ORIGINAL ARTICLE

The prevalence of Factor V Leiden, prothrombin G20210A, MTHFR C677T and MTHFR A1298C mutations in healthy Turkish population

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Abstract

Background: Factor V Leiden (FVL), prothrombin gene (PT G20210A) and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms are the main biomarkers used in the evaluation of tendency to venous thromboembolism. Our study aimed to investigate the distribution frequencies of these polymorphisms in healthy Turks living in the urban Yozgat region.

Material and Methods: This study included 90 blood donor candidates. All the donors were apparently healthy, and there was no family relationship between them. Mutations including FVL, PT G20210A, and MTHFR (C677T, A1298C) were investigated in all participants. Screening of polymorphisms was carried out using the SNaPshot® multiplex system.

Results: There were 42 male and 48 female individuals with age range 17-78 years and mean age 47.5 ± 13.6 years. The heterozygous FVL mutation was noted in 17 (10 male and seven female) donors (19%). FVL mutation was more frequently encountered in males than in females (23.8% vs. 12.5%). The heterozygous PT G20210A mutation was observed in five (5.5%) of the 90 (three male, two female) donors. The prevalence of homozygous polymorphisms of MTHFR C677T was 8.8% and of MTHFR A1298C 13.3%. On the other hand, four of the 90 participants (4.4%) carried none of these polymorphisms.

Conclusion: This study showed that the prevalence of FVL, PT G20210A, MTHFR C677T and MTHFR A1298C polymorphisms is quite high, and the coexistence of FVL with other genotypes is not rare in a healthy Turkish population living in the Yozgat region. Of course, further detailed studies should be performed to support these findings. Hippokratia 2015; 19 (4): 309-313.

Keywords: Factor V Leiden, Prothrombin G20210A, MTHFR, polymorphism

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Introduction

Thromboembolic events occur as a result of the disruption of the balance between fibrinolysis and thrombosis. Thus, it is important to examine the inherited and acquired thrombotic risk factors[1]. In the last five decades, the molecular basis of both coagulation and anticoagulation pathways have been well studied, and some hereditary risk factors have been found responsible for venous thromboembolism (VTE)[2]. Factor V Leiden (FVL), prothrombin gene (PT G20210A), and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms are the common molecular biomarkers used in the evaluation of tendency to venous thromboembolism[1].

In 1993, Dahlback et al[3] uncovered a previously unrecognized mechanism for thromboembolism. It is characterized by poor anticoagulation response to activated protein C (APC) and seems to be related to the polymorphism of factor V. The replacement of glutamine by arginine at codon 506 leads to loss of 506 cleavage sites and reduction in sensitivity to inactivation by APC. The polymorphism of factor V increases the risk of VTE by means of increased thrombin production[4,5].

The mutant FV molecule is known as FVL (Leiden is a city in Holland where FVL was first identified) and named as factor V Q506 or Arg506Gln. Currently, FVL is accepted as the most common inherited risk factor for VTE.

Poorts et al[6] found a single G to A Nucleotide transition at position 20210 in the 3' untranslated region of the prothrombin gene in 18% of selected patients with familial venous thrombosis.

MTHFR enzyme plays a significant role in cellular metabolism of folate and synthesis of nucleotides (DNA, RNA) and is essential in the methyl cycle that converts homocysteine to methionine. To date, two important MTHFR polymorphisms (C677T and A1298C) have been identified. These polymorphisms are related to reduced MTHFR activity. The C677T mutation is known as a point mutation with the substitution of cysteine-thymine at the nucleotide position 677 on the MTHFR gene. It is the only mutation that results in a thermolabile variant and predisposes to mild hyperhomocysteinemia when

the folic acid level is low and has been described as a risk factor for peripheral and coronary arterial disease and also for venous thrombosis[7].

Previous studies have revealed that the prevalence of these mutations (FVL, PT G20210A, MTHFR C677T and A1298C) is different in distinct geographical areas, races and ethnic populations[4]. Our study aimed to investigate the frequency of these mutations in healthy Turks living in the urban Yozgat region.

Material and Methods

This prospective study was carried out in the Cardiovascular Surgery Clinic of Bozok University Hospital, in Yozgat, Turkey between October 2014 and June 2015. The study's protocol was approved by Bozok University clinical research Ethics Committee (14/10/2015, 604/176) and was conducted in accordance with the Helsinki Declaration. Informed consent was obtained from every participant after the aim of the study had been fully elucidated. All the participants were Caucasians living in the same geographical region.

This study included 90 consecutive blood donor candidates. All of the donors were apparently healthy, and there was no family relationship between them. Mutations including FVL, PT G20210A, and MTHFR (C677T, A1298C) were investigated in all participants.

