

RESEARCH ARTICLE

The Possible Association of Some Thrombophilic Gene Polymorphisms with Deep Vein Thrombosis and Pulmonary Thromboembolism

Bazı Trombofilik Gen Polimorfizmlerinin Derin Ven Trombozu ve Pulmoner Tromboemboli ile Olası İlişkisi

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ABSTRACT

Objective: Thrombosis is a multifactorial cardiovascular disorder and its genetic background is controversial. Some genetic variants may have an effect on the development of deep vein thrombosis and pulmonary thromboembolism. We aimed to analyze the distribution of MTHFR C677T and A1298C, PAI-1 -675 4G/5G and ACE I/D single nucleotide polymorphisms (SNP) in these conditions.

Material and Method: A total of 157 cases with deep vein thrombosis, 147 with pulmonary thromboembolism and 140 healthy controls participated in this study. The DNAs obtained from peripheral blood samples were screened for the aforementioned thrombophilic gene variants via reverse hybridization method (Strip Assay).

Results: There were significant differences between the cases of pulmonary thromboembolism and healthy control group for the MTHFR A1298C SNP and C allelic frequency ($p = 0.02$ and $p = 0.03$). The frequency of PAI-1 4G allele was higher in deep vein thrombosis and pulmonary thromboembolism patients than in the control population ($p = 0.002$ and $p = 0.015$). MTHFR C677T and ACE I/D SNPs were not different in both diseases compared to the controls.

Conclusion: It is likely that the MTHFR A1298C variant has an impact on the formation of pulmonary thromboembolism. The PAI-1 4G/5G polymorphism is significantly associated with the development of both deep vein thrombosis and pulmonary thromboembolism. No significant correlation was found between the MTHFR C677T and ACE I/D SNPs and these conditions.

Keywords: Deep Vein Thrombosis, Pulmonary Thromboembolism, Thrombophilic Gene, Polymorphism.

ÖZET

Amaç: Tromboz, multifaktöriyel bir kardiyovasküler bozukluktur ve genetik arka planı tartışmalıdır. Bazı genetik varyantlar derin ven trombozu ve pulmoner tromboemboli gelişiminde etkili olabilir. Biz bu rahatsızlıklarda, MTHFR C677T ve A1298C, PAI-1 -675 4G/5G ve ACE I/D tek nükleotid polimorfizmlerinin (SNP) dağılımını analiz etmeyi amaçladık.

Gereç ve Yöntem: Bu çalışmaya toplam 157 derin ven trombozu, 147 pulmoner tromboemboli ve 140 sağlıklı kontrol katıldı. Periferik kan örneklerinden elde edilen DNA'lar ters hibridizasyon yöntemi (Strip Assay) ile yukarıda belirtilen trombofilik gen varyantları açısından tarandı.

Bulgular: MTHFR A1298C SNP ve C alelik frekansı açısından pulmoner tromboemboli vakaları ile sağlıklı kontrol grubu arasında anlamlı farklılıklar vardı ($p = 0,020$ ve $p = 0,030$). PAI-1 4G alel frekansı derin ven trombozu ve pulmoner tromboemboli hastalarında kontrol popülasyonuna göre daha yüksekti ($p = 0,002$ ve $p = 0,015$). MTHFR C677T ve ACE I/D SNP'leri her iki hastalıkta da kontrollere kıyasla farklı değildi.

Sonuç: MTHFR A1298C varyantının pulmoner tromboembolizm oluşumu üzerinde bir etkisi olması muhtemeldir. PAI-1 4G/5G polimorfizmi hem derin ven trombozu hem de pulmoner tromboembolizm gelişimi ile anlamlı şekilde ilişkilidir. MTHFR C677T ve ACE I/D SNP'leri ile bu rahatsızlıklar arasında anlamlı bir ilişki bulunmadı.

Anahtar Sözcükler: Derin Ven Trombozu, Pulmoner Tromboemboli, Trombofilik Gen, Polimorfizm.

