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*Angiology* 2006; 57; 193

DOI: 10.1177/000331970605700209

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## Factor V Leiden Mutation in Venous Thrombosis in Southeast Turkey

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Venous thrombosis (VT) is a common disease, with an annual incidence in the general population of approximately 1 per 1,000. Factor V Leiden mutation (G1691A) (FVL) is the most common risk factor in venous thrombosis. The prevalence of FVL for thrombosis varies greatly in different regions of the world. FVL mutation has been identified both by conventional method and fluorescence resonance energy transfer (FRET) with the LightCycler. Sixty-one patients with VT, different in age and sex, were consecutively entered into this study to assess the prevalence of FVL in VT in southeast Turkey. FVL mutation was found in 24.6% (15/61). Fourteen individuals were heterozygous and 1 homozygous, a rate of 22.9% and 1.6%, respectively. In conclusion, the authors suggest that FVL mutation is common in patients with venous thrombosis in southeast Turkey.

### Introduction

Venous thrombosis (VT) and pulmonary embolism can cause significant morbidity and mortality. VT is a common disease, with an annual incidence in the general population of approximately 1 per 1,000.<sup>1</sup> Risk factors of VT can be genetic and/or acquired in origin. Some individuals may have 2 or more risk factors simultaneously.<sup>2</sup>

Resistance to activated protein C (APC-R) is characterized by a poor anticoagulant response to activated coagulation protein C.<sup>3</sup> APC-R is

found in more than half of all cases of hereditary thrombophilia. It is the most important cause of hereditary thrombophilia.<sup>4,5</sup> The biochemical and molecular basis for the APC-R can be explained in more than 90% of patients by the inheritance of a single point mutation in the coagulation *factor V* gene (G to A transition at nucleotide position 1691) that renders the protein less readily susceptible to inactivation by APC.<sup>6</sup> The mutation predicts the presence of an abnormal factor V (factor V Leiden [FVL]) molecule in which arginine (R) (CGA) 506 in 1 of the APC cleavage sites has been replaced by glutamine (Q) (CAA).<sup>7</sup>

Deficiencies of protein C, protein S, antithrombin III, and dysfibrinogenemia were together accountable for only 5% to 10% of cases, indicating the FV mutation to be at least 5 times more common than any of the other known genetic defects.<sup>8</sup> The prevalence of the FVL mutation varies by geography and ethnicity, ranging from 2% to 15% in healthy white people.<sup>9,10</sup> Heterozygotes have a 5–10-fold increased risk for

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Angiology 57:193–196, 2006

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thrombosis, while homozygotes have a 50–100-fold increased risk.<sup>5,8,11</sup> Several studies suggested a single origin for FVL mutation. Europe and Turkey could be the geographical areas where the mutation originated. FVL has been found at high prevalence in the healthy Turkish population. However, this high prevalence shows some variation in different regions of Turkey.<sup>9</sup> There are no data about prevalence of FVL in VT in southeast Turkey until now. Therefore, screening studies from different parts of Turkey are important.

The aim of this study was to investigate the prevalence of FVL mutation in patients with venous thrombosis in southeast Turkey.

## Materials and Methods

Between September 1999 and October 2000, 61 patients with VT, different in age and sex, were consecutively entered into our study to determine the prevalence of FVL in southeast Turkey. All of the people who live in this region have health insurance, and our University Hospital has been the best organized health center and the only hematology reference center in southeast Turkey. The patients who have classical predisposing factors for thrombosis, such as pregnancy, malignancy, or use of oral contraceptives, were not enrolled. Deep venous thrombosis (DVT) was diagnosed by