Laboratory study

We collected into tubes containing ethylenediaminetetraacetic acid (EDTA) 5 ml of fasting venous blood from each participant. DNA isolations were performed from 200 µl peripheral blood samples, using QIAamp DNA Blood Mini Kit (Qiagen Inc., Germany) and stored at -20° C until the polymerase chain reaction (PCR) step.

Screening of polymorphisms was carried out using SNaPshot® multiplex system (Applied Biosystems Inc., Switzerland). For this purpose, three primers were designed for all four polymorphisms; two for PCR and one for SNaPshot® reaction. PCRs were checked using 2% agarose gel electrophoresis. All four reactions for each sample were combined and purified using NucleoFast® 96 PCR kit (MACHEREY-NAGEL GmbH, Germany).

SNaPshot® Reactions

Purified PCRs were used in the SNaPshot® assay, which was carried out according to the manufacturer's instructions. Capillary electrophoresis of the SNaPshot® reactions was also carried out according to the manufacturer's recommendations using an ABI 3130 capillary electrophoresis instrument (Applied Biosystems Inc., Switzerland). Genotyping of the polymorphisms was achieved by analyzing the electropherograms obtained during capillary electrophoresis on GeneMapper 4.0 software (Applied Biosystems Inc., Switzerland).

Statistical analysis

Data were expressed as the mean ± standard deviation. The prevalence of mutations was shown as percent (%). The paired sample t-test was used for statistical analysis. A p value of less than 0.05 was considered as statistically significant.

Results

There were 42 male and 48 female individuals ranging in age from 17 to 78 with a mean age of 47.5 ± 13.6. There were no homozygous carriers for FVL and PT G20210A polymorphisms. The heterozygous FVL mutation was noted in 17 (10 male and seven female) of all donors (19%). FVL mutation was more frequently encountered in males than in females (23.8% vs. 12.5%) ($p < 0.001$). Of all heterozygous FVL carriers, 14 had additional defects as follows: four were heterozygous for MTHFR 677, two were homozygous for MTHFR 1298, six were heterozygous for MTHFR 1298, and two were heterozygous for PT G20210A. The remaining three FVL carriers had no additional defects (Table 1). The heterozygous PT G20210A mutation was observed in five (5.5 %) of the 90 (three male, two female) donors. All of them had additional defects (Table 2).

The homozygous and heterozygous MTHFR 677 genotypes were identified in eight (8.8%) and 30 (33.3%) of all donors, respectively. Thirteen (14.4%) participants were double heterozygotes for MTHFR C677T/ MTHFR A1298C genotypes (Table 3). The homozygous and heterozygous MTHFR 1298 genotypes were found in 12 (13.3%) and 30 (33.3%) participants, respectively. One of the 12 homozygous MTHFR 1298 genotypes had associated heterozygous FVL. The remaining homozygous MTHFR 1298 carriers had no additional mutation. On the other hand, there were no polymorphisms in four (4.4%) of 90 participants.

Discussion

Resistance to activated protein C (APCR) is the most frequent hereditary defect associated with venous thromboembolism. The FVL seems to be responsible for more than 90% of APCR cases[8]. APCR resulting from FVL is the most common inherited type of thrombophilia, and its prevalence is higher than the sum of frequencies of all other causes of hereditary thrombophilia[9].

Compared with normal activated factor V, activated FVL is relatively more resistant to inactivation by APC, resulting in a prothrombotic condition[10]. Inactivation of FVL occurs 10-20 times more slowly than the inactivation of the native form of FV. Thus, excessive thrombin generation and a presumed lifelong prothrombotic tendency develop[11]. FVL was found to be a contributory risk factor for the development of DVT in the Iranian population[12]. Also, FVL was found to be significantly associated with recurrent pregnancy loss[13].

Epidemiological and biochemical investigations have suggested that FVL should have occurred as a single event in the past. The prevalence of FVL is considerably higher in the Mediterranean region compared to the rest of the world[14]. It has been estimated that FVL originated in the Middle East thousands of years ago in the Neolithic

Table 1: Distribution of the 17 heterozygote Factor V Leiden carriers according to age, gender and accompanying additional genetic mutations.