Thrombosis is the presence of a blood clot in the arteries or veins that may block the lumen (1), and may cause serious conditions such as stroke, pulmonary embolism, myocardial infarction, or ischemia of various organs and tissues (2). This phenomenon is one of the major health problems worldwide and it is a multifactorial disorder that also includes genetic problems in its pathogenesis. Deep vein thrombosis (DVT) can be defined as the development of a blood clot in the deep veins. It usually occurs in the deep veins of the lower extremities and abdomen, and may resolve spontaneously without being noticed (3). However, DVT is a prevalent cardiovascular disease with an incidence of about 1-2/1000 per year and it can lead to a large number of mortality or significant morbidity (4). A blood clot in the venous system is not so dangerous actually but if a piece of the blood clot detaches from elsewhere and travels in the body through the bloodstream, it can block up the main artery of the lung or one of its branches. It is called pulmonary thromboembolism (PTE). Thrombosis in the venous system is the main cause of the development of pulmonary embolism (5). This condition is a common life-threatening problem in the departments of chest diseases and cardiology. Some cases such as; immobilization, surgery, cancer, trauma, oestrogen therapy, fractures, pregnancy and postpartum period have been declared as a common risk factors for DVT. Pulmonary embolism one of the significant causes of cardiovascular death in the United States and is the third most common cause of cardiovascular events after myocardial infarction and stroke (6). Most patients with pulmonary embolism also have signs of DVT. Some genetic determinants may have a significant role in the pathogenesis of DVT and PTE (7). Inherited thrombophilias can be considered as risk factors for pulmonary embolism, with or without DVT (8). Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in the metabolism of homocysteine and folate by catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. (9). MTHFR variants (C677T and A1298C) reduce enzyme activity to some extent. These polymorphisms may be associated with the development of thrombosis due to increased homocysteine levels. MTHFR 677TT variant causes a moderate increase in homocysteine levels which may have a significance in venous thrombo-

sis and other similar conditions (10). Machado et al. declared that a combined mutation of the MTHFR gene as heterozygous for C677T and A1298C is associated with venous thromboembolism independent of hyperhomocysteinemia (11). The plasminogen activator inhibitor-1 (PAI-1) gene -675 4G/5G polymorphism is linked with PAI-1 level, and the 4G allele may pose a risk for DVT with increased PAI-1 activity (12). Some researchers claimed that people with 4G/4G genotype of PAI-1 have a higher risk of venous thromboembolism (13). On the other hand, angiotensin converting enzyme (ACE) transforms angiotensin-I to angiotensin-II (14) and the ACE gene on chromosome 17q comprises an insertion/deletion (I/D) polymorphism that affects ACE activity (15). There may be a synergistic effect between angiotensin-converting enzyme (ACE) DD genotype and the thrombophilic elements and this position supports the multicausality of recurrent venous thromboembolism (VTE) (16).

We aimed to analyze the association of four gene polymorphisms (MTHFR C677T and A1298C, PAI-1 4G/5G and ACE I/D) with DVT and PTE in this study.

MATERIAL AND METHOD

Study Population

This study was conducted at the Department of Medical Genetics, Faculty of Medicine, Sivas Cumhuriyet University. It was approved by the Cumhuriyet University ethics committee. Study groups were composed of 444 individuals (157 cases of DVT, 147 PTE patients and 140 healthy control subjects). While DVT patients were roughly defined by physical examination and Doppler ultrasonography, pulmonary embolism cases were diagnosed on the basis of clinical examination and radiologic signs obtained from computed tomography and pulmonary angiography. Ages and genders of the patients and controls were recorded. Patients who have Factor V G1691A (Leiden) and Prothrombin G20210A mutations, protein C or S deficiency, structural heart diseases, body mass index (BMI) over 30 kg/m², diabetes, hypertension and the other systemic diseases were excluded. Cigarette smokers and women receiving estrogen hormone therapy were also not allowed to participate in this study.

