Polymorphisms in thrombophilic genes are associated with deep venous thromboembolism in an Iranian population

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ABSTRACT

It has been revealed that the inherited thrombophilia increases the risk of thrombosis in the venous system. To study the association of factor V G1691A, factor V HR2 (4070A/G), prothrombin G20210A, and PAI-1 (−675 I/D, 5G/4G) polymorphisms with deep venous thromboembolism (DVT), these polymorphisms were investigated. A total of 193 patients who presented clinical symptoms of deep venous thromboembolism including 103 men and 90 women, and 500 healthy individuals without both personal and family histories of thromboembolic disorders including 275 men and 225 women were recruited into the study. Genotyping was carried out using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique. Our results showed that the genotype distribution for FV (G1691A and A4070G) and PAI-1 4G/5G polymorphisms in DVT patients were significantly higher than healthy control ($P < 0.05$). Also, the mutant allele frequencies for all studied polymorphisms differed significantly between the case and control groups ($P < 0.05$). We concluded that the prevalence of FV (G1691A and A4070G) and PAI-1 4G/5G polymorphisms increased the risk of DVT occurrence in subjects. These findings provide additional evidence to support the
Introduction

Deep venous thromboembolism (DVT) is a major health problem as a result of thrombosis in the deep venous of the legs (Lopez et al., 2004). The annual incidence of DVT has been estimated between 0.1 and 0.2% of the adult population (Heit, 2006). DVT is a complicated and multi-factorial disorder which can be caused by acquired and genetic risk factors. Acquired risk factors include age, surgery, major trauma, hormone therapy, pregnancy, prolonged immobilization, plaster cast, and some types of cancer that can predispose an individual to an increased risk for DVT (Franco and Reitsma, 2001; Smalberg et al., 2011). According to the results of family and twin studies, genetic factors account for nearly 60% of the risk for DVT (Rahimi et al., 2010).

Three major molecular mechanisms which effectively contribute to DVT are venous stasis, hypercoagulability, and blood vessel wall changes (Lopez et al., 2004; Prevalti et al., 2011). Hypercoagulability states promoting thrombosis, collectively termed “thrombophilias”, may be inherited or acquired, and approximately in 40% of cases are inherited (Coulam et al., 2008). There is much evidence that inherited thrombophilia plays a major role in thrombosis development. To date, several thrombophilic gene polymorphisms have been identified that are involved in the pathogenesis of thromboembolic disorders. Activated protein C resistance (APCR) due to the factor V (FV) G1691A polymorphism and the G20210A polymorphism in the prothrombin gene are well-characterized genetic variants causing thrombophilia. The methylentetrahydrofolate reductase (MTHFR) C677T polymorphism which causes hyperhomocysteinemia has been also considered to be a risk factor for thromboembolism (Sykes et al., 2000). Nevertheless, these genetic variants only explain a fraction of all thromboembolic events. Therefore, it seems that there should be other genetic variants that are also involved in clot formation. Lunghi et al. (1996) have reported novel polymorphisms in the factor V gene. The G4070A polymorphism in FV gene leads to an amino acid substitution His to Arg at position 1299. This polymorphism which is marked by the HR2 haplotype is associated with an increased risk for thromboembolic disease (Alhenc-Gelas et al., 1999). Additionally, previous studies have reported that the increases in plasminogen activator inhibitor-1 (PAI-1) serum level could lead to a thrombotic tendency (Francis, 2002; Sartori et al., 2003; Tsantes et al., 2007). The PAI-1 (−675 I/D, 5G/4G) polymorphism affects the binding of nuclear proteins involved in the regulation of PAI-1 gene transcription. The 4G allele appears to bind only an enhancer, whereas the 5G allele binds both an enhancer and a suppressor. So, the 4G allele is associated with higher rates of PAI-1 synthesis and thromboembolism (Francis, 2002).

This study was design to determine the prevalence of FV G1691A, FV A4070G, prothrombin G20210A and PAI-1 (−675 I/D, 5G/4G) polymorphisms in patients with DVT and healthy control, and to address the question “whether these polymorphisms are associated with DVT” by examining a large study population.