Age and Gender	FVL	PT G20210A	MTHFR 677	MTHFR 1298
33 M	HET	WT	WT	WT
38 F	HET	WT	WT	HET
34 F	HET	WT	HET	WT
32 F	HET	WT	WT	HET
47 F	HET	WT	HET	WT
54 F	HET	WT	WT	HET
56 M	HET	WT	WT	HET
31 M	HET	WT	WT	WT
51 F	HET	WT	HET	WT
51 M	HET	WT	WT	MUT
75 F	HET	WT	WT	WT
55 M	HET	WT	WT	HET
59 M	HET	WT	HET	HET
58 M	HET	WT	WT	MUT
41 M	HET	WT	WT	HET
50 M	HET	WT	HET	HET
56 M	HET	HET	HET	WT

M: male, F: female, FVL: Factor V Leiden, PT 20210A: Prothrombin gene mutation, MTHFR: Methylenetetrahydrofolate reductase, HET: heterozygous, WT: wild type, MUT: homozygous.

Table 2: Distribution of the five heterozygote prothrombin gene mutation (PT G20210A) carriers according to age, gender and accompanying additional genetic mutations.

Age and Gender	PT G20210A	FVL	MTHFR 677	MTHFR 1298
36 F	HET	WT	HET	HET
49 M	HET	WT	HET	WT
56 M	HET	HET	HET	WT
53 M	HET	WT	MUT	WT
50 F	HET	WT	WT	HET

M: male, F: female, FVL: Factor V Leiden, PT 20210A: Prothrombin gene mutation, MTHFR: Methylenetetrahydrofolate reductase, HET: heterozygous, WT: wild type, MUT: homozygous.

Table 3: Distribution of the eight homozygote MTHFR 677 carriers according to age, gender and accompanying additional genetic mutations.

Age and Gender	PT G20210A	FVL	MTHFR 677	MTHFR 1298
40 F	WT	WT	MUT	WT
34 F	WT	WT	MUT	WT
50 M	WT	WT	MUT	WT
52 F	WT	WT	MUT	WT
17 F	WT	WT	MUT	WT
53 M	HET	WT	MUT	WT
48 F	WT	WT	MUT	WT
73 M	WT	WT	MUT	WT

M: male, F: female, FVL: Factor V Leiden, PT 20210A: Prothrombin gene mutation, MTHFR: Methylenetetrahydrofolate reductase, HET: heterozygous, WT: wild type, MUT: homozygous.

period. Thus, the prevalence of FVL mutation is also estimated to be high among Turkish people[15].

FVL is absent in Greenland Inuits and has a low incidence in French and Spanish Basques. Basques are known to be the oldest European ethnic groups remaining from the Paleolithic period. The rarity of FVL in the Basque populations has also suggested that FVL came from outside continental Europe through the migration of Neolithic farmers[14,16]. There is an increasing cline of FVL prevalence from the west to east and from the north to south of continental Europe. Therefore, FVL presumably originated in Turkey and expanded to Europe through farmer migration probably from Anatolia[16].

Anatolia is historically the meeting point of various ethnic groups from the European, Asian and African continents due to its geographical proximity to these continents[17]. Thus, it is logical that the prevalence of FVL changes according to the regions of Anatolia[18]. When

considering the composition of its population, Yozgat is a good sample from which to learn these mutation rates in Turkish society owing to its central location in Anatolia.

The prevalence of FVL also changes according to the geographic region and the ethnicity of the population. The high prevalence of FVL was reported as 9-15% in the southeast of Europe and 13% in the Middle East. However, African and Asian populations have a low incidence of FVL[18]. FVL prevalence has been found high in the healthy Turkish population; nevertheless, there are some variations in different areas of Anatolia[8].

Previous studies showed that the carrier rates of FVL in healthy Turkish people are between 7.1% and 9.1%[8]. In a pooled analysis, the average frequency of FVL carriers was found to be 7.9% in a healthy Turkish population pooled from 26 centers[18]. The prevalence of the FVL ranges from 1% to 15% in healthy European Caucasian populations. Similar results were also reported in Caucasians living outside Europe[14]. FVL reached high frequencies in Europe, with carrier rates of 15.9% in the Greek population and 8.8% in the English Caucasian population[8] and average frequency was estimated to be 3.5%[19]. The previous studies revealed the frequency of FVL to be 13.3% in Greek Cypriots and similarly 12.2% in Turkish Cypriots[18,20]. The frequency of FVL was reported as 1.65% in Hispanic Americans, 0.87% in Black Americans, and no FVL mutation was found in Native Americans and Asian Americans[8]. The prevalence of FVL carriers was reported as 20.1% in Palestinians[21]. In our series FVL genotype prevalence was reported as 19% and is consistent with the prevalence rate reported for healthy Palestinians, but higher than the prevalence rates of European and Turkish populations[14,19].

Aryurachai et al[22] showed that the carrier rate of FVL was higher in men than women (9.9% vs. 2.8%). Our findings also demonstrated a significantly higher prevalence of FVL in men than women (23.8% vs. 12.5%, $p < 0.001$).