Controls were selected from the healthy individuals without any systemic disease.

Laboratory analysis

All venous blood samples were obtained with EDTA tube. Peripheral blood specimens taken from the patients and healthy controls were used for genotyping of thrombophilic genes. The DNAs of patients and controls were isolated using an appropriate DNA isolation kit (Invitex Invisorb Spin Blood Kit, Germany). Obtained DNA samples were analyzed for the gene variants by reverse hybridization method. (Vienna Lab, Strip Assay, Austria). The relevant genes were amplified simultaneously through a single multiplex amplification process labelled with biotin. The amplification steps were adjusted as: 2 min at 95°C for pre-PCR, followed by 35 cycles of (15 s at 95°C for denaturation, 30 s at 56°C for annealing, 30 s at 72°C for extension) and 7 min at 72°C as final elongation. Amplified materials were incubated to the strips, containing oligonucleotide probes. The procedure was completed with color development and determination of signals in an automatic incubator (Auto-LIPA, Innogenetics).

Statistical analysis

The data were analyzed with SPSS version 22 (Chicago, IL, USA). While the t-test was used to com-

pare the mean ages and BMIs of the patients and controls, the Chi-square test was preferred to evaluate the allele and genotype frequencies. The odds ratios for the relationship between allelic frequency and the risk of both DVT and PTE formation were calculated at 95% confidence interval, and a P value less than 0.05 was accepted statistically significant.

RESULTS

The related thrombotic gene variations were detected in DVT and PTE patients and compared with the healthy controls from the same ethnicity. The patients were composed of 82 males (52.2%) and 75 females (47.8%) in DVT and 78 males (53%) and 69 females (47%) in PTE. The mean age of the patients was 55.45 ± 9.58 years (38 to 74 years) in DVT and 55.66 ± 9.45 years (40 to 76 years) in PTE. The healthy controls included 73 males (52.1%) and 67 females (47.9%). The mean age of the control group subjects was 54.88 ± 10.39 years (34 to 73 years). There was no significant difference between the patients and control group in terms of the age and sex. However, in the context of body mass index, both PTE and DVT patients were overweight compared with the healthy population ($p = 0.001$) (Table 1).

Table 1. The characteristics of DVT & PTE patients and the control group.

Characteristics	DVT (n: 157)	PTE (n: 147)	Control (n: 140)	p value
Mean age	55.45 ± 9.58	55.66 ± 9.45	54.88 ± 10.39	NS
Male	82 (52.2%)	78 (53%)	73 (52.1%)	NS
Female	75 (47.8%)	69 (47%)	67 (47.9%)	
BMI	25.64 ± 4.02	25.84 ± 3.89	24.22 ± 3.38	0.01

NS: Nonsignificant.

Although the ratios of 677TT/1298CC genotypes and 677T/1298C alleles in DVT patients were higher (17.8%, 17.8% and 35%, 39.8%, respectively) than in controls, there was no any significant difference in the distribution of MTHFR C677T ($p = 0.056$) and MTHFR A1298C polymorphisms ($p = 0.536$) and allelic frequencies for the DVT patients and controls. While, an association between the MTHFR C677T SNP and the formation of PTE

was not detected ($p = 0.688$), the distribution of MTHFR A1298C SNP and C allelic frequencies were significant in PTE cases compared with controls ($p = 0.020$ and $p = 0.030$). The ratios of MTHFR A1298C AC and CC genotypes and C allelic frequency in PTE patients (57.2%, 15.6% and 44.2%, respectively) were higher than in controls (Table 2).

Table 2. Distribution of MTHFR C677T and A1298C polymorphisms and allele frequencies in DVT & PTE cases and control group.

