The Frequency of Some Thrombophilic Mutations in Eastern Turkey

Doğu Anadolu Bölgesi’nde Bazı Trombofilit Mutasyonlarının Görülme Sıklığı

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Abstract

Objective: Factor V / Factor II / Methylene tetrahydrofolate reductase, gene polymorphisms are closely associated with thrombophilia. Regional frequencies of these mutations may show a characteristic state. The aim of our study was to evaluate the frequency of commonly seen Factor V / Factor II / Methylenetetrahydrofolate reductase gene polymorphisms in Eastern Turkey.

Materials and Methods: In 433 patients sent to the laboratory with the suspicion of thrombophilia, using whole blood samples, an automated Nucleic Acid Test was used for mutation determinations in Verigene System. The kit module was designed to detect the Factor V G1691A / Factor II G20210A / Methylenetetrahydrofolate reductase gene C677T single nucleotide polymorphisms.

Results: In 433 patients, 8.7% for Factor V G1691A polymorphisms were heterozygous genotype, 3.9% for Factor II G20210A polymorphisms were heterozygous genotype, and 43.9% for methylenetetrahydrofolate reductase gene C677T single nucleotide polymorphisms.

Conclusion: Detection of these commonly seen Factor V / Factor II / Methylenetetrahydrofolate reductase single nucleotide polymorphisms can help to identify patients in high risk group and to evaluate the interaction of genetic and acquired risk factors. Our findings suggest that commonly seen thrombophilic allele mutation frequency in our region is the same as the data reported in the literature.

Keywords: Factor II, factor V, MTHFR, polymorphisms

Introduction

Thrombophilia, which is also called hypercoagulability, is a prothrombotic state that increases the risk of thrombosis [1-3]. This clinical condition can be seen in half of people who have an episode of thrombosis that was not associated with another predisposing reason [2]. Although many people have a detectable abnormality, most of these only develop thrombosis when there is an additional risk factor present [1]. Venous thromboembolism (VTE) and pulmonary embolism (PE) have a high ratio of morbidity and mortality [4]. Recently it is widely discussed that venous thromboembolism is a multifactorial disease, and is associated with both genetic and acquired risk factors. Pregnancy, oral contraceptives, lupus
anticoagulants, postoperative states, myeloproliferative diseases, previous thrombosis, and prolonged immobilization are some of the conditions that predispose to VTE [3].

The factor V Leiden (FVL; FV G1691A, FV 1691G>A) and prothrombin (Factor II, FII G20210A, FII 20210G>A) G20210A mutations are the most frequent causes of inherited thrombophilia. An increased risk of venous thrombosis has been considered associated with several genetic defects, which involve genes that control anticoagulant production (antithrombin, protein C, and protein S) and genes responsible for the production of fibrinogen and certain procoagulants. All of these defects result in increased thrombin generation. The FV gene is located in 1q23 of human chromosome. The most common genetic mutation manner is a point mutation in FV gene, which is characterized by a substitution of adenine for guanine at nucleotide 1691, resulting in the substitution of arginine (R) with glutamine (Q) at amino acid 506 (called as FVL) [5]. The FVL mutation is most prevalent in Caucasians, particularly in Europeans and in Americans of European descent, occurring in 5% to 10% of individuals in the general population. It is extremely rare in Asians and Africans. The mutation causes the production of FV protein that is resistant to the action of activated protein C (APC), resulting in over 90% of APC resistance. A fivefold to 10-fold increased risk of thrombosis is seen in heterozygotes, and homozygotes have a 50- to 100-fold increased risk of thrombosis [3].

The FII gene is located in 11p11-q12 of human chromosome, which produces prothrombin. A mutation in the 3'untranslated region of the prothrombin gene, which is a guanine to adenine substitution at nucleotide 20210, is associated with the increases in prothrombin production and an increased risk of venous thrombosis [6]. The prevalence of this mutation has been reported as 2.3% in healthy controls, and as 18% in selected patients with familial thrombosis. This mutation has been reported to be the second most common genetic defect predisposing to venous thrombosis [3, 6].

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) gene is located on human chromosome 1p36.3, and MTHFR is involved in folate metabolism and catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Two common polymorphisms, C677T (thermolabile) and A1298C, have been described in detail. Because the activity of the encoded enzyme is reduced at 37°C by 50 to 60%, and at 46°C by 65%, C677T polymorphism is commonly called thermolabile. C677T polymorphism is known as a point mutation at position 677 of the MTHFR gene that converts a cytosine into a thymine and causes alanine to replace valine in the enzyme [7,8]. Both homozygotes and heterozygotes have increased plasma homocysteine levels if their folate intake is insufficient, but normal blood levels if their folate intake is adequate. The mutation 1298A>C has less effect on plasma homocysteine levels [9]. The frequency of MTHFR C677T homozygosity ranges from 1% to 25.3% among different population [10, 11].

