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Evaluation of the relationship between thrombophilia gene mutations and demographic data, imaging findings and stroke subtypes in young patients followed up with a diagnosis of stroke

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ABSTRACT

Aims: A stroke is defined as a focal or generalized neurological deficit that develops suddenly, lasts for more than 24 hours, or ends in death within this period, with a cause other than a vascular cause. Stroke is among the leading causes of morbidity and mortality in the general population. In our study, we examined the prevalence of all prothrombotic gene mutations observed in patients with and without predisposing risk factors for young patients with stroke and discussed their roles in stroke formation in the context of existing literature.

Methods: In the present study, we conducted a retrospective review of the medical records of 300 patients aged 18 to 49 years who were hospitalized in the neurology clinic of our hospital between June 2023 and June 2024 and diagnosed with acute arterial ischemic stroke based on anamnesis, neurological examination, and radiological imaging (CT and MRI) results. The study cohort comprised 47 patients (23 males, 24 females) aged between 18 and 49 years with a diagnosis of ischemic stroke. In our study, we examined ischemic stroke-related gene polymorphisms in patients with early-onset ischemic stroke (before the age of 50 years). This study aims to investigate the prevalence of factor V-Leiden, prothrombin-G20210A, methylenetetrahydrofolate reductase (MTHFR) C677T, MTHFR A1298C, SERPIN 1, and Factor II polymorphisms in young patients with ischemic stroke.

Results: A total of 47 patients, comprising 23 males and 24 females, were included in the study. The mean age of the patients was 40.34±6.37 years. According to the TOAST classification system, 17 patients (36.2%) were diagnosed with large artery atherothrombosis, one patient (2.1%) had a cardioembolic infarction, and 29 patients (61.7%) had a small artery occlusion. In our study, when evaluating the thrombophilia mutation subtypes in young ischemic stroke patients, 23.4% (n=11) of the patients were found to be heterozygous for the FVL mutation, while 76.6% (n=36) had no mutation. Concerning the SERPIN 1 mutation, 40.4% (n=19) of the patients were heterozygous, 23.4% (n=11) were homozygous, and 36.2% (n=17) exhibited no mutation. The distribution of patients according to MTHFR C677T mutation revealed that 57.4% (n=27) were heterozygous, 19.1% (n=9) were homozygous, and 23.4% (n=11) had no mutation. Considering the distribution of patients according to the MTHFR A1298C gene mutation, a total of 38.3% (n=18) of the patients were heterozygous, 14.9% (n=7) were homozygous, and 46.9% (n=22) had no mutation. Upon analysis of the distribution of patients according to factor XIII mutation, it was identified that 19.1% (n=9) were heterozygous, 2.1% (n=1) were homozygous, and 78.7% (n=37) of the patients exhibited no mutation. Upon analysis of the distribution of patients according to prothrombin (Factor II) mutation, it was identified that 8.5% (n=4) were heterozygous, while 91.5% (n=43) exhibited no mutation.

Conclusion: Although stroke is less prevalent in young adults, the underlying etiology is highly varied. Further research, including the investigation of genetic and prothrombotic mutations, is vital for the prevention of recurrent strokes in young adults.

Keywords: Stroke, protrombotic mutation, young patient

INTRODUCTION

A stroke is defined as a focal or generalized neurological deficit that develops suddenly, lasts for more than 24 hours, or ends in death within this period, with a cause other than a vascular cause. Stroke is among the leading causes of morbidity and mortality in the general population.[1,2] Stroke is the second leading cause of mortality and the third leading cause of disability worldwide. Annually, more than 11 million individuals worldwide experience an ischemic stroke. Although it is well known that the prevalence of ischemic stroke increases with age, it is notable that 10-15% of cases occur in young individuals between the ages of 18 and 50.[3] The incidence of stroke in the young population is variable, with rates ranging from 2.5-40/100,000 individuals,[4,5] with a notable increase in incidence with advancing age, with a higher prevalence observed in females between the ages of 18-44 compared to males.[2] Among the etiologic causes, age, gender, race, and family history are non-modifiable risk factors, while hypertension, diabetes mellitus, heart disease, hypercholesterolemia, smoking, and carotid stenosis are definitively modifiable factors. However, despite comprehensive investigations, a definitive cause cannot be identified in 23-25% of cases.[6]