MTHFR C677T Genotypes & Alleles	DVT/PTE n: 157/147	Control n: 140	p value
CC	75 (47.8%)/66 (44.9%)	70 (50%)	p= 0.056 p >0.05
CT	54 (34.4%)/67 (45.6%)	58 (41.4%)	p= 0.688 p >0.05
TT	28 (17.8%)/14 (9.5%)	12 (8.6%)	
C	204 (65%)/199 (67.7%)	198 (70.7%)	p= 0.135 p >0.05
T	110 (35%)/95 (32.3%)	82 (29.3%)	p= 0.432 p >0.05
Odds ratios	0.768 (0.54-1.09) %95 CI & 0.868 ((0.61-1.24) %95 CI		
MTHFR A1298C Genotypes & Alleles	DVT/PTE n: 157/147	Control n: 140	p value
AA	60 (38.2%)/40 (27.2%)	60 (42.8%)	p= 0.536 p >0.05
AC	69 (44%)/84 (57.2%)	61 (43.6%)	p= 0.020 p <0.05
CC	28 (17.8%)/23 (15.6%)	19 (13.6%)	
A	189 (60.2%)/164 (55.8%)	181 (64.6%)	p= 0.264 p >0.05
C	125 (39.8%)/130 (44.2%)	99 (35.4%)	p= 0.030 p <0.05
Odds ratios	0.827 (0.59-1.15) %95 CI & 0.690 (0.49-0.96) %95 CI		

The comparison of DVT cases and control group was significant in relation to the distribution of PAI-1 4G/5G SNP ($p = 0.003$). The 4G/4G genotype ratio and 4G allelic frequency in DVT patients (31.8% and 57.3%, respectively) were statistically higher than in healthy controls. PAI-1 4G/5G SNP was also associated with PTE. The differences

between PTE patients and healthy controls in relation to PAI-1 4G/5G polymorphism and 4G allelic frequency were significant ($p = 0.020$ and $p = 0.015$). The PAI-1 4G/4G genotype ratio and 4G allelic frequency in patients with PTE (23.8% and 54.8%, respectively) were higher than in healthy controls (Table 3).

Table 3. Comparison of PAI-1 4G/5G genotypes and allele frequencies between DVT & PTE cases and control group.

PAI-1 4G/5G Genotypes & Alleles	DVT/PTE n: 157/147	Control n: 140	p value
5G/5G	27 (17.2%)/21 (14.3%)	37 (26.4%)	p= 0.003 p <0.05
4G/5G	80 (51%)/91 (61.9%)	81 (57.9%)	p= 0.020 p <0.05
4G/4G	50 (31.8%)/35 (23.8%)	22 (15.7%)	
5G	134 (42.7%)/133 (45.2%)	155 (55.4%)	p= 0.002 p <0.05
4G	180 (57.3%)/161 (54.8%)	125 (44.6%)	p= 0.015 p <0.05
Odds ratios	0.600 (0.43-0.83) %95 CI & 0.666 (0.48-0.92) %95 CI		

The distributions of ACE I/D SNP in DVT cases and control group were similar. Although the ratios of ACE DD genotypes and D allelic frequency were higher in the cases with DVT (32.5% and 60.5%, respectively) than in controls, no significant differences were found between DVT patients and controls in terms of ACE I/D SNP and D allelic frequency

($p = 0.278$ and $p = 0.148$). Likewise, while the number of ACE DD genotype and D allelic frequency were higher in the patients with PTE (35.4% and 60.2%, respectively) than in controls, these data were not statistically significant ($p = 0.150$ and $p = 0.178$) (Table 4).

Table 4. Comparison of ACE I/D genotypes and allele frequencies between DVT & PTE cases and control group.