The PT G20210A mutation results in elevated levels of prothrombin due to increased prothrombin synthesis. Similar to FVL, our study showed that the prevalence of PT G20210A carriers is higher than that of the white population of European origin (Caucasians) and that those from Asian and African backgrounds have almost none of these mutations[23,24]. The distribution of the PT G20210A genotype has a north-south gradient in Europe. The incidence of carriers has been reported as 3% in Southern Europe, nearly twice of that in Northern Europe[25]. The prevalence of the PT G20210A genotype (7.8%) in the Greek-Cypriot population was found to be 2.2 times higher than in Europeans[20]. In a previous study conducted in the Turkish population, this mutation was found to be 6.5% in patients with venous thrombosis and 1.2% in healthy Turkish people[26]. A high frequency of PT G20210A mutation (5.5%) in the healthy Turkish population was observed in this study. PT G20210A mutation was found in 9.3% of healthy Palestinians[21]. It is higher than those values reported from other countries[20].

The frequency of MTHFR C677T polymorphism is variable in different countries. In the healthy Greek Cypriot population, the rates of homozygous and heterozygous MTHFR C677T carriers were found to be 17.8% and 44%, respectively[20]. In the healthy Turkish population, the frequencies of homozygosity and heterozygosity were found to be 9.6% and 47.4%, respectively[27]. In our series, homozygous and heterozygous MTHFR C677T carriers were found to be 8.8% and 33.3%, respectively. In European Caucasians, the rate of homozygous MTHFR C677T carriers ranges between 7.8% and 18%[27]. The prevalence of homozygous MTHFR C677T carriers was reported as 11.5% in Japan and similarly 9.6% in Turkey[27]. In the current study, it was found to be 8.8%. The prevalence of MTHFR C677T carriers was found to be 42.2% of our participants, which is higher than the values reported for the same genotype among healthy Palestinians (13.8%) and Lebanese (39.7%)[21,28].

In Turkish DVT patients, the rate of MTHFR C677T polymorphism was reported as 56.8% (46.7% heterozygous and 10% homozygous) in men and 62.6% (47.7% heterozygous and 15% homozygous) in women[1]. In this series, the frequency of MTHFR C677T polymorphism was found to be 40.5% (33.3% heterozygous and 7.1% homozygous) in men and 43.7% (32.5% heterozygous and 10.4% homozygous) in women.

Actually, it is widely accepted that the MTHFR 677 genotype alone is not a risk factor for thrombosis. In the homozygous state, however, this variant can be considered as a risk factor when combined with other thrombophilic conditions[24]. The carriers of the heterozygote MTHFR 677 genotype have no evidence of increased homocysteinemia levels or increased risk of venous thrombosis. Homozygosity for the mutation (677 TT) is associated with moderately increased homocysteinemia levels, especially in folate deficiency. In individuals with normal homocysteinemia concentration, the determination of MTHFR mutation may not be indicated in the clinical assessment of thrombophilia[9].

The A1298C polymorphism occurs in the C-terminal region and could only influence the allosteric enzyme regulation through the S-adenosylmethionine-binding site. Although MTHFR A1298C mutation has decreased enzymatic activity, neither the heterozygous or homozygous A1298C genotypes are associated with elevated homocysteinemia concentration, or a lowered folic acid concentration. Conversely, the presence of the homozygous C677T allele increases the risk of thrombolytic events. This discrepancy could be explained by the fact that the C677T polymorphism found in exon 4 directly affects the N-terminal catalytic domain of MTHFR enzyme[7]. The frequency of the MTHFR A1298C genotype is not as well studied as that of the C677T genotype[27]. The prevalence of the homozygous MTHFR A1298C genotype was found to be 10% of the Turkish population[27], similar to our series (13.3%). The frequency of C677T/A1298C compound heterozygosity was reported as 17% in the USA, 20% in Holland, 21.6% in Turkey[27] and 15.5% in this series.

Conclusion

Our study showed that the prevalence of FVL, PT G20210A, MTHFR C677T and MTHFR A1298C polymorphisms is quite high and the coexistence of FVL with other genotypes is not rare in a healthy Turkish population. One of the advantages of our study is that all participants lived in the same region and were of the same origin and race. This study also showed that FVL and PT G20210A genotypes were more frequently encountered in populations living in the middle of Anatolia compared with those of the other geographic regions of Turkey and Eurasia. Of course, the sample size of this study compared to the population size of the Yozgat region is small and the results may be altered if further studies take place.

Conflict of interest

The authors declared no conflicts of interest.

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