The etiologic evaluation of young patients with stroke involves a different set of investigations than those employed in elderly patients. While classical atherosclerotic risk factors become apparent after the age of 35 years, cardioembolism, dissection, non-atherosclerotic vasculopathy, and prothrombotic conditions have been reported to be important in patients aged 18-35 years.[7] The prevalence of hypercoagulability in young patients with stroke is between 6-15%.[8] Evaluation of Factor V Leiden (FVL), prothrombin G20210A gene, methylenetetrahydrofolate reductase (MTHFR) genes, protein C (PC), protein S (PS), antithrombin 3 (ATIII), fibrin, activated protein C resistance (APCR), homocysteine antinuclear antibody (ANA), and anticardiolipin antibody (ACA) tests in qualified laboratories, as well as their availability in all centers for young patients with stroke, present a significant challenge in clinical practice. However, these tests offer a comprehensive perspective.

In our study, we examined the prevalence of all prothrombotic gene mutations observed in patients with and without predisposing risk factors for young patients with stroke and discussed their roles in stroke formation in the context of existing literature.

METHODS

The study was approved by the Sakarya University Faculty of Medicine Clinical Researches Ethics Committee (Date: 02.05.2023 Decision No: 108). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In the present study, we conducted a retrospective review of the medical records of 300 patients aged 18 to 49 years who were hospitalized in the neurology clinic of our hospital between June 2023 and June 2024 and diagnosed with acute arterial ischemic stroke based on anamnesis, neurological examination, and radiological imaging (CT and MRI) results. The study cohort comprised 47 patients (23 males, 24 females) aged between 18 and 49 years with a diagnosis of ischemic stroke. Patients with sinus venous thrombosis, intracranial hemorrhage, and subarachnoid hemorrhage were excluded from the study. The medical history of patients was reviewed to identify potential risk factors for stroke, including hypertension, diabetes mellitus, coronary heart disease, history of stroke, atrial fibrillation, smoking, hyperlipidemia, heart valve replacement, hyperthyroidism/hypothyroidism, vasculitic disease, hematologic disease, chronic alcohol consumption, and oral contraceptive (OCS) drug use.

The etiology of patients' stroke was determined according to the TOAST and BAMFORD classification systems.[9] The results of the analysis of genetic polymorphisms associated with thrombophilia, as recorded in the patient files, were also documented.

In our study, we examined ischemic stroke-related gene polymorphisms in patients with early-onset ischemic stroke (before the age of 50 years). This study aims to investigate the prevalence of FVL, prothrombin-G20210A, methylenetetrahydrofolate reductase (MTHFR) C677T, MTHFR A1298C, SERPIN 1, and Factor II polymorphisms in young patients with ischemic stroke.

RESULTS

A total of 47 patients, comprising 23 males and 24 females, were included in the study. The mean age of the patients was 40.34±6.37 years. The age range was from 24 to 49 years. The number of female patients was 24 (51.1%), and the number of male patients was 23 (48.9%). Six patients (12.8%) had accompanying hypertension, five (10.6%) had diabetes mellitus, four (8.5%) had coronary artery disease, eight (17%) had hyperlipidemia, and two (4.3%) had atrial fibrillation. Twenty-eight of the patients (59.6%) were identified as current smokers.

The demographic data of the patients are presented in Table 1.

Table 1. Demographic data

	Min-Max	Mean±SD
Age	24-49	40.34±6.37
	n (%)	
Male gender	23 (48.9)	
Hypertension	6 (12.8)	
Diabetes mellitus	5 (10.6)	
Smoking	28 (59.6)	
Coronary artery disease	4 (8.5)	
Hyperlipidemia	8 (17)	
Atrial fibrillation	2 (4.3)	

Min: Minimum, max: maximum, SD: Standart deviation

Doppler ultrasonography of the carotid vertebral arteries revealed that 42 patients (89.3%) exhibited normal vasculature, 2 patients (4.3%) demonstrated symptomatic ICA stenosis exceeding 50%, and 3 patients (6.4%) exhibited vertebrobasilar insufficiency. Upon evaluation of the transthoracic echocardiograms performed on admission, it was determined that 45 patients (95.7%) showed normal echocardiographic findings, while segmental hypokinesia was identified in two patients (4.3%) (Table 2).