ACE (I/D) Genotypes&Alleles	DVT/PTE n: 157/147	Control n: 140	p value
II	18 (11.5%)/22 (15%)	22 (15.7%)	p= 0.278 p >0.05 p= 0.150 p >0.05
ID	88 (56%)/73 (49.6%)	83 (59.3%)	
DD	51 (32.5%)/52 (35.4%)	35 (25%)	
I	124 (39.5%)/117 (39.8%)	127 (45.4%)	p= 0.148 p >0.05
D	190 (60.5%)/177 (60.2%)	153 (54.6%)	p= 0.178 p >0.05
Odds ratios	0.786 (0.56-1.09) %95 CI & 0.796 (0.57-1.10) %95 CI		

DISCUSSION

Deep vein thrombosis and pulmonary embolism are significant morbidity and mortality problems. Thrombophilic disorders may be congenital or acquired and just about 40% of cases with thrombosis are inherited (17). Congenital thrombophilia may result from the overactivity of coagulation factors through different mutations. Hereditary thrombophilia is a condition associated with the formation of thrombosis in the venous system. Some thrombophilic markers (FV G1691A, FII G20210A, XIII V34L, MTHFR C677T and A1298C, PAI-1 -675 4G/5G, etc.) may be effective in the pathogenesis of both DVT and PTE. The aim of this current study was to analyze the possible association of four thrombotic gene polymorphisms (MTHFR C677T and A1298C, PAI-1 -675 4G/5G, ACE I/D) with the development of DVT and PTE.

MTHFR is the enzyme that contributes to the conversion of homocysteine to methionine in homocysteine metabolism. MTHFR variants cause an increase in homocysteine levels by reducing enzyme activity. High homocysteine level poses a significant risk for vascular thrombosis. There are conflicting results for the relation between MTHFR gene polymorphisms and thromboembolic disorders in the literature. Guzmán et al. detected a significant difference between DVT cases and healthy controls in terms of the MTHFR C677T polymorphism (18). In a study by Ghaznavi et al. (19) the MTHFR C677T SNP was not significantly associated with the development of DVT. On the other hand, a case-control study asserted no association between the MTHFR A1298C mutation and DVT (20). Basol et al. (21) claimed that despite no relation between MTHFR gene polymorphisms (C677T and A1298C) and pulmonary embolism, there was an association between combined mutations of MTHFR C677T-MTHFR A1298C and pulmonary embolism in Turkish population. According to Ekim et al. (22) the MTHFR C677T gene polymorphism in the homozygous state can be considered as a risk factor for thrombosis especially when combined with other

thrombophilic elements. Bezgin et al. (23) stated that the MTHFR A1298C mutation rate was higher in PTE cases compared with DVT patients and control group. In our analysis, the allelic frequencies of both MTHFR C677T and MTHFR A1298C SNPs in DVT patients were not significant in comparison with controls. There was no association of the MTHFR C677T SNP with PTE patients but the data obtained suggest that the MTHFR A1298C is effective in the formation of PTE. We expected the C677T variant to be more effective than the A1298C in both conditions. However, MTHFR A1298C polymorphism alone was found to be associated only with pulmonary thromboembolism.

PAI-1 can be roughly defined as one of the serine proteinase inhibitors with a molecular weight of approximately 50 kD (24). It is the major suppressor of both tissue-type and urokinase-type plasminogen activator. The PAI-1 4G/5G polymorphism influences gene expression and the 4G/4G variant has an association with high levels of PAI-1 leading to a hypofibrinolytic condition and therefore an increased thrombosis risk (25). While there are some studies linking the 4G/5G SNP of the PAI-1 gene to the risk of thrombosis, the basic role of this variant for the development of thrombotic diseases is still controversial. Bezgin and his colleagues emphasized that high levels of PAI-1 cause a disruption in the fibrinolytic system and thereby increase the risk of thrombotic events (23). A correlation was found between the 4G/5G SNP of PAI-1 gene and the occurrence of pulmonary thromboembolism in a Chinese population (26). Conversely, Oguzulgen et al. could not find any relationship between this polymorphism and PTE (27). Kaya et al. (28) did not find any significant relationship between the 4G/5G SNP of PAI-1 and the formation of venous thromboembolism. Our study suggests that there is a strong link between the PAI-1 4G/5G variant and both DVT and PTE. The PAI 1 4G allele is very likely to be effective in the formation of DVT and PTE. We know that this polymorphism is also associated with recurrent miscarriages.

