

Genetic factors that may affect the risk of developing thrombosis in patients with myeloproliferative disorders

Type

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Keywords

polymorphism, thrombosis, genetic factor, myeloproliferative disorders

Abstract

Introduction

Patients with Philadelphia-negative (Ph-) myeloproliferative neoplasms: primary myelofibrosis, essential thrombocythemia and polycythemia vera; often develop thrombotic events. It was suggested that the genetic variants that are responsible for blood coagulation and elevated homocysteine level play causal role in the occurrence of thrombosis. Our aim was to evaluate the single nucleotide polymorphisms in PROS1, EPCR, PROC, MTHFR, MS genes and their associations with the risk of developing thrombosis as well as clinical characteristics in patients with myeloproliferative disorders.

Material and methods

The screening of PROS1 g.66847T>C, EPCR c.4678G>C, EPCR c.6936A>G, PROC c.565C>T, MTHFR c.677C>T, MTHFR c.1298A>C, MS c.2756A>G polymorphisms was performed at Lithuanian University of Health Sciences, Kaunas, Lithuania. The PCR-RFLP method was applied.

Results

After genotyping 88 patients with Ph- myeloproliferative disorders, the association was found between venous thrombosis and MTHFR c.1298A>C ($p=0.019$). Regression analysis revealed that carriers of PROS1 66847TC, EPCR 4678GC, 6936AG or GG, PROC 565CT or TT, MTHFR 677CT, MS 2756AG genotypes were associated with a lower risk of developing venous thrombosis. EPCR 6936AG genotype could be considered as a protective factor against arterial thrombosis. Genotypes PROC 565CT and 565TT were associated with a lower risk of decreased levels of MPV, 565TT carriers were less likely to develop arterial or venous thrombosis, compared with those carrying the CC or CT genotype.

Conclusions

It can be concluded that more research into these polymorphisms needs to be performed, since there are many conflicting results published regarding the complexity of the possible interactions between these gene variants and predisposition to thrombotic events.

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Running title: SNPs AFFECTING THROMBOSIS IN PMF, ET, PV

Preprint

ABSTRACT

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Materials and methods: The screening of *PROS1* g.66847T>C, *EPCR* c.4678G>C, *EPCR* c.6936A>G, *PROC* c.565C>T, *MTHFR* c.677C>T, *MTHFR* c.1298A>C, *MS* c.2756A>G polymorphisms was performed at Lithuanian University of Health Sciences, Kaunas, Lithuania. The PCR-RFLP method was applied.

Results: After genotyping 88 patients with Ph- myeloproliferative disorders, the association was found between venous thrombosis and *MTHFR* c.1298A>C (p=0.019). Regression analysis revealed that carriers of *PROS1* 66847TC, *EPCR* 4678GC, 6936AG or GG, *PROC* 565CT or TT, *MTHFR* 677CT, *MS* 2756AG genotypes were associated with a lower risk of developing venous thrombosis. *EPCR* 6936AG genotype could be considered as a protective factor against arterial thrombosis. Genotypes *PROC* 565CT and 565TT were associated with a lower risk of decreased levels of MPV, 565TT carriers were less likely to develop arterial or venous thrombosis, compared with those carrying the CC or CT genotype.

Conclusions: It can be concluded that more research into these polymorphisms needs to be performed, since there are many conflicting results published regarding the complexity of the possible interactions between these gene variants and predisposition to thrombotic events.

Keywords: genetic factor; polymorphism; myeloproliferative disorders; thrombosis.

Preprint

INTRODUCTION

Myeloproliferative neoplasms (MPNs), by definition, are clonal hematologic diseases that occur by overproduction of active blood cells [1]. The group of Philadelphia-negative (Ph-, non *BCR-ABL*) myeloproliferative neoplasms, polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are known for their different phenotypes but similar complications [2]. Primary myelofibrosis specifically is a clonal stem cell disorder that is characterized by chronic myeloproliferation, megakaryocytic hyperplasia and the presence of the myeloid series immature cells in the circulating blood [3]. PMF is a relatively rare disease and affects approximately 1 in 500.000 people worldwide. Essential thrombocythemia is specifically defined by the thrombocytosis and thrombotic or hemorrhagic events. This disease can also progress into secondary myelofibrosis and occurs in approximately 2 cases in 100 000 per year [4]. Polycythemia vera is characterized by uncontrollable blood cells proliferation and accumulation (white blood cells, red blood cells, platelets) [5]. The incidence of PV is at least 2 per 100 000 [6]. Common complications for these diseases are thrombotic events, which can often be the main cause of death [7, 8]. The incidence of thrombotic complications varies between disease types. They occur in 7.2–15.0% patients with PMF, in 19.0–32.0% patients with ET, and in 30.0–41.0% patients with PV [9–12]. It is known that thrombotic complications may be genetic or acquired. Pathogenesis of thrombosis in MPNs is complex, but not fully understood yet [13].