### Table 1

The mean and range of age in all studied groups.

| Group                              | Mean age ± SD (years) | Range (years) |
|------------------------------------|-----------------------|---------------|
| Case group (n = 193)               | 46.18 ± 4.72          | 38–57         |
| Control group (n = 500)            | 46.27 ± 5.82          | 36–59         |
| Men of the case group (n = 103)    | 45.38 ± 4.44          | 38–54         |
| Women of the case group (n = 90)   | 47.10 ± 4.89          | 39–57         |
| Men of the control group (n = 275) | 45.79 ± 6.23          | 36–59         |
| Women of the control group (n = 225)| 46.85 ± 5.23          | 37–58         |

Abbreviation: SD, standard deviation.
Material and methods

Study population and inclusion criteria

In this case control study, 193 individuals (103 male and 90 female) with DVT clinical symptoms were selected as case group, and 500 healthy subjects (275 male and 225 female) without both personal and family histories of thromboembolic disorders were included as control group. The mean and range of age for the case and control groups were summarized in Table 1. All case subjects were referred by clinician after confirmation of the existence of thrombus using Doppler ultrasonography (ALOKA, SSD-1700, Japan). Recognized environmental factors that may increase the risk of DVT, including surgery, major trauma, hospitalization, prolonged immobilization and cancer were excluded from criteria. None of the healthy control had any evidence of thrombotic events. The present study was approved by the Ethics and Human Rights Committee of Tabriz University of medical sciences, Tabriz, Iran under number 217/d/3324 and the informed consents were filled out by all participants.

DNA extraction and genotype screening

5 ml of peripheral blood samples were taken from all participants and genomic DNA was extracted from leukocytes using GIAamp DNA Blood Mini kit (Qiagen, USA), according to the manufacturer’s instruction. Detections of FV G1691A, FV A4070G, prothrombin G20210A and PAI-1 (−675 I/D, 5G/4G) polymorphisms were performed by Amplification Refractory Mutation System-PCR (ARMS-PCR) technique. Amplification of DNA was carried out using the three primer sets for each polymorphism, one forward and two reverse specific primers for the wild type and mutant alleles (Hoppe et al., 2003). PCR amplifications were performed on total volume 25 μl solution containing 100 ng genomic DNA, 1× PCR buffer, 10 pmol of each primer, 10 nmol each deoxyribonucleotide triphosphates, 1.5 mM Mg2+ and 1 U Taq DNA polymerase. The PCR conditions consisted of an initial denaturation step (94 °C, 2 min) was followed by 10 cycles of denaturation (94 °C, 15 s) and annealing/extension (65 °C, 60 s), followed by a final 20 cycles of denaturation (94 °C, 10 s), annealing (62 °C, 50 s), and extension (72 °C, 30 s) (Hoppe et al., 2003). The PCR products were separated on 2% agarose gel and visualized with ethidium bromide staining and UV illumination with a gel documentation system (Gel Doc 2000; Bio-Rad, Hercules, USA).

Statistical analysis

Statistical analysis was performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). The Pearson’s chi-square ($\chi^2$) and Fisher’s exact tests were used for evaluation of genotype and allele frequencies. The homozygote and heterozygote genotypes of each group were unified as a new group and then odds ratios and 95% confidence intervals were calculated. $P$ values less than 0.05 were considered statistically significant.

Table 2
The genotype distribution of four thrombophilic gene polymorphisms in the case and control groups.

| Polymorphism | Case (n = 193) | Control (n = 500) | $P$ value$^a$ |
|--------------|----------------|------------------|--------------|
|              | Normal (%)     | Heterozygote (%) | Homozygote (%) | Normal (%) | Heterozygote (%) | Homozygote (%) |
| FV 1691G/A$^b$ | 88.6 | 11.4 | 0.0 | 95.8 | 4.2 | 0.0 | 0.001 |
| FV HR2 4070 A/G$^c$ | 89.11 | 9.33 | 1.56 | 94.4 | 5.6 | 0.0 | 0.004 |
| F II 20210 G/A$^d$ | 88.6 | 10.36 | 1.04 | 94 | 6 | 0.0 | 0.010 |
| PAI-1 (−675 I/D, 5G/4G)$^e$ | 25.9 | 43 | 31.1 | 70.6 | 18.2 | 11.2 | 0.000 |

Abbreviations: FV; Factor V, FV HR2; Factor V-His1299 Arg, F II; Factor II or Prothrombin, PAI-1; Plasminogen Activator Inhibitor-1.

$^a$ $P$ value calculated by chi-square test.