ACE is a dipeptidyl carboxypeptidase responsible for the transformation of angiotensin I to angioten-

sin II, a significant vasopressor (29) and it is the main component of the renin-angiotensin system. A possible correlation between insertion/deletion polymorphism of the ACE gene and the risk of venous thrombosis is not clear in the literature. Ay et al. suggest that although the ACE I/D is a SNP associated with ACE level, D/D variant does not contribute deep venous thrombosis and thromboembolism (30). In a multivariate analysis by von Depka et al. (31) the DD genotype of ACE was evaluated as an intermediate risk of hereditary thrombophilia. The prevalence of this genotype was higher in cases with venous thromboembolism than the control group. Some investigators state that the D/D genotype poses a risk in terms of cardiovascular pathology because of increased ACE plasma levels and enhances the likelihood of venous thromboembolism in thrombogenic conditions. (32). Our study does not support the contribution of ACE DD genotype to the formation of DVT or venous thromboembolism.

Clinical surveillance of cases with thrombosis or clinical suspicion of thrombophilia involves a combined assessment of genetic and other relevant risk factors, as thrombosis is a multifactorial condition. On the other hand, standard guidelines are used for the treatment of venous and arterial thrombosis. Treatment protocols for thrombophilia may comprise low molecular weight heparin injections and low dose acetylsalicylic acid (33). In the case of a pulmonary embolism, adjusting its treatment may be somewhat complicated. Although non-surgical approaches (anticoagulation or systemic thrombolysis etc.) have replaced surgery as the primary treat-

ment, surgical pulmonary embolectomy remains an important alternative for selected patients (34).

Conclusions

In conclusion, we detected a strong association between the 4G/5G polymorphism of PAI-1 and both DVT and PTE. The 4G/4G variant is likely to be involved in the development of these diseases. We also suggest that the A1298C polymorphism of MTHFR may be effective in the development of PTE. Screening of these polymorphisms for prophylaxis may be useful in risk groups (obesity, malignancy, congestive heart failure, immobility etc.) and in cases with a family history of PTE and DVT. Our research is limited to a certain region. More studies should be done to strengthen these findings.

Disclosure

The authors have not any conflicts of interest to notify related to the authorship and publication of this article.

Ethical Statement

This research was approved by the Ethics Committee of Sivas Cumhuriyet University (Decision number: 2016-03/37) . Informed consent forms were obtained from the individuals participating in the study. All processes was conducted in compliance with the Helsinki Declaration.

Author contributions

Design: MEY. Literature search: MEY, HKK. Laboratory analysis: MEY, HKK, OO. Drafting of the article: MEY, HK. Statistics: HK. Review and revision of the article: OO. Supply of patients: OB, YKT

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REFERENCES

1. Wang X, Ziegler M, McFadyen JD, Peter K. Molecular imaging of arterial and venous thrombosis. *Br J Pharmacol*. 2021; 178: 4246-69.
2. Ayyoub S, Orriols R, Oliver E, Ceide OT. Thrombosis Models: An Overview of Common In Vivo and In Vitro Models of Thrombosis. *Int J Mol Sci* 2023; 24: 2569.
3. Wenger N, Sebastian T, Engelberger RP, Kucher N, Spirk D. Pulmonary embolism and deep vein thrombosis: Similar but different. *Thromb Res* 2021; 206: 88-98.
4. Liu X, Cao P, Li Y et al. Safety and efficacy of pharmacomechanical thrombolysis for acute and subacute deep vein thrombosis patients with relative contraindications. *Medicine (Baltimore)* 2018; 97: e13013.
5. Zhao B, Hao B, Xu H et al. Predictive Model For Pulmonary Embolism In Patients With Deep Vein Thrombosis. *Ann Vasc Surg* 2020; 66: 334-43.
6. Jenab Y, Hosseini K, Esmaeili Z, Tofighi S, Ariannejad H, Sotoudeh H. Prediction of in-hospital adverse clinical outcomes in patients with pulmonary thromboembolism, machine learning based models. *Front Cardiovasc Med* 2023; 10: 1087702.

