Inherited Thrombophilic Risk Factors in Venous Thromboembolism: Factor V Leiden and Prothrombin 20210 A

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Abstract

This case-control study was designed to investigate the prevalence of mutations in the prothrombin gene (20210A) and the factor V Leiden in unselected patients with venous thromboembolism (VTE). One hundred consecutive patients with objectively documented VTE (49 isolated pulmonary embolism, 45 pulmonary embolism + deep vein thrombosis, and 6 deep vein thrombosis) and 256 unmatched control subjects were included in the study.
Seven percent of the 100 patients were found to be carriers of the prothrombin 20210A allele. This mutation was present in 2.3 % of the 256 controls (Odds ratio 3.13; 95% CI 1.02 – 9.57) (p=0.05). Twenty-four percent of the patients had the mutation of factor V Leiden while this mutation was present in 9.8 % of the 132 controls (OR:2.89; 95% CI 1.38 – 6.02) (p=0.006). Six patients were homozygous carriers of the factor V Leiden mutation and 4 patients shared both mutations. Patients with isolated pulmonary embolism (n=49) and patients with pulmonary embolism + deep vein thrombosis (n=45) showed similar prevalences for factor V Leiden mutation (24.4% and 17.8% respectively) and for prothrombin 20210A allele (8.1% and 4.4% respectively).
It was concluded that the 20210A allele of the prothrombin gene and factor V Leiden mutation are significantly higher in Turkish patients with VTE.

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Introduction

Venous thromboembolism (VTE) is a common disease, with an annual incidence in the general population of approximately 1 per 1000. Risk factors for VTE include both hereditary and acquired conditions (1). Inherited thrombophilia is a multigenic disorder, where the predisposition towards thrombosis increases with the number of risk factors present in the patient (2-4).
In 1993, Dahlback described resistance to activated protein C that was subsequently linked to a single base-pair mutation in the factor V gene, known as the factor V Leiden mutation (5,6). This mutation is found in 11 % to 29 % of patients with VTE (7,8). Heterozygous carriers of factor V Leiden have a 3 to 8-fold increased risk for VTE (7,9). Another hereditary risk factor was identified in 1996 by Poort et al as a genetic variant in the 3-untranslated region (a G to A transition at position 20210) of the

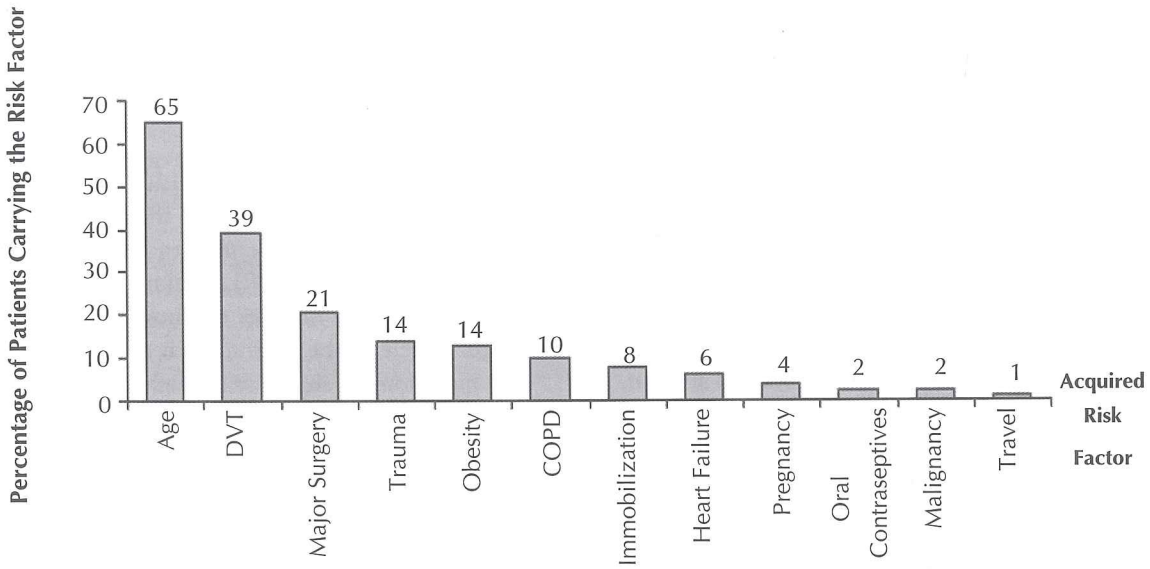

Figure 1. *Acquired risk factors in patients with PE.*

prothrombin gene, which was associated with an increase in prothrombin levels (10). The mutation prothrombin 20210A allele was found in 4 % to 17 % of patients with VTE (11,12) and has been linked to a 1.3 to 8-fold increased risk for VTE (9,11).

The aim of this present study was to investigate the prevalence of the 20210A allele of the prothrombin gene and of factor V Leiden mutation in patients with VTE in Turkey.

Materials and Methods

The study was carried out with the approval of the local ethics committee and in accordance with the principles of the Helsinki Declaration. Informed consent was obtained from all subjects.