$^b$ FV, at nucleotide position 1691 on the gene a G was exchanged by an A.

$^c$ FV HR2, at nucleotide position 4070 on the gene an A was exchanged by a G.

$^d$ F II, at nucleotide position 20210 on the gene a G was exchanged by an A.

$^e$ PAI-1, at nucleotide position −675 on the gene promoter a GGGGG was exchanged by a GGGG.
Table 3
Comparison of genotype distribution of four thrombophilic gene polymorphisms between men and women of each group.

| Polymorphism        | Case (n = 193) | Control (n = 500) | P valuea |
|---------------------|---------------|-------------------|---------|
|                     | Men of case (n = 103) | Women of case (n = 90) | Men of control (n = 275) | Women of control (n = 225) |         |
|                     | Normal | Heterozygote | Homozygote | Normal | Heterozygote | Homozygote | Normal | Heterozygote | Homozygote | P valuea |
| FV 1691G/A<sup>b</sup> | 86.4 | 13.60 | 0.0 | 91.11 | 8.89 | 0.0 | 0.367 | 95.63 | 4.37 | 0.0 | 0.000 |
| FV HR2 4070 A/G<sup>c</sup> | 93.2 | 5.82 | 0.98 | 84.44 | 13.33 | 2.23 | 0.150 | 94.18 | 5.82 | 0.0 | 0.141 |
| F II 20210 G/A<sup>d</sup> | 95.14 | 4.86 | 0.0 | 81.11 | 16.66 | 2.23 | 0.007 | 93.81 | 6.19 | 0.0 | 0.000 |
| PAI-1 (<−675 I/D, 5G/4G)<sup>e</sup> | 26.21 | 54.37 | 19.42 | 25.55 | 30 | 44.45 | 0.000 | 70.54 | 17.82 | 11.64 | 70.66 | 18.67 | 10.67 | 0.926 |

Abbreviations: FV; Factor V, FV HR2; Factor V-His1299 Arg, F II; Factor II or Prothrombin, PAI-1; Plasminogen Activator Inhibitor-1.

<sup>a</sup> P value calculated by chi-square test.

<sup>b</sup> FV, at nucleotide position 1691 on the gene a G was exchanged by an A.

<sup>c</sup> FV HR2, at nucleotide position 4070 on the gene an A was exchanged by a G.

<sup>d</sup> F II, at nucleotide position 20210 on the gene a G was exchanged by an A.

<sup>e</sup> PAI-1, at nucleotide position −675 on the gene promoter a GGGGG was exchanged by a GGGG.
Results

The genotype frequencies of the FV (G1691A and A4070G), prothrombin G20210A and PAI-1 4G/5G polymorphisms in the case and control groups were displayed in Table 2. Our results showed that the frequency of FV (G1691A and A4070G) and PAI-1 4G/5G polymorphisms in the case group was significantly higher than those in healthy control group \( (P < 0.05) \). Nevertheless, the frequency of prothrombin G20210A polymorphism was higher in patients with DVT than those in the healthy control group but with no significant difference \( (P > 0.05) \) (see Table 2).

The frequencies of the FV (G1691A and A4070G), prothrombin G20210A and PAI-1 4G/5G polymorphisms were compared between men and women subjects in each group. We found that the frequency of prothrombin G20210A and PAI-1 4G/5G polymorphisms was statistically different between men and women in the case group \( (P < 0.05) \) (see Table 3). We also observed that the genotype frequencies of FV G1691A and PAI-1 4G/5G polymorphisms in men of the case group were significantly higher when compared with those in healthy control groups \( (P < 0.05) \) (see Table 4), whereas, FV A4070G, prothrombin G20210A and PAI-1 4G/5G polymorphisms in women of the case group were significantly higher than those in healthy control \( (P < 0.05) \) (see Table 4).

The heterozygosity, homozygosity and mutant allele frequencies for all studied polymorphisms are given in Table 5. Significant differences in mutant allele frequencies were observed between DVT patients and control groups \( (P < 0.05) \) (see Table 5). When the mutant allele frequencies were compared between men and/or women of cases and controls, we found that FV 1691A and PAI-1 4G alleles in men \( (P < 0.05) \) and FV A4070G, prothrombin 20210A and PAI-1 4G in women of the case are statistically significant and different than those in men and women of the healthy group \( (P < 0.05) \), respectively (see Table 5).

