Molecular Analysis of Factor V Leiden, Factor V Hong Kong, Factor II G20210A, Methylenetetrahydrofolate Reductase C677T, and A1298C Mutations Related to Turkish Thrombosis Patients

Bilgen Dölek, PhD,*† Serpil Eraslan, PhD,† Sevim Eroğlu, BSc,†
Belgin Eroglu Kesim, MD,† Turgut Ulutin, PhD,§ Altan Yalçıner, MD,†
Yahya R. Laleli, MD,† and Nermin Gözükırmızı, PhD*‖

*Department of Molecular Biology and Genetics, Istanbul University, Istanbul, Turkey †Department of Molecular Genetics, Duzen Laboratory, Istanbul, Turkey ‡Obstetrics and Gynecology Clinic, Sisli Etfal Training and Research Hospital, Istanbul, Turkey; §Department of Medical Biology, Istanbul University, Istanbul, Turkey; and ‖ TUBITAK, Research Institute for Genetic Engineering and Biotechnology, Gebze, Kocaeli, Turkey

Summary: Inherited gene disorders related to the hemostatic system have been documented as risk factors for thrombosis. The roles of factor V Hong Kong (FV Hong Kong), factor V Leiden (FV Leiden), factor II G20210A (FII G20210A), methylenetetrahydrofolate reductase (MTHFR) C677T, and MTHFR A1298C mutations in Turkish patients with thrombosis (270 patients) compared with healthy controls (114 subjects) were evaluated. Polymerase chain reaction–based restriction enzyme analysis was carried out to screen these mutations, and single-strand conformation analysis was established to identify variations using the primers selected for restriction enzyme analysis studies. As a result, a significant relationship was determined among FV Leiden, FII G20210A, and thrombosis. The FV Hong Kong mutation was observed in only 2 patients with pulmonary vein thrombosis who are FV Leiden/FV Hong Kong compound heterozygous for FV gene. MTHFR C677T and A1298C were equally distributed in the patient group compared with the control group. All named mutations were also identified with single-strand conformation analysis, but a new variant/polymorphism during studies was not found. Because some inherited abnormalities are associated with thromboembolic disorders, determining the mutations and gene-to-gene interactions in patients with thrombosis history has a great impact on diagnosis and treatment of these diseases.

Key Words: Deep vein thrombosis—Inherited coagulation factor—Mutation—Turkish population.

Inherited disorders related to hemostatic system are known as risk factors for thromboembolic events such as myocardial infarction, stroke, pulmonary embolism, pregnancy complications, and especially recurrent deep vein thrombosis (DVT) (1,2). The most common identified mutations are factor V Leiden (FV Leiden), prothrombin 20210G>A (FII G20210A), methylenetetrahydrofolate reductase 677 C>T (MTHFR C677T), and 1298A>C (MTHFR A1298C) (3-5). FV Leiden and FV Hong Kong mutations of FV gene can render FVa resistant to activated protein C because of a loss of 306 and 506 cleavage sites, respectively (6). FV Leiden predisposes the patient to thrombosis[7]; however, the FV Hong Kong mutation is a substitution of A>G in the 1090th nucleotide of factor V gene (6) and is still a matter of contention. The transition at nucleotide position 20210 in 3́ untranslated region of the factor II gene is associated with elevated

Address correspondence to Bilgen Dölek, PhD, Duzen Laboratory; Department of Molecular Genetics, II. Tasocagı cad. Dr. Orhan Birman Kosku, No:8, Mecidiyekoy-Istanbul, 34383, Mecidiyekoy-Istanbul, Turkey; e-mail: bilgen@duzen.com.tr.

Clinical and Applied Thrombosis/Hemostasis
Vol. 13, No. 4, October 2007 435-438
DOI: 10.1177/1076029607303341
© 2007 Sage Publications

prothrombin levels and an increased risk for venous thrombosis (5,8). MTHFR A1298C and MTHFR C677T point mutations result in reduced enzyme activity that leads to elevated homocysteine concentrations and decreased folate concentration in plasma, which may represent important risk factors in thromboembolic diseases (9). We conducted this study to determine the prevalence of FV Leiden, FV Hong Kong, FII G20210A, MTHFR C677T, and MTHFR A1298C in Turkish patients with a history of venous thromboembolic events and healthy control subjects, as well as to name unknown nucleotide variations in factor V, factor II, and MTHFR genes.