7. Zhu Zhang, Haobo Li, Haoyi Weng et al. Genome- wide association analyses identified novel susceptibility loci for pulmonary embolism among Han Chinese population. *BMC Med* 2023; 21: 153.
8. Turan O, Undar B, Gunay T, Akkoclu A. Investigation of inherited thrombophilias in patients with pulmonary embolism. *Blood Coagul Fibrinolysis* 2013; 24: 140-9.
9. Nie F, Yu M, Zhang K et al. Association of MTHFR gene polymorphisms with pancreatic cancer: meta-analysis of 17 case-control studies. *Int J Clin Oncol* 2020; 25: 312-21.
10. Prasun P, Vermeire K, Alali A. Cerebral venous sinus thrombosis and SCN1A, a novel association? *Mol Biol Rep* 2024; 51: 883.
11. Machado M, Neto D, Nunes S et al. The Importance of MTHFR C677T/A1298C Combined Polymorphism in Deep Vein Thrombosis: A Case Report. *Cureus* 2022; 14: e29265.
12. Akhter MS, Biswas A, Ranjan R et al. Plasminogen activator inhibitor-1 (PAI-1) gene 4G/5G promoter polymorphism is seen in higher frequency in the Indian patients with deep vein thrombosis. *Clin Appl Thromb Hemos*. 2010; 16: 184-8.
13. Zhang Q, Jin Y, Li X et al. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphisms and risk of venous thromboembolism-a meta-analysis and systematic review. *Vasa* 2020; 49: 141-6.
14. Saleh NY, Salem SS, Abo-El Fotoh WM, Soliman SE, Abo-Haded HM. Angiotensin converting enzyme insertion/deletion (ACE I/D) gene polymorphism in Egyptian children with congenital heart disease. *Birth Defects Res* 2020; 112: 963-9.
15. Zhang Y, Zhao Z, Wang C et al. Association Between the Angiotensin Converting Enzyme Gene Insertion/Deletion Polymorphism and Avascular Necrosis of the Femoral Head. *Genet Test Mol Biomarkers* 2019; 23: 778-82.
16. Fatini C, Gensini F, Sticchi E et al. ACE DD genotype: An independent predisposition factor to venous thromboembolism. *Eur J Clin Invest* 2003; 33: 642-7.
17. Coulam CB, Wallis D, Weinstein J, DasGupta DS, Jeyendran RS. Comparison of Thrombophilic Gene Mutations Among Patients Experiencing Recurrent Miscarriage and Deep Vein Thrombosis. *Am J Reprod Immunol* 2008; 60: 426-31.
18. Guzmán N, Salazar LA. Frequency of prothrombotic risk factors in patients with deep venous thrombosis and controls: their implications for thrombophilia screening in Chilean subjects. *Genet Test Mol Biomarkers* 2010; 14: 599-602.
19. Ghaznavi H, Soheili Z, Samiei S, Soltanpour MS. Association of Methylenetetrahydrofolate Reductase C677T Polymorphism with Hyperhomocysteinemia and Deep Vein Thrombosis in the Iranian Population. *Vasc Specialist Int* 2015; 31: 109-14.
20. Soltanpour MS, Soheili Z, Pourfathollah AA, Samiei S, Meshkani R, Kiani AA, et al. The A1298C Mutation in Methylenetetrahydrofolate Reductase Gene and Its Association With Idiopathic Venous Thrombosis in an Iranian Population. *Lab Medicine* 2011; 42: 213-6.
21. Basol N, Karakus N, Savas AY, Kaya I, Karakus K, Yigit S. The importance of MTHFR C677T/A1298C combined polymorphisms in pulmonary embolism in Turkish population. *Medicina (Kaunas)* 2016; 52: 35-40.
22. Ekim M, Ekim H, Yılmaz YK. The prevalence of Factor V Leiden, prothrombin G20210A, MTHFR C677T and MTHFR A1298C mutations in healthy Turkish population. *Hippokratia* 2015; 19: 309-13.
23. Bezgin T, Kaymaz C, Akbal O, Yılmaz F, Tokgoz HC, Ozdemir N. Thrombophilic Gene Mutations in Relation to Different Manifestations of Venous Thromboembolism: A Single Tertiary Center Study. *Clin Appl Thromb Hemost* 2018; 24: 100-6.
24. Jacobs A, Schutte AE, Ricci C, Pieters M. Plasminogen activator inhibitor-1 activity and the 4G/5G polymorphism are prospectively associated with blood pressure and hypertension status. *J Hypertens* 2019; 37: 2361-70.
25. Kollabathula A, Sharma S, Kumar N, Ahluwalia J, Das R, Varma N, et al. Plasminogen Activator Inhibitor-1 4G/5G Promoter Polymorphism in Adults with Splanchnic Vein Thrombosis: A Case-Control Study. *Indian J Hematol Blood Transfus* 2022; 38: 169-72.
26. Wang C, Zhai ZG, Shen YH, Zhao L. Clinical and genetic risk factors for venous thromboembolism in Chinese population. *Front Med* 2010; 4: 29-35.
27. Oguzulgen IK, Yilmaz E, Demirtas S, Erkekol FO, Ekim N, Demir N, et al. The role of Plasminogen activator inhibitor-1 polymorphism, factor-V-Leiden, and prothrombin- 20210 mutations in pulmonary thromboembolism. *Clin Appl Thromb Hemost* 2009; 15: 73-7.
28. Kaya H, Karkucak M, Salifoğlu H, Torun D, Kozan S, Tunca Y. The investigation of angiotensin converting enzyme I/D and plasminogen activator inhibitor-1 4G/5G polymorphisms in venous thromboembolism patients. *Tuberk Toraks* 2013; 61: 88-95.