Finally, When the heterozygote and homozygote genotypes of each group were unified into a new group (carrier group), and then odds ratios and 95% confidence intervals were calculated we found an association between FV G1691A and PAI-1 4G/5G gene polymorphisms and DVT (see Table 6).

Discussion

Previous literatures have discussed that thrombophilic gene polymorphisms might be related with an increased tendency for intravascular coagulation. Hence, to our best knowledge, the association between FV G1691A, FV A4070G, prothrombin G20210A, and PAI-1 (−675 I/D, 5G/4G) polymorphisms with DVT in Northwestern Iran was investigated in the current study for the first time. Taken together, our results showed that the group with a history of DVT has an increase in the frequency of FV (G1691A and A4070G) and PAI-1 4G/5G polymorphisms when compared with healthy controls \( (P < 0.05) \). However, a higher prevalence of prothrombin G20210A polymorphism was also seen in DVT patients, but did not show a statistically significant difference. These findings are in agreement with most of the previously published data.

Previous studies on FV G1691A and prothrombin G20210A polymorphisms in DVT patients proposed that both polymorphisms are major inherited risk factors associated with DVT. Meyer et al. (2001) showed that the prevalence of FV G1691A polymorphism is higher in DVT patients Meyer et al. (2001). Arsov et al. (2006) reported that the prevalence of the FV G1691A polymorphism among patients with venous thromboembolic disease was 21.1%, compared with that in the healthy controls (5.5%) Arsov et al. (2006). Juul et al. (2004). have also reported the positive association of FV G1691A polymorphism with DVT Juul et al. (2004). De Stefano et al. (1999) found that carriers of FV G1691A and prothrombin G20210A polymorphisms have an increased risk of deep venous thrombosis De Stefano et al. (1999). Conversely, Lijfering et al reported that these polymorphisms do not have any effect on the risk of venous thrombosis Lijfering et al., 2010). The study of Bouaziz-Borgi et al. (2006) revealed that the frequency of prothrombin G20210A polymorphism was higher in DVT patients compared with controls, but the difference was not statistically significant Bouaziz-Borgi et al. (2006).Perez-Ceballos et al. (2002) did not find any association between prothrombin G20210A polymorphism and DVT Perez-Ceballos et al. (2002).

Bouaziz-Borgi et al. (2007) studied 126 patients who are suffering from DVT and 197 healthy controls for FV A4070G polymorphism and reported that this polymorphism had a potential role in deep venous thromboembolism Bouaziz-Borgi et al. (2007). In addition, Gohil et al. (2009) reported that there is an
### Table 4
Comparison of genotype distribution of four thrombophilic gene polymorphisms between men and women of the case and healthy control groups.

| Polymorphism          | Men (n = 378) |          |          |          | Women (n = 315) |          |          |          |          |          |          |        |        |
|-----------------------|---------------|----------|----------|----------|-----------------|----------|----------|----------|----------|----------|----------|--------|--------|
|                       | Case (n = 103)| Control (n = 275) | Case (n = 90) | Control (n = 225) |                       |
|                       | Normal % | Heterozygote % | Homozygote % | Normal % | Heterozygote % | Homozygote % | Normal % | Heterozygote % | Homozygote % | P valuea | Normal % | Heterozygote % | Homozygote % | P valuea |
| FV 1691G/Ab          | 86.4  | 13.6  | 0.0  | 95.63  | 4.37  | 0.0  | 0.005  | 91.11  | 8.89  | 0.0  | 96.0  | 4.0  | 0.0  | 0.099  |
| FV HR2 4070 A/Gc     | 93.2  | 5.82  | 0.98 | 94.18  | 5.82  | 0.0  | 0.262  | 84.44  | 13.33 | 2.23 | 94.66 | 5.34 | 0.0  | 0.004  |
| F II 20210 G/Ad      | 95.14 | 4.86  | 0.0  | 93.81  | 6.19  | 0.0  | 0.806  | 81.11  | 16.66 | 2.23 | 94.22 | 5.78 | 0.0  | 0.001  |
| PAI-1 (−675 I/D, 5G/4G)e | 26.21 | 54.37 | 19.42 | 70.54  | 17.82 | 11.64 | 0.000  | 25.55  | 30.0  | 44.45 | 70.66 | 18.67 | 10.67 | 0.000  |