A total of 100 patients (47 males and 53 females) with objectively documented venous thromboembolism (pulmonary embolism and /or deep vein thrombosis) were included in the study. Mean age was 56.2±16.9 years, ranging from 16 to 88 years. All patients had been admitted to the Department of Pulmonary Disease hospital of the Istanbul Faculty of Medicine within the last 10 years. The diagnosis of pulmonary embolism (PE) was confirmed by ventilation/perfusion lung scan, spiral CT or pulmonary angiography. To confirm lower limb deep vein thrombosis (DVT), all patients underwent Doppler Ultrasonography or venography. In this group (the study group) there were 49 patients with isolated PE, 45 patients with PE + DVT, and 6 patients with only DVT. The 20210A allele and factor V Leiden mutation were tested in all patients.

The control group consisted of 256 unmatched individuals (127 males, 128 females, with a mean age 41.5±14.4 years, ranging from 14 to 85 years). None of the control subjects had a past or familial history of VTE, of cardiopulmonary disease or acquired risk factors for VTE (except for age). The 20210A allele was tested in all control subjects, but factor V Leiden mutation could be investigated only in 132 of the controls.

Genetic analysis

Blood samples were collected into vacutainer® tubes containing 1/10 volume of 0.105-M trisodium citrate and were centrifuged at 3000 g for 15 min to obtain platelet-poor plasma. Genomic DNA was extracted from white blood cells and amplified by polymerase chain reaction (PCR). Prothrombin 20210A polymorphism and factor V Leiden 1691G → A mutation were determined by Light-Cycler prothrombin (G20210A) Mutation Detection Kit® and Light-Cycler Factor V Leiden Mutation Detection Kit® (Roche). PCR is performed pursuant to an agreement with Roche Molecular Systems, Inc. Identification of mutations was loaded with Light Cycler computer program (Roche Diagnostics GmbH Mannheim, Germany).

Statistical analysis

The Chi-square-Fisher's exact test was used in the statistical analysis. Odds ratios (OR) were calculated in terms of relative risk of VTE; of risk of VTE in the presence of the 20210A allele and also of risk of VTE in the presence of factor V Leiden mutation. Additionally, 95% confidence intervals (95% CI) were calculated.

Results

The most commonly present acquired risk factors in our patients with PE were older age (>40 years) and DVT (Figure 1). There were only four patients without any acquired risk factors for VTE and two of these patients were carriers of factor

Table 1. Prevalence of factor V Leiden and prothrombin 20210A mutation				
	Patients	Controls	OR	P
Factor V Leiden m.	24% (n:24/100)	9.8% (n:13/132)	2.89	p=0.006
Prothrombin 20210A m.	7% (n: 7/100)	2.3% (n:6/256)	3.13	P=0.05

V Leiden mutation (one homozygous and one heterozygous). While 7% of the 100 patients were carriers of the 20210A allele, the mutation was present in 2.3% (n=6) of the 256 controls (OR: 3.13, 95% CI 1.02-9.57) (P=0.05), and while 24% of the 100 patients had the mutation of factor V Leiden, the mutation was present in only 9.8% (n=13) of 132 controls (OR: 2.89, 95% CI 1.38-6.02) (P=0.006) (Table 1).

Six of 24 patients with factor V Leiden mutation (25%) were homozygous carriers and four of these six patients had a history of recurrent VTE. There were no homozygous carriers among the patients with the 20210A allele. Of 100 patients, only four (4%) shared both mutations and two of them had a history of recurrent VTE.

Patients with isolated pulmonary embolism (n=49) and patients with pulmonary embolism + deep vein thrombosis (n=45) showed a similar prevalence for factor V Leiden mutation (24.4% and 17.8% respectively) and for prothrombin 20210A allele (8.1% and 4.4% respectively).

Discussion

We detected 7 individuals (7%) carrying the prothrombin gene 20210A variant in patients with VTE. In contrast, 2.3% of the control group were carriers of the 20210A allele. These figures are close to those reported from Sweden (7.1%; 1.8%) and from United Kingdom (5.5%; 1.2%), but less than those reported from Spain (17.2%; 6.5%) (13,14,12).

In our study, carriers of factor V Leiden mutation comprised 24% of the patient group and 9.8% of the control group. The prevalence of the Leiden V mutation in our control group is similar to the prevalence of healthy subjects reported from other centers in Turkey (15,16).

The prevalence of factor V Leiden mutation is high among European populations (Greece 15%, U.K. 8.8%, Germany 4.0%). On the other hand, several studies have shown that this mutation is rare among people from Asia, Africa and the far eastern countries. Although, a big part of Turkey is geographically located in Asia, the prevalence of factor V Leiden mutation is as high as that in Europe (17-20).

Heterozygotes for factor V Leiden mutation usually have a 5- to 10 fold increased risk of VTE. This risk is significantly increased in homozygotes and may be as high as 50 to 100 fold (21,22). In our study, 6 out of 24 factor V Leiden carriers were homozygous and 18 were heterozygous.

The incidence of VTE among factor V Leiden carriers increases with age at a significantly greater rate than among subjects without this mutation (23). In our study, 66% of carriers of factor V Leiden mutation were older than 40 years of age.

The prevalence of factor V Leiden mutation is reported to be significantly higher in patients with PE + DVT than in patients with isolated PE (24,25). In this present study, patients with isolated PE and patients with PE + DVT showed similar prevalences of factor V Leiden mutation.

In conclusion, our findings confirm that the prevalences of 20210A allele of the prothrombin gene and of factor V Leiden mutation are significantly higher in patients with VTE than in healthy controls.

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