Table 2. Doppler and echocardiography findings

		n (%)
Doppler	Normal	42 (89.3)
	ICA stenosis below 50%	0 (0.0)
	ICA stenosis over 50%	2 (4.3)
	Vertebrobasilar insufficiency	3 (6.4)
Echocardiography	Normal	45 (95.7)
	Valve disease	0 (0.0)
	Segmentary hypokinesia	2 (4.3)

According to the TOAST classification system, 17 patients (36.2%) were diagnosed with large artery atherothrombosis, one patient (2.1%) had a cardioembolic infarction, and 29 patients (61.7%) had a small artery occlusion. Following the BAMFORD classification, 40 (85.1%) of the patients included in the study exhibited anterior circulation strokes, while 7 (14.9%) demonstrated posterior circulation strokes.

Table 3 presents the ischemic stroke subtypes according to the TOAST and BAMFORD classifications.

In our study, when evaluating the thrombophilia mutation subtypes in young ischemic stroke patients, 23.4% (n=11) of the patients were found to be heterozygous for the FVL mutation, while 76.6% (n=36) had no mutation. Concerning the SERPIN 1 mutation, 40.4% (n=19) of the patients were heterozygous, 23.4% (n=11) were homozygous, and 36.2% (n=17) exhibited no mutation. The distribution of patients according to MTHFR C677T mutation revealed that 57.4% (n=27) were heterozygous, 19.1% (n=9) were homozygous, and 23.4% (n=11) had no mutation. Considering the distribution of patients according to the MTHFR A1298C gene mutation, a total of 38.3% (n=18) of the patients were heterozygous, 14.9% (n=7) were homozygous, and 46.9% (n=22) had no mutation. Upon analysis of the distribution of patients according to factor XIII mutation, it was identified that 19.1% (n=9) were heterozygous, 2.1% (n=1) were homozygous, and 78.7% (n=37) of the patients exhibited no mutation. Upon analysis of the distribution of patients according to prothrombin (Factor II) mutation, it was identified that 8.5% (n=4) were heterozygous, while 91.5% (n=43) exhibited no mutation.

Table 3. Prevalence rates of stroke subtypes according to TOAST and BAMFORD classification

TOAST		**n (%)**
	Large vessel disease	17 (36.2)
	Cardioembolic	1 (2.1)
	Lacunar infarction	29 (61.7)
	Due to other causes	0 (0.0)
	Unspecified cause	0 (0.0)
BAMFORD		
	Anterior circulation stroke	40 (85.1)
	Posterior circulation stroke	7 (14.9)

Table 4 and Table 5 present the incidence of prothrombotic gene polymorphisms and the distribution of gene polymorphisms in subgroups according to the TOAST classification.

Table 4. Frequency of prothrombotic gene polymorphisms

	Normal n (%)	Heterozygous n (%)	Homozygous n (%)
mthfrC667T	11 (23.4)	27 (57.4)	9 (19.1)
SERPIN1	17 (36.2)	19 (40.4)	11 (23.4)
mthfrA1298C	22 (46.8)	18 (38.3)	7 (14.9)
Factor XIII	37 (78.7)	9 (19.1)	1 (2.1)
Factor II	43 (91.5)	4 (8.5)	0 (0.0)
Factor V Leiden	36 (76.6)	11 (23.4)	0 (0.0)

Table 5. Rates of genetic polymorphisms in stroke subgroups according to TOAST classification

