ASCO classification.

Activated FV, together with factor Xa, forms the prothrombinase complex, which markedly accelerates the conversion of prothrombin to thrombin. The subsequent inactivation of FV by activated protein C (APC) serves as a critical regulatory mechanism to prevent excessive and uncontrolled clotting.²¹ FV mutations can exert prothrombotic effects through two main mechanisms: by inducing resistance to APC and by acting as modifying variants that alter the functional balance of FV activity.^{21,22} The FV H1299R mutation causes a slight increase in APC resistance and elevates the concentration of the more thrombogenic FV isoform in plasma.²² A more direct but mild prothrombotic tendency is observed with the FV Cambridge mutation, which affects the Arg306 cleavage site, causing partial

impairment of inactivation. Most importantly, FVL affects the primary Arg506 cleavage site, leading to profound APC resistance and a strong prothrombotic effect.^{21,23} The FVL mutation is the most common prothrombotic genetic mutation.²⁴ It is associated with venous thromboembolism (VTE) in patients across all age groups and ischemic stroke in children;²⁵ however, no association has been found between FVL mutation and ischemic stroke in the general adult population.^{26,27} Anadure et al. conducted a study comparing the frequency of FVL mutation between a group with large vessel atherosclerosis and a healthy control group in South India. The results showed no statistically significant difference between groups.²⁸ The Genetics of Early Onset Stroke (GEOS) study investigated the relationship between ischemic stroke and FVL mutation in young adults (aged 15–49 years) and in stroke subgroups according to TOAST classification. However, the study found no significant difference in young ischemic stroke patients or between subgroups.²⁹ In their meta-analysis, Hamedani et al. found a significant increase in FVL mutation among young ischemic stroke patients, based on 18 studies and 2045 cases.³⁰ In our study, we did not observe a significant association between FV mutations and stroke etiology.

The factor II (G20210A) mutation is associated with elevated prothrombin levels, thereby predisposing to a procoagulant state. Although studies have reported heterogeneous results, accumulating evidence suggests a significant association between the prothrombin G20210A mutation and ischemic stroke, particularly in young patients (aged < 57 years).^{31,32} In contrast, although large-scale studies have demonstrated that the factor XIII V34L variant is protective against VTE and CAD,^{33,34} its association with ischemic stroke remains inconsistent in the literature. Some evidence suggests that the variant may not affect stroke occurrence but could increase stroke severity.^{35,36} The FGB -455 G>A polymorphism affects FGB-chain expression and plasma fibrinogen levels, potentially contributing to a prothrombotic state; nonetheless, its clinical significance is controversial and varies among ethnic groups.³⁷ The glycoprotein IIb/IIIa (GPIIb/IIIa) integrin complex, which is found on platelets, consists of subunits from the ITGB3 gene. Genetic variations in the ITGB3 gene may both increase an individual's risk of ischemic stroke and reduce the effectiveness of aspirin.^{38,39} Our study

found no association between the factor II, factor XIII, FGB, or ITGB3 and ischemic stroke or its etiological subtypes.

Endothelial protein C receptor (EPCR) is a protein which binds specifically to protein C on endothelial cells. It is found in plasma as soluble EPCR (sEPCR), which competes with APC. When protein C binds to sEPCR, its affinity for the thrombin-thrombomodulin complex is increased, thereby stimulating its activation. Plasma levels of sEPCR have been linked to variations in the EPCR gene (PROC1), including haplotypes H1, H2, H3, and H4. The H3 haplotype has been linked to elevated plasma sEPCR levels and an increased risk of venous thrombosis.^{40,41} In contrast, the H1 haplotype is associated with increased APC and decreased sEPCR levels and has been reported to have a protective effect against venous thrombosis and myocardial infarction.^{42,43} Our study found no significant difference between the control group and patients with ischemic stroke in terms of H1 and H3 haplotypes.

NOS3 synthesizes nitric oxide (NO), which plays a crucial role in vasodilation, blood pressure regulation, and inhibition of platelet and leukocyte adhesion to the endothelium. Additionally, it limits the oxidation of atherogenic low-density lipoproteins (LDLs) and reduces vascular smooth muscle cell migration and proliferation.⁴⁴ In a case-control study with 265 ischemic stroke patients, MacLeod et al. found no significant association between the NOS3 G298A polymorphism and ischemic stroke.⁴⁵ Likewise, in a study conducted by Anliçak et al., no significant association was found between NOS3 G894T and T786C polymorphisms and ischemic stroke in a Turkish population.⁴⁶ Saluja et al., in a case-control study of 153 patients with ischemic stroke, found that the NOS3 G298A TT genotype was associated with an increased risk of ischemic stroke (OR: 2.43).⁴⁷ In the present study, no significant associations were observed between NOS3 polymorphisms and ischemic stroke. However, the NOS3 E298D polymorphism was found to be significantly higher in stroke patients with HTN, and the NOS3 T-786C polymorphism was significantly elevated in stroke patients with CAD compared to the control group with the same conditions.