Abbreviations: FV; Factor V, FV HR2; Factor V-His1299 Arg, F II; Factor II or Prothrombin, PAI-1; Plasminogen Activator Inhibitor-1.

a *P* value calculated by chi-square test.
b FV, at nucleotide position 1691 on the gene a G was exchanged by an A.
c FV HR2, at nucleotide position 4070 on the gene an A was exchanged by a G.
d F II, at nucleotide position 20210 on the gene a G was exchanged by an A.
e PAI-1, at nucleotide position −675 on the gene promoter a GGGGG was exchanged by a GGGG.
Table 5
The distribution of heterozygote and homozygote genotypes and mutant allele frequencies of the four thrombophilic gene polymorphisms in all studied groups.

|                        | Case group (n = 193) | Control group (n = 500) | Men of the case group (n = 103) | Women of the case group (n = 90) | Men of the control group (n = 275) | Women of the control group (n = 225) | %    | %    | P valuea | %    | %    | P valuea | %    | %    | P valuea | %    | %    | P valuea |
|------------------------|----------------------|-------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|------|------|---------|------|------|---------|------|------|---------|------|------|---------|
| **FV 1691G/A**         |                      |                         |                                 |                                 |                                 |                                 |      |      |         |      |      |         |      |      |         |      |      |         |
| GA                     | 11.40                | 4.20                    | 0.001                           | 13.60                           | 8.89                            | 0.367                           | 4.37 | 0.00 | 4.37    | 4.00 | 1.00 | 4.37    | 8.89 | 4.37 | 0.005   | 8.89 | 4    | 0.099   |
| AA                     | 0.00                 | 0.00                    | NS                              | 0.00                            | 0.00                            | NS                              | 0.00 | 0.00 | NS      | 0.00 | 0.00 | NS      | 0.00 | 0.00 | NS      |      |      |         |
| Frequency of A allele  | 5.69                 | 2.1                     | 0.001                           | 6.79                            | 4.40                            | 0.382                           | 2.18 | 0.00 | 2.18    | 2.00 | 1.00 | 2.18    | 4.40 | 2.18 | 0.006   | 4.40 | 2    | 0.103   |
| **FV HR2 4070 A/G**    |                      |                         |                                 |                                 |                                 |                                 |      |      |         |      |      |         |      |      |         |      |      |         |
| AG                     | 9.33                 | 3.60                    | 0.089                           | 5.82                            | 13.33                           | 0.086                           | 5.82 | 0.00 | 5.82    | 5.34 | 0.84 | 5.82    | 5.82 | 1.00 | 5.34    | 13.33| 5.34 | 0.031   |
| GG                     | 1.56                 | 0.00                    | 0.021                           | 0.98                            | 2.23                            | 0.599                           | 0.00 | 0.00 | 0.00    | NS  | 0.00 | 0.98    | 0.00 | 0.00 | 0.272   | 2.23 | 0.00 | 0.081   |
| Frequency of G allele  | 6.21                 | 2.8                     | 0.004                           | 3.88                            | 8.88                            | 0.056                           | 2.90 | 0.00 | 2.90    | 2.66 | 0.85 | 2.88    | 8.88 | 2.66 | 0.002   |      |      |         |
| **F II 20210 G/A**     |                      |                         |                                 |                                 |                                 |                                 |      |      |         |      |      |         |      |      |         |      |      |         |
| GA                     | 10.36                | 6.00                    | 0.051                           | 4.86                            | 16.66                           | 0.009                           | 6.19 | 5.78 | 6.19    | 5.86 | 1.00 | 6.19    | 5.78 | 1.00 | 0.806   | 16.66| 5.78 | 0.004   |
| AA                     | 1.04                 | 0.00                    | 0.077                           | 0.00                            | 2.23                            | 0.216                           | 0.00 | 0.00 | 0.00    | NS  | 0.00 | 0.00    | NS  | 0.00 | 0.272   | 2.23 | 0.00 | 0.081   |
| Frequency of A allele  | 6.21                 | 3.00                    | 0.008                           | 2.42                            | 10.55                           | 0.001                           | 3.09 | 2.88 | 3.09    | 2.42 | 1.00 | 3.09    | 2.88 | 1.00 | 0.809   | 10.55| 2.88 | 0.000   |
| **PAI-1 (−675 I/D, 5G/4G)** |                  |                         |                                 |                                 |                                 |                                 |      |      |         |      |      |         |      |      |         |      |      |         |
| 5G/4G                  | 5.69                 | 2.1                     | 0.001                           | 6.21                            | 4.60                            | 0.014                           | 20.54| 20   | 20.54   | 20   | 0.53 | 20.54   | 4.60 | 20   | 0.000   | 4.60 | 20   | 0.000   |
| Frequency of 4G allele  | 52.59                | 20.3                    | 0.000                           | 54.37                           | 30                              | 0.001                           | 17.82| 18.67| 17.82   | 18.67| 0.81 | 17.82   | 18.67| 0.00 | 18.67   | 18.67| 0.00 | 18.67   |