The protein C (PROC) anticoagulant pathway plays a key role in controlling thrombosis. PROC is activated on endothelium by the thrombin-thrombomodulin-endothelial protein C receptor (EPCR) complex [14]. In the presence of cofactor protein S (PROS1), activated protein C proteolytically inactivates coagulation factors VIIIa and Va, thereby inhibiting clot formation. By binding to EPCR and protease-activated receptor-1, PROC also exerts anti-inflammatory and cytoprotective effects on a variety of cell types [15]. It is known that

deficiencies in one of the three natural anticoagulants, antithrombin, protein C or protein S are inherited forms of thrombophilia, which have been linked to venous thrombosis (VT).

Moreover, mutations and polymorphisms in the *PROC* and *PROS1* genes are associated with thrombotic disease [16-20].

As mentioned, EPCR, a transmembrane protein expressed on the endothelium of large vessels, is involved in the activation of protein C anticoagulant pathway by binding PROC and enhancing its activation [21]. It increases 5-fold the activation efficiency of PROC leading to markedly elevated anticoagulant activity [22]. Thus, the involvement of EPCR in the anticoagulant pathway of hemostasis suggests a potential role in the pathogenesis of thrombosis [23]. Blocking of EPCR seems to accelerate thrombus development *in vivo* [24]. Furthermore, polymorphisms in *EPCR* gene have been studied regarding their involvement in the development of thrombosis [25], but conclusions were questioned [26, 27].

Several key enzymes, including 5,10-methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MS*) and methionine synthase reductase (*MTRR*), are important in homocysteine metabolism and therefore in methylation reactions [28-30]. Disturbed function of the enzymes results in hyperhomocysteinemia and may cause thrombophilia [31]. It has been suggested that *MTHFR*, *MS* and *MTRR* genes are involved in the aetiology of malignancies. The substitutions in *MTHFR* gene may be associated with myeloproliferative neoplasms risk and disease progression. *MTHFR* has been described as one of the candidate genetic risk factors for thrombosis. *MTHFR* association with thrombotic events has been extensively studied over the past decade, however, the results were inconclusive [31-35]. Moreover, polymorphisms in the *MS* gene have been associated with haematological malignancies [36]. Many studies were conducted to investigate the relationship between substitutions in the *MS* gene and blood cancers [37-40]. Nevertheless, the results still remain conflicting.

To our knowledge, the relationship between MPNs and polymorphisms in *PROS1*, *EPCR*, *PROC*, *MTHFR*, *MS* genes is not fully understood yet. The main purpose of our study was to evaluate the effects of different SNPs: *PROS1* g.66847T>C, *EPCR* c.4678G>C, *EPCR* c.6936A>G, *PROC* c.565C>T, *MTHFR* c.677C>T, *MTHFR* c.1298A>C, *MS* c.2756A>G on the risk of thrombosis development and clinical characteristics in patients with PV, ET, and PMF.

MATERIALS AND METHODS

Editorial Policies and Ethical Considerations

This study was approved by Kaunas Regional Ethics Committee for Biomedical Research (protocol no. BE-2-9). Signed informed consent forms for participation in the study were obtained from all participants.

Patients

The study included 88 patients who were diagnosed with ET, PV or PMF between 2000 and 2014 at the Department of Hematology of the Institute of Oncology, the Lithuanian University of Health Sciences, Kaunas, Lithuania. The patient group consisted of men (42%) and women (58%), with the mean age of 64.2 years (standard deviation 14,436), the range 27—87 years. Diagnosis was conformed as ET in 45 patients (51.1%), PV in 36 (40.9%) and PMF in seven (8%) patients of the studied group. Medical information included the patient's age, sex, history of arterial or venous thrombosis, mean platelet volume, platelet count, mean corpuscular volume. Clinical characteristics are presented in Table I.

Genotyping

Venous blood samples were collected in vacutainers with EDTA as anticoagulant. Samples were stored at -20°C until further processing. Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA extraction kit, according to the instructions provided by the manufacturer (Thermo Fisher Scientific Baltics, Vilnius,

Lithuania). Genomic variants in all of the cases were detected by employing a polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method. Primer sequences and restriction enzymes used for the detection of *PROS1* g.66847T>C, *EPCR* c.4678G>C, *EPCR* c.6936A>G, *PROC* c.565C>T, *MTHFR* c.677C>T, *MTHFR* c.1298A>C, *MS* c.2756A>G SNPs are presented in Table II. The digested fragments were electrophoresed in a 2% agarose gel.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) 20.0 for Windows was used for the association analysis. To compare percentages the Chi-squared and Fisher's tests were performed. The logistic regression analysis was used to distinguish thrombotic risk factors significantly affecting the possibility of thrombosis and changes in MPV and MCV among the studied patients. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated from the logistic model. A two-tailed p value <0.05 was taken as statistically significant.