METHODS

The patient group consisted of a total 270 unrelated cases, 84 men and 186 women, who presented acute clinical symptoms of DVT, such as pain, edema, and venous distention. The DVT diagnosis was made clinically and confirmed by color Doppler ultrasonography. In a total of 270 patients, 131 patients had spontaneous DVT and a family history. Sixty of the remaining patients had DVT during pregnancy or in the postpartum period. Fifty-seven patients were diagnosed after a trauma and 22 in the postoperative period. Control samples were selected from 114 healthy individuals with nonvascular diseases and a nonthrombosis history. Informed consent forms were fulfilled and signed by all patients and controls. Na_2EDTA anticoagulated peripheral blood samples were collected from both patients and controls. Genomic DNAs were isolated from blood leukocytes with a modified salting-out procedure (10). The purity and concentration of isolated DNA were determined by spectrophotometer and observed with 0.8% agarose gel electrophoresis. The primer selection, polymerase chain reactions, and DNA analyses were carried out with slight modifications described in previous studies (2,5,6). All individuals were subject to DNA analyses for FV Leiden, FV Hong Kong, FII G20210A, MTHFR C677T, and MTHFR A1298C mutations. Single-strand conformation analysis (SSCA) was conducted using the primers selected for restriction enzyme analysis, and electrophoreses were performed on 37.5:1 native acrylamide gels using the appropriate gel concentrations and electrophoresis conditions for each region. Statistical analyses were performed using χ^2-compared baseline characteristics of cases and control individuals. The crude odds ratios and confidential intervals were estimated in patient and control groups.

RESULTS

A total number of 270 unrelated patients, 84 men and 186 women, and 114 healthy individuals were subjected to restriction enzyme analysis and SSCA analyses. The homozygous and heterozygous states of each mutation and their combinations were calculated to determine the genotype frequencies. The genotype frequencies of the mutations are given in Table 1. The outcome showed that FV Leiden was found in 76 (28.3%) patients and 4 (3.5%) controls, and FII G20210A was found in 23 (8.6%) patients and 2 (1.8%) healthy controls. The MTHFR C677T mutation was detected in 134 patients and 45 controls and MTHFR A1298C in 170 patients and 64 controls. The calculated genotype frequencies for FV Leiden and FII G20210A mutations differed significantly between patient and control groups. MTHFR C677T and A1298C mutations were equally distributed between patient and control groups. The substitution at position 1090 of factor V gene was detected in 2 patients and in none of the control subjects. The genotype frequencies of these mutations in conjunction with at least 1 of the other variations were also calculated, and the results are summarized in Table 2. The combinations of FV Leiden with FII G20210A were not found to affect the prevalence of thrombosis in statistical analyses. In our study, the combination of FV Leiden with MTHFR C677T was observed in 35 patients and no controls, and with MTHFR A1298C, it was observed in 46 patients and 4 controls. The mobility differences of FV Leiden, FV Hong Kong, FII G20210A, MTHFR C677T, and MTHFR A1298C mutations in their homozygous, heterozygous, and compound heterozygous states were carefully assessed using the SSCA technique. The results showed that all cases, which were found positive for the mutations mentioned previously here, gave a significant pattern, but unfortunately, there were no other variations detectable with the optimized conditions.