Abbreviations: FV; Factor V, FV HR2; Factor V-His1299 Arg, F II; Factor II or Prothrombin, PAI-1; Plasminogen Activator Inhibitor-1, NS; Nonsignificant.

a P value calculated by chi-square test.
b FV, at nucleotide position 1691 on the gene a G was exchanged by an A.
c FV HR2, at nucleotide position 4070 on the gene an A was exchanged by a G.
d F II, at nucleotide position 20210 on the gene a G was exchanged by an A.
e PAI-1, at nucleotide position −675 on the gene promoter a GGGGG was exchanged by a GGGG.
evidence to support an association of FV A4070G polymorphism with DVT Gohil et al. (2009). Some other studies, however, suggested that there were no statistical differences in the prevalence of this polymorphism between the patient and control groups. Benson et al. (2001) demonstrated that the prevalence of this polymorphism is similar in the case and control groups Benson et al. (2001). Also, these findings are consistent with the study published by Castaman et al. (2003).

In accordance with our data which shows the association of the increased risk of DVT with PAI-1 4G/5G polymorphism, there are other studies that confirm the association between this variant and venous thromboembolism. In a case–control study by Stegnar et al. (1998), they indicated an association between the 4G/5G polymorphism in the promoter of the PAI-1 gene and plasma PAI-1 levels in patients with venous thromboembolism Stegnar et al. (1998). This result was confirmed by Seguí et al. (2000) who reported that the presence of the 4G allele significantly increased the risk of thrombosis in patients with thrombophilic defects Seguí et al. (2000). Akar et al. (2000) demonstrated that the 4G/5G polymorphism is more prevalent in patients compared with healthy controls Akar et al. (2000). Conversely, no differences in the frequencies of heterozygous and homozygous PAI-I polymorphism were observed when patients with DVT were compared with controls in a study by (Hooper et al., 2000).

In conclusion, our study demonstrated that the FV (G1691A and A4070G) and PAI-1 4G/5G polymorphisms are more frequently found among patients with DVT, compared with healthy controls. This finding raises the possibility that these polymorphisms increase venous thromboembolism susceptibility.

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References

Akar, N., Yilmaz, E., Akar, E., Avcu, F., Yalcin, A., Cin, S., 2000. Effect of plasminogen activator inhibitor-1 4G/5G polymorphism in Turkish deep vein thrombotic patients with and without FV1691 G-A. Thromb. Res. 97 (4), 227–230 [Feb 15, PubMed PMID: 10674409. Epub 2000/02/16. eng].

Alhenc-Gelas, M., Nicaud, V., Gandrille, S., van Dreden, P., Amiral, J., Aubry, M.L., et al., 1999. The factor V gene A4070G mutation and the risk of venous thrombosis. Thromb. Haemost. 81 (2), 193–197 [Feb, PubMed PMID: 10063990. Epub 1999/03/04. eng].

Arsow, T., Miladinova, D., Spiroski, M., 2006. Factor V Leiden is associated with higher risk of deep venous thrombosis of large blood vessels. Croat. Med. J. 47 (3), 433–439 [Jun, PubMed PMID: 16758522. Pubmed Central PMCID: 2080416. Epub 2006/06/08. eng].

Benson, J.M., Ellingsen, D., El-Jamil, M., Jenkins, M., Miller, C.H., Dilley, A., et al., 2001. Factor V Leiden and factor V R2 allele: high-throughput analysis and association with venous thromboembolism. Thromb. Haemost. 85 (5), 1188–1192 [Nov, PubMed PMID: 11816705. Epub 2002/01/31. eng].