29. Hočevár K, Peterlin A, Jovanović AM et al. Association between angiotensin-converting enzyme gene insertion/deletion polymorphism and susceptibility to preterm birth: A case-control study and meta-analysis. *Eur J Obstet Gynecol Reprod Biol* 2018; 231: 122-8.
30. Ay C, Bencur P, Vormittag R et al. The angiotensin- converting enzyme insertion/deletion polymorphism and serum levels of angiotensin converting enzyme in venous thromboembolism. Data from a case control study. *Thromb Haemost* 2007; 98: 777-82.
31. von Depka M, Czwalińska A, Wermes C et al. The deletion polymorphism in the angiotensin-converting enzyme gene is a moderate risk factor for venous thromboembolism. *Thromb Haemost* 2003; 89: 847-52.
32. Annunziata A, Coppola A, Di Spirito V, Cauteruccio R, Marotta A, Micco PD, Fiorentino G. The Angiotensin Converting Enzyme Deletion/Deletion Genotype Is a Risk Factor for Severe COVID-19: Implication and Utility for Patients Admitted to Emergency Department. *Medicina (Kaunas)* 2021; 57: 844.
33. A L Miranda-Vilela. Role of polymorphisms in factor V (FV Leiden), prothrombin, plasminogen activator inhibitor type-1 (PAI-1), methylenetetrahydrofolate reductase (MTHFR) and cystathionine β -synthase (CBS) genes as risk factors for thrombophilias. *Mini Rev Med Chem* 2012; 12: 997-1006.
34. Martinez Licha CR, McCurdy CM, Maldonado SM, Lee LS. Current Management of Acute Pulmonary Embolism. *Ann Thorac Cardiovasc Surg* 2020; 26: 65-71.