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Original Research Article

# The importance of *MTHFR* C677T/A1298C combined polymorphisms in pulmonary embolism in Turkish population

*Nursah Basol* [a,*], *Nevin Karakus* [b], *Asli Yasemen Savas* [a], *Ilker Kaya* [c], *Kayhan Karakus* [d], *Serbulent Yigit* [b]

[a] *Department of Emergency Medicine, Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey*
[b] *Department of Medical Biology, Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey*
[c] *Department of Cardiovascular Surgery, Tokat State Hospital, Tokat, Turkey*
[d] *Department of Radiology, Tokat State Hospital, Tokat, Turkey*

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ABSTRACT

*Background and objective:* Pulmonary embolism (PE) is an important cardiovascular emergency with high mortality. There are still problems related to the diagnosis of PE and genetic research may play a key role on diagnosis as well as determining risk stratification. In the present study, the aim was to evaluate *MTHFR* C677T and A1298C polymorphisms that play a role on folate metabolism in PE patients.

*Materials and methods:* A total of 118 PE patients and 126 controls were enrolled in the current study. Genomic DNA was isolated and genotyped using polymerase chain reaction (PCR) analyses for the *MTHFR* C677T and A1298C polymorphisms.

*Results:* There was no association between clinical and demographic characteristics of PE patients and both *MTHFR* C677T and A1298C polymorphisms. Allele frequencies showed a significant difference between patients and controls. T allele frequency was significantly higher in the patients' group than the control group. There was an association between PE and combined *MTHFR* C677T and A1298C polymorphisms.

*Conclusion:* We found an association between *MTHFR* C677T/A1298C combined mutations and PE in the Turkish population. Future genetic studies investigating combined mutations could be very helpful to identify risk population in PE.



* *Corresponding author at*: Department of Emergency Medicine, Faculty of Medicine, Gaziosmanpasa University, 60100 Tokat, Turkey. Tel.: +90 3562129500/3418; fax: +90 356 2133179.
E-mail addresses: nursahbsl@gmail.com, drnursahbs@hotmail.com (N. Basol).

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## 1. Introduction

Pulmonary embolism (PE), a serious clinical manifestation of venous thromboembolism (VTE), is commonly seen cardiovascular disease with high mortality rate [1]. PE is a multifactorial disease that includes genetic and environmental factors together on the pathogenesis. The importance of genetic factors was presented in recent years [2]. As an example; protein C, protein S and antithrombin deficiencies were identified in 1965 [3]. However, genetic screening has not been exactly clarified yet in clinics according to our knowledge.

The increased level of homocysteine (hyperhomocysteinemia) is a strong risk factor for thrombosis and it is influenced by genetic factors. Methylene-tetrahydrofolate reductase (*MTHFR*) is an important enzyme that regulates folate metabolism due to affect DNA methylation and nucleic acid synthesis [4]. *MTHFR* catalyzes the reduction of 5,10 methylenetetrahydrofolate (5,10-MTHF) to 5 methyltetrahydrofolate (5-MTHF). 5-MTHF is a methyl donor for the homocysteine-to-methionine remethylation and circulatory form of folate. The reduction of *MTHFR* activity causes hyperhomocysteinemia [5]. The gene for *MTHFR* is located on chromosome 1 at 1p36.3 and consists of 11 exons [6]. *MTHFR* gene includes two common polymorphisms – C677T (ALA222VAL) and A1298C (GLU429ALA) polymorphisms [7]. These polymorphisms are correlated with reduction on *MTHFR* enzyme activity. With this way, these polymorphisms play a role on pathogenesis of thrombosis. There are several studies that present influence of MTHFR polymorphisms on diseases such as ischemic stroke [8], obstetrical pathologies [9], arterial and venous thrombosis [10,11] and metabolic diseases [12].