DISCUSSION

In this study, the differences in prevalence of genetic risk factors for thrombosis in both patient and control groups in the Turkish population were studied. The observed genotype frequencies of FV Leiden and FII G20210A mutations were found to be 28.3% and 8.6%, respectively, pointing to a significant association between these alleles and

TABLE 1. Frequencies of Factor V Leiden, Factor V Hong Kong, Factor II G20210A, Methylenetetrahydrofolate Reductase C677T, and Methylenetetrahydrofolate Reductase A1298C Genotypes in 270 Patients With Thrombosis and 114 Controls

Gene Mutation	Total Number of Patients With Homozygous or Heterozygous Genotype	Calculated Frequencies (%)	Total Number of Controls With Homozygous or Heterozygous Genotype	Calculated Frequencies (%)	Odds Ratio (95% Confidence Interval)	χ^2 Test
Factor V Leiden	76	28.3	4	3.5	10.77 (3.84-30.25)	29.5
Factor V Hong Kong	2	0.7	0	0		0.85
Factor II G20210A	23	8.6	2	1.8	5.21 (1.22-22.5)	6.03
Methylenetetrahydrofolate reductase A1298C	170	63.2	64	56.1	1.33 (0.85-2.4)	1.57
Methylenetetrahydrofolate reductase C677T	134	49.8	45	39.5	1.51 (0.97-2.36)	3.32

TABLE 2. The Genotype Frequencies of Factor V Leiden, Factor V Hong Kong, Factor II G20210A, Methylenetetrahydrofolate Reductase C677T, and Methylenetetrahydrofolate Reductase A1298C Mutations in Conjunction With at Least 1 of the Other Variation

Gene Mutation	Total Number of Patients With Homozygous or Heterozygous Genotype	Calculated Frequencies (%)	Total Number of Controls With Homozygous or Heterozygous Genotype	Calculated Frequencies (%)	Odds Ratio (95% Confidence Interval)	χ^2 Test
Factor V Leiden + factor IIG20210A	4	1.5	0	0		1.77
Factor V Leiden + methylenetetrahydrofolate reductase C677T	35	13	0	0		16.3
Factor V Leiden + methylenetetrahydrofolate reductase A1298C	46	17.1	4	3.5	5.65 (1.98-16.1)	12.95
Factor V Leiden + factor V Hong Kong	1	0.4	0	0		0.42
Factor II G20210A + methylenetetrahydrofolate reductase C677T	14	5.2	1	0.9	6.18 (0.8-47.6)	3.96
Factor II G20210A + methylenetetrahydrofolate reductase A1298C	11	4.1	1	0.9	4.8 (0.61-37.6)	2.71
Factor II G20210A + factor V Hong Kong	1	0.4	0	0		0.42
Methylenetetrahydrofolate reductase C677T + methylenetetrahydrofolate reductase A1298C	68	25.3	24	21.1	1.26 (0.74-2.14)	0.75
Methylenetetrahydrofolate reductase 677T + factor V Hong Kong	1	0.4	0	0		0.42
Methylenetetrahydrofolate reductase A1298C + factor V Hong Kong	1	0.4	0	0		0.42

thrombosis. These findings were in concordance with the study published by Gurgey et al (11). We observed higher frequencies for FV Leiden and FII G20210A mutations in Turkish thrombosis patients compared with other studies in other populations (12). Although Grossman et al concluded that FV Leiden mutation frequencies were similar in both patient and control groups, recent research data with more detailed analyses contradict their findings (13). We observed that the genotypes MTHFR C677T and A1298C do not appear to be important risk factors for thromboembolic disorders when present alone. The combination of MTHFR mutations with FV Leiden increased thrombosis risks according to statistical analyses and the clinical findings of these patients. Lu et al showed that MTHFR C677T was a common mutation in the Chinese population, thus correlating with our findings. In their study, FV Leiden and FII G20210A mutations were determined as risk factors (14). FV Leiden and FII G20210A mutations were also found to be relevant risk factors partly for pulmonary vein thrombosis (15). Chan et al determined a genetic abnormality at exon 7 (Arg306) of the FV gene, named FV Hong Kong, and found 2 positive cases in the thrombosis group and only 1 in the control group in that study (6). We could not observe an abnormal DNA pattern/variation using selected primers for FV Leiden, FV Hong Kong, FII G20210A, MTHFR C677T, and MTHFR A1298C mutations by SSCA and DNA sequencing analysis. The thrombosis mutations that we screened in this study make up 25% to 50% of venous thromboembolism patients (16,17). Our aim focused on determining other thrombosis factor genes and gene polymorphism–gene polymorphism interactions that play a role in families with thrombosis history. Associations between inherited abnormalities and thromboembolic disorders will be helpful for diagnosis and treatment of thrombosis.