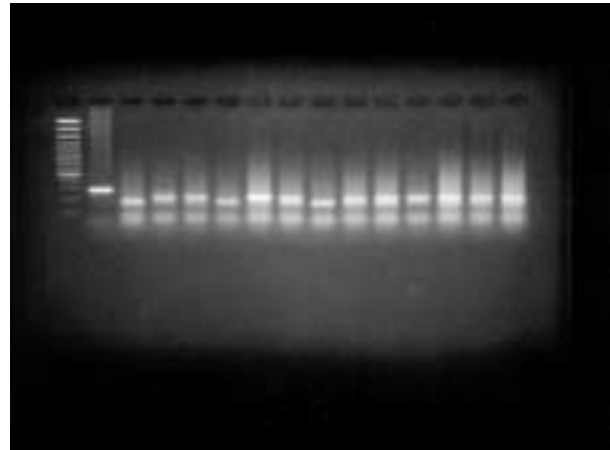
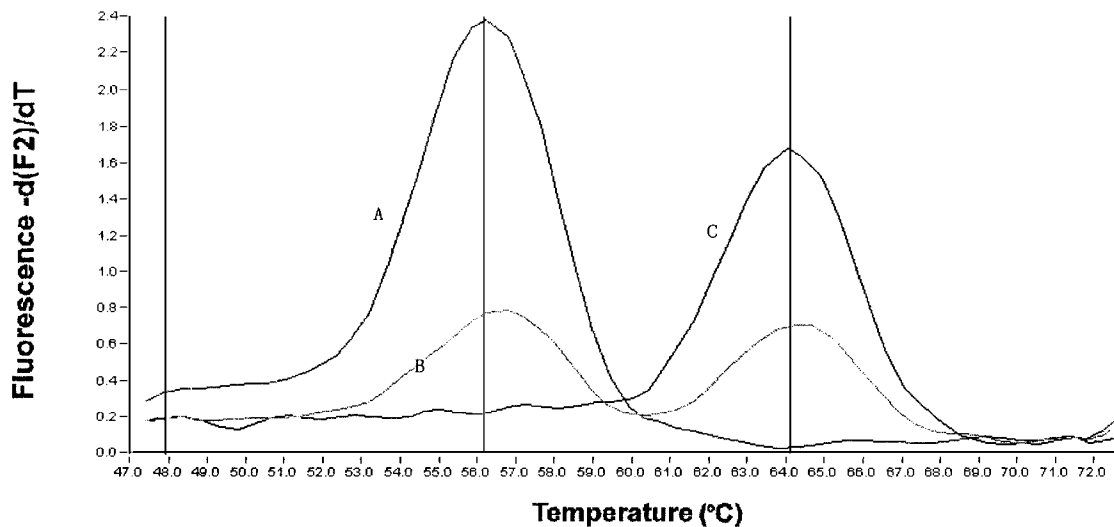


Figure 1. MnlI digest of 267 bp fragment of *factor V* gene. Lane 1, molecular weight marker; lane 2, the 267 bp amplified product; lanes 3, 6, 9, normal genotype; lanes 4, 5, 8, 10–15, heterozygous genotype; lane 7, homozygous genotype.

ultrasonography, pulmonary embolism by clinical and scintigraphic findings, and cerebral thrombosis by computed tomography scanning.

The laboratory investigation was performed at least 3 months after the most recent thrombotic episode. APC-R was determined as described.<sup>3</sup> The results were expressed as the APC ratio, which was calculated by dividing the clotting time obtained with APC/CaCl<sub>2</sub> solution by clotting

Figure 2. Factor V genotyping shows a homozygous wild type (C), a heterozygous (B), and a homozygous mutation (A).



time obtained with calcium chloride alone. FVL mutation was detected with both conventional method and fluorescence resonance energy transfer (FRET) with the LightCycler (Roche Molecular Biochemicals)<sup>12</sup> (Figures 1, 2). Genomic DNA was prepared from EDTA-blood by standard procedures. The region in exon 10 that encodes 1 of the APC cleavage sites in FV was PCR amplified from genomic DNA with 2 primers 5'TGCCAGT-GCTTACAAGACCA3' and 5'TGTTATCACACTG-GTGCTAA3'.<sup>7</sup> The conditions for polymerase chain reaction (PCR) for 40 cycles of amplification were as follows: 40 s denaturation at 94°C, 40 s annealing at 59°C, and 40 s extension at 72°C. The 267 base pair (bp) amplified product was subjected to MnlI digestion: the 1691 G fragment will give fragments of 37, 67, and 163 bp, whereas the 1691A fragment will give fragments of 67 and 200 bp. The fragments were separated on ethidium bromide stained 2% agarose gels.