In the present study, we tried to investigate in Eastern Turkey the frequencies of human FVL, FII G20210A, and MTHFR C677T single nucleotide polymorphisms (SNPs) mentioned above and to make genotyping in patients with probable thrombophilia.

**Materials and Methods**

This study aimed to analyse the routine laboratory results of a cohort of patients with thrombophilia. Data for the study was obtained from the laboratory information system. A total of 433 results were analysed. All patients included in the study were sent to the laboratory with suspicion of thrombophilia. Whole blood samples were obtained from the patients, and analyses were performed daily in fresh samples. A Nucleic Acid Test in the form of Verigene Hypercoagulation Panel was used for mutation determinations in Verigene System (Nanosphere Inc.; Northbrook, IL, USA). The Verigene Processor SP had the combinations of automated nucleic acid extraction, purification, amplification and hybridization in one module. In other words, a nanotechnology based on nucleic acid and protein determinations was used for molecular diagnostic testing. The kit insert was followed for testing procedure. The kit module was designed to detect the 1691G>A, G20210A and C677T SNPs in FV, FII and MTHFR genes, respectively. The result reports were obtained for each polymorphism as wild-type (homozygote), heterozygote or mutant (homozygote).

**Results**

The number of the patients results included in the study was 433 (male: 170 and female: 263; age mean: 38.4 ± 12.1 and range: from 18 to 75). In the study population of 433 patients genotyped between May 2012 and April 2014, for FII G20210A mutation, 17 patients were heterozygous (3.9 %), and 416 with wild-type (96.1%); for FVL mutation, 38 patient was heterozygous (8.7%), 1 patient homozygous mutation (0.23%) and 394 with wild-type (91.1%); and for MTHFR C677T mutation, 190 patients were heterozygous (43.9%), 230 with wild-type (53.1%) and 13 homozygous mutation (3.0%). In 433 patients, 8.7% for FVL 1691G>A mutations were heterozygous genotype, 3.9% for FII 20210G>A mutations were heterozygous genotype, 43.9% for MTHFR 677C>T mutations were heterozygous genotype and 3.0% homozygous mutation genotype. Table 1 summarizes the distribution of FV/FII/MTHFR gene polymorphism frequency in both sexes and in total.
Discussion

As mentioned above, the FV and FII genes encode FV protein and prothrombin, which both play a key role in the formation of blood clots (coagulation or fibrin production). The MTHFR gene encodes an enzyme that catalyses the conversion of 5,10 methylenetetrahydrofolate to 5- methyltetrahydrofolate, the primary circulating form of folate. The aetiology of most common congenital abnormalities has been described as multifactorial. Inherited changes in the MTHFR gene may contribute to the development of some of these congenital anomalies. The same thing is true for thrombotic disorders. For this reason, a population-based study of FVL/ FII G20210A/ MTHFR C677T polymorphisms in Eastern Turkey was conducted.

Haghighatgoo et al. [11] reported MTHFR C677T genotype frequency in patients of Middle Eastern descent, living in USA, as follows: 47.6% wild type, 40.5% heterozygous, and 11.9% homozygous genotype. When compared with the present data, it can be seen that Haghighatgoo’s reported homozygous and heterozygous percentages are a little bit higher. A good comparison of the results of our study could be made with those of Sazci et al. [9], a study conducted in Turkey. For MTHFR C677T mutation, they detected a frequency of 42.9% as heterozygous, and of 9.6% as homozygous genotype, which were very similar to those of the present study. In a study carried out by Friedline et al. [3], the combined genotyping for the FVL and prothrombin mutations in patients presenting with thromboembolic episodes was evaluated, and the genotypic findings were correlated with clinical characteristics. Their result associated with FVL mutations was very similar to ours, and that associated with prothrombin mutations was higher.

Detection of FVL/ FII G20210A/ MTHFR C677T polymorphisms can help to identify patients at high risk and help evaluate the interaction of genetic and acquired risk factors. The study data showed that no regionality of these mutations is present for Eastern Turkey. It was determined in 433 patients with suspected thrombophilia that 8.7% for FV 1691G>A mutations were heterozygous genotype, that 3.9% for FII 20210G>A mutations were heterozygous genotype, and that 43.9% for MTHFR 677C>T mutations were heterozygous genotype and 3.0% homozygous mutation genotype.

A few studies (12, 13) have been carried out in our region, in which the relation of any disease to the frequency of a given polymorphism has been investigated. However, it has been stated the more population screening, the more credible the results in molecular biological testing. The same is true for our study: more population screening is needed for results that are more reliable.

Ethics Committee Approval: This study doesn’t need to be approved by ethical committee, because this is a retrospective study conducted by the analysis of routine laboratory data.

Informed Consent: This study doesn’t include an informed consent, because this study is performed by the analysis of routine laboratory data.

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References

Ozturk et al. Thrombophilic Mutations Frequency