The role of ACE is to convert angiotensin I into angiotensin II, which is a peptide that stimulates aldosterone production in the renin-angiotensin system (RAS). In addition, ACE breaks down bradykinin, a potent vasodilator, into kinin

breakdown products. The RAS is activated by the reaction between renin and AGT, which determines the rate of angiotensin II synthesis.⁴⁸ The RAS pathway is tightly intertwined with a cascade of inflammatory, thrombotic, and fibrotic factors, with angiotensin II playing a central role. It causes vasoconstriction and stimulates sodium and water retention through AGTR1 and AGTR2.⁴⁹ The ACE insertion/deletion polymorphism is the most common genetic polymorphism in the RAS, with an insertion in the ACE gene leading to a decrease in ACE level. According to Zhang et al., individuals homozygous for the I allele (I/I) had 31% lower ACE levels than I/D heterozygotes and 65% lower ACE levels than individuals homozygous for the D allele (D/D). In their meta-analysis of 10070 patients with stroke, the D/D genotype was associated with a 37% higher rate of ischemic stroke than the I/I and I/D genotypes.⁵⁰ The ACE gene polymorphism has been associated with various etiologies of stroke, such as small vessel disease and cardioembolic stroke. In small vessel disease, this polymorphism contributes to endothelial dysfunction, leading to microvascular damage and an increased risk of lacunar infarctions.⁵¹ Additionally, ACE polymorphisms may exacerbate small vessel disease by impairing cerebral blood flow regulation, highlighting its specific role in lacunar strokes.⁵² In cardioembolic stroke, the role of ACE polymorphism is indirect, primarily mediated by HTN and left ventricular hypertrophy (LVH), both of which are associated with an increased risk of stroke.⁵³ These findings emphasize the genetic underpinnings of stroke subtypes and the crucial role of ACE polymorphisms in stroke etiology. Our study found no significant differences in ACE, AGT, and AGTR1 polymorphisms between patients with ischemic stroke and the control group. However, based on both ASCO and TOAST classifications, ACE polymorphism was significantly associated with cardioembolic stroke etiology. Furthermore, among individuals with CAD who experienced a stroke, the prevalence of AGT polymorphism was significantly higher compared to the CAD control group.

PAI-1 is involved in regulating fibrinolysis. Deficiency of PAI-1 has been linked to increased susceptibility to bleeding, while increased levels have been associated with thrombotic diseases such as myocardial infarction and deep vein thrombosis (DVT).⁵⁴ Despite these known associations, the relationship between stroke and

PAI-1 polymorphism remains unclear, warranting further studies.⁵⁵ In the present study, we found no significant association between PAI-1 genetic variants and the risk of ischemic stroke.

Limitations: This study has several limitations that should be acknowledged. First, the research was conducted at a single center with a relatively modest sample size, which may limit the generalizability of the results to the broader Turkish population. Second, due to the low frequency of mutation carriers and the complete absence of certain variants in our study, statistical analyses could not be performed for the APOE, APOB, LTA, and PAI-1 (rs1799889) variants. Third, the number of healthy controls was smaller than that of the patient group, which may have influenced the statistical power of certain comparisons. Finally, the lack of consideration for potential confounding factors such as diet, lifestyle, and medication adherence may affect the comprehensive interpretation of stroke risk. Future research should aim to address these limitations.

Conclusion

This study investigated the role of genetic polymorphisms as risk factors for stroke, as well as their influence on stroke etiology (ASCO and TOAST) and associated comorbidities. The MTHFR A1298C polymorphism was found to be significantly associated with an increased risk of ischemic stroke and with specific etiological subtypes. Although NOS3, ACE, and AGT polymorphisms were not associated with overall stroke risk, the NOS3 E298D variant was significantly associated with stroke among patients with HTN, while the NOS3 T-786C and AGT M268T variants were significantly associated with stroke in patients with CAD. In addition, the ACE polymorphism was significantly associated with cardioembolic stroke according to both the ASCO and TOAST classifications.

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