RESULTS

***PROS1* g.66847T>C.** The wild 66847TT genotype was observed in 67 of the patients (76.1%) and the heterozygous 66847TC genotype was found in 21 of the patients (23.9%) (Table III). No homozygous patients with the 66847CC genotype were detected. Fisher's exact test showed no significant associations between *PROS1* g.66847T>C polymorphism and clinical characteristics (Table IV). According to logistic regression analysis, the carriers of the 66847TC genotype had a 0.063-fold lower chance of developing venous thrombosis (p=0.007; OR 0.063; 95% CI 0.008 – 0.471) (Table V).

***EPCR* c.4678G>C.** Out of 88 patients, 31 patients were observed to have the wild 4678GG genotype (35.2%). The heterozygous 4678GC genotype was found in 43 patients (48.8%) and the homozygous mutant 4678CC genotype – 14 (16.0%) (Table III). Chi-squared association analysis was performed and no significant associations were found between the studied

polymorphism and clinical characteristics (Table VI). As shown in Table V, carriers of 4678GC genotype were less susceptible to venous thrombosis development ($p < 0.001$; OR 0.226; 95% CI 0.099-0.513).

EPCR c.6936A>G. After applying the PCR-RFLP method, out of 88 patients, 31 had the wild 6936AA genotype (14.8%). The heterozygous 6936AG genotype was observed in 60 cases (68.2%) and the homozygous mutant 6936GG genotype was detected in 15 patients (17.0%) (Table III). No significant associations were found after performing Chi-squared analysis (Table VII). Logistic regression analysis revealed that carriers of the 6936AG genotype had a lower risk of developing venous ($p < 0.001$; OR 0.171; 95% CI 0.077 – 0.381) or arterial thrombosis ($p = 0.026$; OR 0.515; 95% CI 0.287 – 0.925). The results showed that 6936GG genotype was also associated with reduced risk of venous thrombosis development ($p = 0.017$; OR 0.083; 95% CI 0.011 – 0.641) (Table V).

PROC c.565C>T. After genotyping our studied group, 44 patients (50%) were found to have the wild 565CC genotype. The heterozygous 565CT genotype was observed in 25 (28.4%) patients and the homozygous 565TT genotype was found in 19 (21.6%) (Table III). No significant associations were found after performing Chi-squared analysis (Table VIII). Logistic regression analysis revealed that there is a lower chance of developing venous thrombosis for carriers of the 565CT genotype ($p = 0.003$; OR 0.158; 95% CI 0.047 – 0.534) and 565TT genotype ($p = 0.009$; OR 0.067; 95% CI 0.009 – 0.505). Occurrence of thrombosis overall was lower to those who had the 565TT genotype ($p = 0.048$; OR 0.357; 95% CI 0.129 – 0.992). The carriers of 565CT ($p = 0.034$; OR 0.389; 95% CI 0.162 – 0.931) and 565TT ($p = 0.019$; OR 0.267; 95% CI 0.089 – 0.803) genotypes had a lower chance of decreased mean platelet volume (Table V).

MTHFR c.677C>T. After applying the PCR-RFLP method, 45 patients were confirmed to have the wild 677CC genotype (51.1%), the heterozygous 677CT genotype was observed in

37 (42.0%) and the 677TT genotype was confirmed in 6 patients (6.9%) of our studied group (Table III). Chi-squared analysis showed no significant associations between the studied polymorphism and clinical characteristics (Table IX). Binary logistic regression analysis revealed that the 667CT genotype was associated with the lower the risk of venous thrombosis development ($p=0.001$; OR 0.214; 95% CI 0.089 – 0.518) (Table V).

MTHFR c.1298A>C. The wild 1298AA genotype was discovered in 37 (42%) patients, the heterozygous 1298AC genotype was found in 34 (38.6%) patients and the homozygous CC genotype – 17 (19.4%) patients (Table III). Chi-squared analysis showed the significant association between the studied polymorphism and venous thrombosis (χ^2 (2,N=88)=7.278,p=0.019) (Table X).