Bouazziz-Borgi, L., Almawi, W.Y., Mitraoui, N., Nsiri, B., Keleshian, S.H., Kreidy, R., et al., 2006. Distinct association of factor V-Leiden and prothrombin G20210A mutations with deep venous thrombosis in Tunisia and Lebanon. Am. J. Hematol. 81 (8), 641–643 [Aug, PubMed PMID: 16823828. Epub 2006/07/11. eng].
Bouaziz-Borgi, L., Nguyen, P., Hezard, N., Mesharrafieh, U., Almawi, W.Y., Majhoub, T., 2007. A case control study of deep venous thrombosis in relation to factor V G1691A (Leiden) and A4070G (HR2 Haploype) polymorphisms. Exp. Mol. Pathol. 83 (3), 480–483 (Dec, PubMed PMID: 17555744. Epub 2007/06/09. eng).

Castaman, G., Faioni, E.M., Tosetto, A., Bernardi, F., 2003. The factor V HR2 haplotype and the risk of venous thrombosis: a meta-analysis. Haematologica 88 (10), 1182–1189 (Oct, PubMed PMID: 14553116. Epub 2003/10/14. eng).

Coulam, C.B., Wallis, D., Weinstein, J., DasGupta, D.S., Jeyendran, R.S., 2008. Comparison of thrombophilic gene mutations among patients experiencing recurrent miscarriage and deep vein thrombosis. Am. J. Reprod. Immunol. 60 (5), 426–431 (Nov, PubMed PMID: 18803625. Epub 2008/09/23. Eng).

De Stefano, V., Martinelli, I., Mannucci, P.M., Paciaroni, K., Chiusolo, P., Casorelli, I., et al., 1999. The risk of recurrent deep vein thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N. Engl. J. Med. 341 (11), 801–806 (Sep 9, PubMed PMID: 10477778. Epub 1999/09/09. eng).

Francis, C.W., 2002. Plasminogen activator inhibitor-1 levels and polymorphisms. Arch. Pathol. Lab. Med. 126 (11), 1401–1404 (Nov, PubMed PMID: 12421149. Epub 2002/11/08. eng).

Franco, R.F., Reitsma, P.H., 2001. Genetic risk factors of venous thrombosis. Hum. Genet. 109 (4), 369–384 (Oct, PubMed PMID: 11702218. Epub 2001/11/10. eng).

Gohil, R., Peck, G., Sharma, P., 2009. The genetics of venous thromboembolism. A meta-analysis involving approximately 120,000 cases and 180,000 controls. Thromb. Haemost. 102 (2), 360–370 (Aug).

Heit, J.A., 2006. The epidemiology of venous thromboembolism in the community: implications for prevention and management. J. Thromb. Thrombolysis 21 (1), 23–29 (Feb, PubMed PMID: 16475038. Epub 2006/02/14. eng).

Hooper, W.C., Lally, C., Austin, H., Renshaw, M., Dilley, A., Wenger, N.K., et al., 2000. The role of the t-PA I/D and PAI-1 4G/5G polymorphisms in African–American adults with a diagnosis of myocardial infarction or venous thromboembolism. Thromb. Res. 99 (3), 223–230 (Aug 1, PubMed PMID: 10942788. Epub 2000/08/16. eng).

Hoppe, B., Heymann, G.A., Koscielny, J., Hellstern, P., Kiesewetter, H., Salama, A., 2003. Screening for multiple hereditary hypercoagulability factors using the amplification refractory mutation system. Thromb. Res. 111 (1–2), 115–120 (PubMed PMID: 14640489. Epub 2003/12/04. eng).

Juul, K., Tybjerg-Hansen, A., Schnohr, P., Nordestgaard, B.G., 2004. Factor V Leiden and the risk for venous thromboembolism in the adult Danish population. Ann. Intern. Med. 140 (5), 330–337 (Mar 2, PubMed PMID: 14996674. Epub 2004/03/05. eng).

Lijfering, W.M., Middeldorp, S., Veeger, N.J., Hamulyak, K., Prins, M.H., Buller, H.R., et al., 2010. Risk of recurrent venous thrombosis in homozygous carriers and double heterozygous carriers of factor V Leiden and prothrombin G20210A. Circulation 121 (15), 1706–1712 (Apr 20, PubMed PMID: 20368522. Epub 2010/04/07. eng).