		Large vessel disease	Cardioembolic stroke	Lacunar infarct
		n (%)	n (%)	n (%)
MTHFRC667T	Normal	4 (8.5)	0 (0.0)	7 (14.9)
	Heterozygous	9 (19.1)	1 (2.1)	17 (36.2)
	Homozygous	4 (8.5)	0 (0.0)	5 (10.6)
SERPIN1	Normal	4 (8.5)	0 (0.0)	13 (27.7)
	Heterozygous	9 (19.1)	0 (0.0)	10 (21.3)
	Homozygous	4 (8.5)	1 (2.1)	6 (12.8)
MTHFRA1298C	Normal	8 (17.0)	1 (2.1)	13 (27.7)
	Heterozygous	6 (12.8)	0 (0.0)	12 (25.5)
	Homozygous	3 (6.4)	0 (0.0)	4 (8.5)
Factor XIII	Normal	12 (25.5)	1 (2.1)	24 (51.1)
	Heterozygous	4 (8.5)	0 (0.0)	5 (10.6)
	Homozygous	1 (2.1)	0 (0.0)	0 (0.0)
Factor II	Normal	13 (27.7)	1 (2.1)	29 (61.7)
	Heterozygous	4 (8.5)	0 (0.0)	0 (0.0)
	Homozygous	0 (0.0)	0 (0.0)	0 (0.0)
Factor V Leiden	Normal	16 (34.0)	1 (2.1)	19 (40.4)
	Heterozygous	1 (2.1)	0 (0.0)	10 (21.3)
	Homozygous	0 (0.0)	0 (0.0)	0 (0.0)

DISCUSSION

Thrombophilia can be defined as a disturbance in the equilibrium of the coagulation system, which predisposes the individual to the formation of thrombi. This tendency can be caused by acquired factors or genetic variations that affect clotting. Hereditary thrombophilia is a condition in which these genetic variations affect the amount or function of a protein in the coagulation system, thereby creating a tendency to clot.[9] The two most common causes of hereditary thrombophilia worldwide are FVL and prothrombin G20210A gene variations.

Ischemic stroke is a complex multifactorial disorder in which genetic and environmental factors play a role in its etiopathogenesis. Since strokes cause severe health and socio-economic losses, it is crucial to identify stroke risk factors in detail and implement precautions.[10] Some studies have demonstrated that prothrombotic gene mutations are a risk factor in the etiopathogenesis of ischemic stroke.

Although ischemic stroke is less prevalent in young adults than in the elderly, it is diverse with respect to its underlying pathogenesis and risk factors. Approximately 10-15% of all strokes occur between the ages of 18 and 50.[11-13]

Some types of hereditary thrombophilia are clearly considered as a risk factor for ischemic stroke.[14]

The protein 5,10-methylene tetrahydrofolate reductase, encoded MTHFR, is a folate-dependent enzyme that catalyzes the rate-limiting step in the methylation of homocysteine to methionine. The C677T polymorphism in MTHFR results in the conversion of alanine to valine at amino acid 222, and is associated with elevated circulating homocysteine levels.[10,15] Elevated homocysteine levels have been identified as a risk factor for atherosclerosis and atherothrombosis, due to induced endothelial dysfunction. The C677T transition has been linked to an increased risk of ischemic stroke. These polymorphisms have also been observed in healthy individuals.[16]

A meta-analysis of 24 studies with a total of 900 patients that investigated the effect of MTHFR C677T mutations on ischaemic stroke showed that stroke patients had elevated homocysteine levels compared with controls.[17] A study by Alkanlı[18] and colleagues investigating genetic risk factors for ischaemic stroke in 82 patients with ischemic stroke and 92 controls reported that the MTHFR A1298C gene polymorphism is a genetic risk factor for ischemic stroke in the Thrace region, while the MTHFR C677T gene polymorphism is a risk factor for the unspecified subtype. In our study, the MTHFR C677T mutation was identified in 76.5% of patients with stroke. Of these, 57.4% were heterozygous and 19% were homozygous.

The role of the FVL mutation in the etiology of arterial thrombosis and ischemic stroke remains unidentified. The FVL mutation may modestly increase the risk of arterial thrombotic events relative to venous thromboembolism, with a more pronounced effect observed in individuals younger than 55 years and females.[19] The increased prevalence of the FVL mutation in ischemic stroke patients suggests its potential role in the pathogenesis of ischemic stroke, but it does not increase the risk in the presence of other risk factors.