MS c.2756A>G. Out of 88 patients with myeloproliferative disorders, 54 (51.4%) patients were confirmed as carriers of the wild 2756AA genotype, heterozygous 2756AG genotype was observed in 30 (34.1%) patients and the homozygous 2756GG – in 4 (4.5%) patients (Table III). Chi-squared analysis revealed no associations (Table XI). Logistic regression analysis showed that carriers of the 2675AG genotype had a lower risk of developing venous thrombosis ($p=0.001$; OR 0.091; 95% CI 0.021 – 0.387). These results are summarised in Table V.

DISCUSSION

The selected SNPs for this study have a tendency to occur in patients with Ph-myeloproliferative neoplasms, but their significance to thrombosis development is not fully evaluated yet. The objective of this study was to assess the importance of *PROS1* g.66847T>C, *EPCR* c.4678G>C, *EPCR* c.6936A>G, *PROC* c.565C>T, *MTHFR* c.677C>T, *MTHFR* c.1298A>C, *MS* c.2756A>G polymorphisms to the development of thrombotic events and clinical characteristics related to them.

Polymorphisms in *PROC* (coding for protein C) and *PROS1* (coding for protein S) can be the cause of a dysfunctional component in the coagulation cascade and could be associated with various thrombotic events. In this study, it was observed that a nucleotide change from T to C in the 66847 position of *PROS1* gene can lead to lower probability of developing venous thrombosis. Although, in a study, performed by Reiner et al., there was no evidence of associations between the studied polymorphism and incidence of cardiovascular diseases [45]. Apart from this study there are no more researches performed with this SNP and hematological complications. This information suggests that the polymorphism is not widely studied and needs more investigation, hence, there are signs of possible importance. Protein C, which is coded by *PROC* gene, is a key component of the coagulation cascade and it is known that the lack of protein C cause a predisposition to venous thrombosis. Tang and his colleagues reported that the carriers of 565CT genotype had an 8.8-fold increased risk of venous thrombosis in Chinese population with hereditary protein C deficiency [43]. This study contradicts our results, since our data suggests that the 565CT or 565TT genotypes can be considered as a protective factors against venous thrombosis. This incongruity could be caused by mismatched studied group ethnicity, since there was a report by Yin et al. that Asian populations have higher rates of venous thromboembolism (4 – 31.76%) compared to the general population (0,29—4%) and that the *PROC* c.565C>T SNP has a higher frequency of occurrence in the Chinese population, compared to populations in the west [46]. Another predictor with diagnostic importance is mean platelet volume which increases with the overproduction of thrombocytes [47]. Our study revealed that patients harboring the 565CT and 565TT genotypes may have a lower risk of decrease in MPV. In a study performed by Ayer et al. it was reported that there are no significant associations between mean platelet volume and thromboembolic events in ET (p=0.483) and PV (p=0.221) groups [48]. This suggests that MPV may not contribute to thrombotic events in myeloproliferative disorders.

It was previously reported that the *EPCR* c.4678G>C SNP was identified as a protective factor against venous thrombosis because of its association with high levels of circulating activated protein C (APC). The data in Medina et al. study suggests that carriers of FV Leiden with the 4678CC genotype may have a lower risk of venous thrombosis development and may experience first thrombotic events significantly later than those with 4678GG or 4678GC genotypes [26]. Karabjyk et al. also reported that this polymorphism may offer protection against thrombosis, where out of 111 patients 79 were observed to have the 4678GC genotype and 19 patients had the 4678GG genotype [49]. This information agrees with our findings, although the obtained results that another studied polymorphism – *EPCR* c.6936A>G – could be considered as a protective factor against thrombosis are inconsistent with the previous researches. Besbes et al. also reported a significant association between this polymorphism and thrombotic events, although they reported different findings. After genotyping 110 patients with hematological complications they calculated that the 6936AG genotype may increase the risk of thrombosis, compared to the 6936AA genotype [50]. That was in agreement with a newer study performed by Zoheir et al. where it was established that the G allele increases the risk of thrombosis by two-fold (studied patient group consisted of 90 people) [51]. This difference could be explained by the fact that these genotypes showed different distribution among different studied group ethnicity.

Higher levels of homocysteine are thought to play an important role in the occurrence of venous thrombosis [52]. A common genetic variant that affects homocysteine levels exists in the gene that encodes the methylenetetrahydrofolate reductase (MTHFR) enzyme. This polymorphism, in which a substitution from C to T occurs at position 677, induces a change of alanine to valine and impaired enzyme activity [53]. Although, its correlation with venous thrombosis remains controversial. In this study the only significant association between the 677CT genotype and lower risk of VT occurrence was found.