Lopez, J.A., Kearon, C., Lee, A.Y., 2004. Deep vein thrombosis. Hematol. Am. Soc. Hematol Educ. Program. 439–456 (PubMed PMID: 15561697. Epub 2004/11/25. Eng).

Luconi, S., Iacoviello, L., Gemmatti, D., DiLasio, M.G., Castoldi, E., Pinotti, M., et al., 2016. Detection of new polymorphic markers in the factor V gene: association with factor V levels in plasma. Thromb. Haemost. 75 (1), 45–48 (Jan, PubMed PMID: 8713778. Epub 1996/01/01. eng).

Meyer, G., Emmerich, J., Helley, D., Arnaud, E., Nicaud, V., Alhenc-Gelas, M., et al., 2001. Factors V Leiden and II 20210A in patients with symptomatic pulmonary embolism and deep vein thrombosis. Am. J. Med. 110 (1), 12–15 (Jan, PubMed PMID: 11152859. Epub 2001/01/12. eng).

Perez-Ceballos, E., Corral, J., Alberca, I., Ayma, A., Llamas, M., Montes, R., et al., 2002. Prothrombin A19911G and G20210A polymorphisms role in thrombosis. Br. J. Haematol. 118 (2), 610–614 (Aug, PubMed PMID: 12139755. Epub 2002/07/26. eng).

Previtali, E., Bucciarelli, P., Pasamonti, S.M., Martinelli, I., 2011. Risk factors for venous and arterial thrombosis. Blood Transfus. 9 (2), 120–138 (Apr, PubMed PMID: 21084000. Pubmed Central PMID: 3096855. Epub 2010/11/19. Eng).

Rahimi, Z., Mozafari, H., Shahriari-Ahmadi, A., Alimogaddam, K., Ghavamzadeh, A., Aznab, M., et al., 2010. Deep vein thrombosis and thrombophilic mutations in western Iran: association with factor V Leiden and prothrombin G20210A. Haematologica 95 (5), 806 (Sep, PubMed PMID: 19946141. Epub 2009/05/12. eng).

Sartori, M.T., Danesin, C., Saggiorato, G., Gormene, D., Simioni, P., Spiezia, L., et al., 2003. The PAI-1 gene 4G/5G polymorphism and deep vein thrombosis in patients with inherited thrombophilia. Clin. Appl. Thromb. Hemost. 9 (4), 299–307 (Oct, PubMed PMID: 14653439. Epub 2003/12/05. eng).

Segui, R., Estellés, A., Mira, Y., España, F., Villa, P., Falcó, C., et al., 2000. PAI-1 promoter 4G/5G genotype as an additional risk factor for venous thrombosis in subjects with genetic thrombophilic defects. Br. J. Haematol. 111 (1), 122–128.

Smalberg, J.H., Kruip, M.J., Janssen, H.L., Rijken, D.C., Leebeek, F.W., de Maat, M.P., 2011. Hypercoagulability and hypofibrinolysis and risk of deep vein thrombosis and splanchic vein thrombosis: similarities and differences. Arterioscler. Thromb. Vasc. Biol. 31 (3), 485–493 (Mar, PubMed PMID: 21325670. Epub 2011/02/18. Eng).

Stegnæ, M., Uhrin, P., Peternel, P., Mavri, A., Salobir–Pajnic, B., Stare, J., et al., 1998. The 4G/5G sequence polymorphism in the promoter of plasminogen activator inhibitor-1 (PAI-1) gene: relationship to plasma PAI-1 level in venous thromboembolism. Thromb. Haemost. 79 (5), 975–979 (May, PubMed PMID: 9609232. Epub 1998/06/03. eng).

Sykes, T.C., Fegan, C., Mosquera, D., 2000. Thrombophilia, polymorphisms, and vascular disease. Mol. Pathol. 53 (6), 300–306 (Dec, PubMed PMID: 11193048. Pubmed Central PMID: 1186984. Epub 2001/02/24. eng).

Tsantes, A.E., Nikolopoulos, G.K., Bagos, P.G., Rapti, E., Mantzios, G., Kapsimali, V., et al., 2007. Association between the plasminogen activator inhibitor-1 4G/5G polymorphism and venous thrombosis. A meta-analysis. Thromb. Haemost. 97 (6), 907–913 (Jun, PubMed PMID: 17549286. Epub 2007/06/06. eng).