ACKNOWLEDGMENTS

This work was supported by the Research Foundation of the University of Istanbul, Project No. T-29/23072002.

REFERENCES

1. Handin RI. Disorders of coagulation and thrombosis. In: Braunwals E, Fauci AS, Kasper DL, et al, eds. *Harrison's Principles of Internal Medicine*. New York, NY: McGraw-Hill; 2001:751.
2. Gerhardt A, Scharf RE, Beckman MW, et al. Prothrombin and factor V mutations in women with history of thrombosis during pregnancy and the puerperium. *N Engl J Med.* 2000;342:374-380.
3. Tirado I, Mateo J, Soria JM, et al. Contribution of prothrombin 20210A allele and factor V Leiden mutation to thrombosis risk in thrombophilic families with other hemostatic deficiencies. *Haemotologica.* 2001;86:1200-1208.
4. Chango A, Boisson F, Barbe F, et al. The effect of 677 C to T and 1298 A to C mutations on plasma homocysteine and 5,10-metylenetetrahydrofolate reductase activity in healthy subjects. *Br J Nutr.* 2000;83:593-596.
5. Poort SR, Rosendaal FR, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood.* 1996;88:3698-3703.
6. Chan WP, Lee CK, Kwong YL, et al. A novel mutation of Arg306 of factor V gene in Hong Kong Chinese. *Blood.* 1998;91:1135-1139.
7. Rosendaal FR, Koster T, Vanderbroucke JP, et al. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood.* 1995;85:1504-1508.
8. Soria JM, Almasy L, Souto JC, et al. Linkage analysis demonstrates that the prothrombin G20210A mutation jointly influences plasma prothrombin levels and risk of thrombosis. *Blood.* 2000;95:2780-2785.
9. Kang SS, Passen EL, Ruggie N, et al. Thermolabile defect of methylenetetrahydrofolate reductase in coronary artery disease. *Circulation.* 1993;88:1463-1469.
10. Miller M, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleate cells. *Nucleic Acids Res.* 1988;16:1215-121.
11. Gurgey A, Haznedaroglu IC, Egesel T, et al. Two common genetic thrombotic risk factors: factor V Leiden and prothrombin G20210A in adult Turkish patients with thrombosis. *Am J Hematol.* 2001;67:107-111.
12. Varela MI, Adamczuk YP, Forastiero RR, et al. Major and potential prothrombotic genotypes in a cohort of patient with venous thromboembolism. *Thromb Res.* 2001; 104:317.
13. Grossman R, Schwender S, Geisen U, et al. CBS 844 ins68, MTHFR TT677 and EPCR 4031 ins 23 genotypes in patients with deep-vein thrombosis. *Thromb Res.* 2002; 107:13-15.
14. Lu Y, Zhao Y, Liu G, et al. Factor V gene G1691A mutation, prothrombin gene G20210A mutation and MTHFR C677T mutation are not risk factors for pulmonary thromboembolism in Chinese population. *Thromb Res.* 2002;106:7-12.
15. Ordonez AJG, Carreira JMF, Alvarez CRF, et al. Comparison of the risk of pulmonary embolism and deep vein thrombosis in the presence of factor V Leiden or prothrombin G20210A. *Thromb Haemost.* 2000;83:352-354.
16. Schafer AI, Levine MN, Konkle BA, et al. Thrombotic disorders: diagnosis and treatment. *Am Soc Hematol.* 2003;520.
17. Murrin S, Marelich GP, Arroliga AC, et al. Hereditary thrombophilia and venous thromboembolism. *Am J Respir Crit Care Med.* 1998;158:1369-1373.