There are still some problems in the diagnosis stage of PE due to variety of symptoms particularly in emergency departments [3]. CT scans are reliable on diagnosis but it includes some risks as allergic reactions or contrast-induced nephropathy. Also, it includes downsides as its cost and exposure of radiation [13]. Besides variety of symptoms, subclinical cases may be seen in PE [14]. Both of them lead to an increase suspicion of pulmonary embolism in ED. Some risk scoring systems as Wells and Geneva are identified due to risk stratification for PE [15]. Additionally, especially the negativity of D-dimer may estrange from doubt of PE [16,17]. Nonetheless, there is an increase to use CT scans in ED [13]. All these mentioned factors increase importance of genetic researches due to finding possible diagnosis marker or determine patients with genetic predisposition to PE. Additionally, it may provide prophylaxis to the risk population and it will help decrease the incidence of PE. To the best of our knowledge, in literature, the effects of *MTHFR* polymorphisms in PE were shown mostly in case reports and thus, there is limited information about this issue [18]. This study aimed to evaluate these polymorphisms in PE patients in our population.

## 2. Materials and methods

### 2.1. Subjects

The study group consisted of 118 unrelated patients with PE (61 male and 57 female; mean age: 58.21 ± 10.531 standard deviation [SD] years), and 126 (74 male and 52 female; mean age: 55.98 ± 7.773 SD years) unrelated healthy controls. PE patients were recruited consecutively and prospectively from those whom were treated and followed up in the Emergency Medicine Department of Gaziosmanpasa University Research Hospital, Tokat, Turkey. The diagnosis of PE was confirmed with thorax computed tomography pulmonary angiography (CTPA) by experienced emergency physicians and also radiologists. All control subjects were confirmed to be free from VTE, coronary artery disease, malignancy, pregnancy, previous surgery and stroke. Controls with family history of any evidence for thrombosis and women controls with prior history of abortions or other obstetric complications were excluded from the study. All participants, patients and healthy controls, were of Turkish origin, from the inner Central Black Sea region of Turkey. The healthy controls matched for age and gender with PE patients. The study protocol was approved by the Local Ethics Committee of Gaziosmanpasa University, Faculty of Medicine and written informed consent was obtained from the study participants.

### 2.2. Genotyping

Genomic DNA was extracted from whole venous blood samples using a commercial DNA isolation kit (Sigma–Aldrich, Taufkirchen, Germany). The *MTHFR* gene C677T (Ala222Val, rs1801133) and A1298C (Glu429Ala, rs1801131) polymorphisms were analyzed by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) assay. The *MTHFR* C677T polymorphism was analyzed by using forward (F) 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and reverse (R) 5′-AGG ACG GTG CGG TGA GAG TG-3′ primers. The amplification conditions consisted of an initial melting step of 5 min at 94 °C; followed by 35 cycles of 30 s at 94 °C, 30 s at 61 °C, and 30 s at 72 °C; and a final elongation step of 5 min at 72 °C. After amplification, the 198 bp PCR product was digested with HinfI. The digestion products were separated on 3% agarose gels, and fragments stained with the ethidium bromide were photographed on an ultraviolet transilluminator. Wild type (CC) individuals were identified by only a 198 bp fragment, heterozygotes (CT) by both the 175/23 bp and 198 bp, and homozygote variants (TT) by the 175/23 bp. For *MTHFR* A1298C polymorphism, amplification was carried out using primers (F: 5′-CTT TGG GGA GGT GAA GGA CTA CTA C-3′ and R: 5′-CAC TTT GTG AGC ATT CCG GTT TG-3′) and the protocol described previously [19]. The amplified 256 bp product was digested with MboII. Wild type (AA) was identified by 4 fragments (176, 30, 28, and 22 bp), heterozygous AC by 5 fragments (204, 176, 30, 28, and 22 bp) and homozygous variant by 3 fragments (204, 30, and 22 bp). The major visible bands were 204 and 176 bp. Second PCR was performed to confirm samples whose results were not clear.

### 2.3. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics, version 20) and OpenEpi Info software package version 3.01 (www.openepi.com). The chi-square ($\chi^2$) test was used to evaluate the Hardy–Weinberg equilibrium (HWE) for the distribution of