## Results

The study group consisted of 61 patients with DVT (34 women, 27 men). The mean age was 40 years (range 16–75). The mutation was found in 15 (24.6%) cases. Fourteen individuals were heterozygous and 1 homozygous, a rate of 22.9% and 1.6% respectively. The results of FVL from the LightCycler were in complete agreement with those obtained from the standard method involving restriction enzyme digestion of PCR products and electrophoretic analysis. The presenting thrombotic events were DVT in a leg (57 patients), pulmonary embolism with DVT (3 patients), and thrombosis in cerebral vessels (1 patient). Twenty-five (41%) of the patients had previous thrombotic events. The mean age at the time of the first thrombotic episode was 40 years (range 17–75). Nine (15%) of the patients had a family history of thrombosis. FVL was not found in pulmonary embolism cases. The characteristics of patients are summarized in Table I.

## Discussion

Activated protein C (APC) resistance has been shown to be the most frequent inherited defect associated with venous thrombosis.<sup>8</sup> The FVL has

been found to be responsible for more than 90% of the APC resistance cases.<sup>6</sup> The prevalence of genetic risk factors for thrombosis varies greatly in different parts of the world, both in patients with thrombosis and in general populations.<sup>10</sup> Several studies have been published on the occurrence of thrombosis due to FVL. Initial studies reported a carrier rate for FVL of 3% in the Netherlands.<sup>5</sup> FVL reached high frequencies in Europe, with carrier rates of 15% among Greeks, and 8.8% in UK whites.<sup>10</sup> In the UK study, the mutation was not found in 800 individuals from native populations of Africa, Southeast Asia, Australia, and America.<sup>10</sup> In North America, the Physicians' Health Study, made up of predominantly Caucasian physicians, found the frequency to be 6%.<sup>13</sup>

These findings establish the FVL mutation in the FV gene as one of the most common monogenic disorders in North American and European Caucasian individuals.<sup>8</sup> Among Caucasian populations, FVL is the most common genetic defect causing thrombosis currently known.<sup>5,10,14</sup> Outside Europe, the mutation is very rare, with an allele frequency of 1.65% in Hispanic Americans, and 0.87% in African-Americans. No FVL mutation was found in Asian Americans or Native Americans tested.<sup>8</sup> In addition, Chaa et al<sup>15</sup> reported that FVL was rarely found in the Hong

Table I. Characteristics of patients.

No. of patients	61
Female	34
Male	27
Mean age (range)	40 (16–75)
Deep venous thrombosis (DVT)	57
DVT + pulmonary embolism	3
Cerebral thrombosis	1
Family history	9 (15%)
Previous thrombotic events	25 (41%)
FVL mutation	15 (24.6%)
Mean age at first thrombotic episode	40 (17–75)

Kong Chinese population; a different mutation site such as A 1090→G in exon 7 of the *FV* gene (Arg 306) may be of clinical importance.<sup>15</sup> The prevalence of FVL in VT shows variation in different regions of Turkey.<sup>9</sup> The carrier rate for FVL in Turkey was shown to be about 7.1% to 9.1%, and the rate of FVL in VT was about 30% in previous publications.<sup>9,16</sup> In our study, the rate of FVL in VT was found to be equal to the highest value from the other parts of Turkey. We suggest the prevalence of FVL in 320 healthy individuals in our region to be about 9.1%.<sup>17</sup> APC resistance was also measured; 34 of the subjects had an APC-R positive (10.6%) and 29 (5 homozygous and 24 heterozygous) of them had a mutation for FVL. Our data support findings from a previous study on the association between VT and FVL carrier status.<sup>9,10,16</sup> Regarding the high prevalence of FVL in our region, family members of any subject with FVL mutation should be screened to identify this genetic mutation. Further investigations are needed to determine an exact reason for the regional variations of genetic thrombophilic factors and its importance in the thrombogenesis.

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