In a study conducted in the Turkish population, the prevalence of the FVL mutation was reported to be 10%. It accounts for 25% of all hereditary thrombophilia cases.[20] While the presence of a heterozygous FVL mutation has been shown to increase the risk of thrombosis by a factor of 5-10, the presence of a homozygous mutation increases the risk by a factor of 50-100.[21] In a study by Grossmann et al.[22] 93 patients with a history of ischemic stroke under the age of 50 were compared with 186 healthy individuals. The FVL mutation was identified in 13 patients in the control group and 15 patients in the patient group (6.9% in the control group and 16.1% in the patient group). These findings led to the conclusion that Factor V Leiden mutation is a significant risk factor for stroke in young adults.[22]

Slooter et al.[23] performed a comparative analysis of 193 female patients aged 20-49 years with ischemic stroke and 767 healthy individuals. This study found that women who use contraceptive pills and carry the FVL mutation have an increased risk of ischemic stroke. In our study, the heterozygous FVL mutation was identified in 23.4% of patients with stroke, while no homozygous mutation was observed. The prothrombin G20210A mutation has been shown to increase the amount of plasma prothrombin by affecting the synthesis of prothrombin at the mRNA and protein levels. The prothrombin G20210A mutation has been shown to result in serum prothrombin levels that are 30% higher than those observed in normal controls.[24] In a study of 72 patients who had suffered an ischemic stroke before the age of 50 and had no other risk factors, the risk of stroke was found to be 4-5 times higher in individuals with the prothrombin G20210A mutation.[25] The results of this study suggest that the prothrombin G20210A mutation contributes to the increased risk of cerebral ischemia. The prevalence of the heterozygous form of the prothrombin mutation in the general population is 1-2%. In our study, the heterozygous prothrombin G20210A mutation was identified in 8.5% of young patients with ischemic stroke, and no homozygous mutation was detected.

PAI-1 (SERPIN 1) functions to inhibit the activity of tissue plasminogen activator and uricinase, which act as activators in the conversion of plasminogen to plasmin. Serum levels of PAI-1 are associated with genetic factors, and elevated serum levels contribute to the development of a hypofibrinolytic state by increasing the risk of thrombosis.[26] The prevalence of PAI-1 heterozygosity was 44.0%, while homozygosity was observed in 24.0%.[27] The presence of the PAI-1 4G allele is associated with elevated PAI-1 levels. Several studies have investigated the association between the PAI-1 4 guanosine/5 guanosine (4G/5G) polymorphism and the development of VTE. The results of these studies have been inconsistent. In our study, we identified a SERPIN 1 mutation in 63.8% of patients with stroke. Of these mutations, 40.4% were heterozygous and 23.4% were homozygous.

Factor XIII is a transglutaminase enzyme that plays a critical role in the final step of the coagulation system. The Factor XIII Valine34Losine (FXIII V34L) mutation is the result of leucine to valine substitution at position 34 of the Factor XIII gene, resulting in a change in the region three amino acids from the thrombin cleavage site. Recent reports suggest that the FXIII V34L mutation paradoxically may have a mild protective effect against arterial and venous thrombosis. The presence of the FXIII V34L mutation has been observed to provide some protection against cerebral infarction, but at the same time increase the risk of hemorrhagic stroke.[28] Thrombophilia, also known as a coagulation disorder, can lead to thromboembolic processes, contributing to the development of stroke. Therefore; the thrombophilia panel is studied to elucidate this etiology, especially in patients with stroke at a young age. The thrombophilia tests evaluated in our study included Factor II prothrombin G20210A, FVL, MTHFR C677T, MTHFR A1298C, and Factor XIII, PAI mutations. In light of these data; the etiology of young ischemic stroke is multifactorial, and prothrombotic gene mutations may increase the occurrence of ischemic stroke only in combination with risk factors such as hypertension, diabetes, smoking, and alcohol consumption.

CONCLUSION

Although stroke is less prevalent in young adults, the underlying etiology is highly varied. Further research, including the investigation of genetic and prothrombotic mutations, is vital for the prevention of recurrent strokes in young adults.

The present study is limited by the absence of a control group, the relatively small sample size, and inadequate data on biochemical parameters (homocysteine and fibrinogen).

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was approved by the Sakarya University Faculty of Medicine Clinical Researches Ethics Committee (Date: 02.05.2023 Decision No: 108).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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