There are a lot of studies with inconsistent results regarding this polymorphism. Two studies carried out in Iranian and Northwestern Greece populations showed no significant associations between the *MTHFR* c.677C>T polymorphism and venous thrombosis [54, 55]. A meta-analysis, performed by M. den Heijer and colleagues, showed that the 677TT genotype relates with 20% increased risk of VT. Also, a difference between increased risk of thrombosis in studies carried out in Europe and North America (lower risk in Europe) was noticed, although not significant, which may be explained by dietary differences [56]. Another polymorphism in this gene – c.1298A>C. According to our study this SNP seems to be associated with venous thrombosis. Akar et al. research revealed that this polymorphism alone does not contribute to thrombotic events, although they found out that carriers of *MTHFR* c.677C>T and c.1298A>C in double heterozygous or homozygous states have an impact on higher thrombotic risk in Turkish population [57]. This information partially could explain the controversial results published by other authors regarding these SNPs but further investigations need to be done to establish clear prognostic value.

One of the other factors that may predispose to thrombosis is the nucleotide change from A to G in the 2756 position of the methionine synthase (*MS*) gene which leads to changes in the enzyme activity and increased homocysteine levels [58]. From our data, it is apparent that the 2756AG genotype may be considered as a protective factor against venous thrombosis. That was in agreement with the results of Z. Yates et al. study, which reported that the G allele may offer significant protection against thromboembolic events (OR=0.39, p=0.0065) [59]. There is convincing evidence that the *MS* c.2756A>G and *MTHFR* c.677C>T polymorphisms could provide possible health benefits.

As interesting as these findings are, more research into these polymorphisms needs to be performed, since there are many conflicting results published regarding the complexity of

the possible interactions between these gene variants and predisposition to thrombotic events. This kind of research into possible biomarkers for thrombosis in myeloproliferative patients is the first in Lithuania and could lead to the new insights when determining disease treatment and outcomes.

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Table I. Clinical characteristics.

| Characteristics | Patients (n=88) |
|--|------------------------|
| Age: mean (SD) | 64.2 (14.44) |
| Males: n (%) | 37 (42) |
| Females: n (%) | 51 (58) |
| Arterial thrombosis: n (%) | 29 (33) |
| Venous thrombosis: n (%) | 10 (11.4) |
| Incidence of thrombosis: n (%) | 39 (44) |
| Decreased mean platelet volume: n (%) | 22 (25) |
| Normal mean platelet volume: n (%) | 22 (25) |
| Increased mean platelet volume: n (%) | 1 (1.1) |
| Decreased mean corpuscular volume: n (%) | 22 (25) |
| Normal mean corpuscular volume: n (%) | 54 (61.3) |
| Increased mean corpuscular volume: n (%) | 3 (3.4) |

Note: n, number of individuals; SD, standard deviation.

Table II. Primer sequences and restriction enzymes used for genotyping, length of DNA fragments after applying the PCR-RFLP method.

| Substitution | Primer sequence | Restriction enzyme | DNA fragment (bp) | |
|------------------------------------|--|--------------------|-------------------|--------------|
| | | | WTA | PA |
| <i>PROS1</i> g.66847T>C [41] | F:5'-GAGTCACAGTGTCTGCT-3' R:5'-AGGCACATATCATCACTCCT-3' | <i>AccI</i> | 394 | 394, 340, 54 |
| <i>EPCR</i> c.4678G>C [26] | F:5'-GCTTCAGTCAGTTGGTAAAC-3' R:5'-TCTGGCTTCACAGTGAGCTG-3' | <i>DdeI</i> | 314 | 314, 252, 62 |
| <i>EPCR</i> c.6936A>G [42] | F:5'-CCTACACTTCGCTGGTCCTGGGCGTCCTGGTCTGC-3' R:5'-CCTACACTTCGCTGGTCCTGGGCGTCCTGGTCTGC-3' | <i>PstI</i> | 290 | 290,254 |
| <i>PROC</i> c.565C>T [43] | F:5'-TCCTTGAACCCTGCACTGTGGCAA-3' R:5'-TTTCAGGTGACTACGCTTCTTCTCCATGC-3' | <i>Hin6I</i> | 143, 30 | 173,143,30 |
| <i>MTHFR</i> c.677C>T [44] | F:5'-TGAAGGAGAAGGTGTCTGCGGGA-3' R:5'-AGGACGGTGCGGTGAGAGTG-3' | <i>HinfI</i> | 198 | 198,175,23 |

| | | | | |
|-----------------------------------|---|---------------|-----------|-----------------|
| <i>MTHFR</i> c.1298A>C [44] | F:5'-GCAAGTCCCCCAAGGAGG-3' R:5'-GGTCCCCACTTCCAGCATC-3' | <i>MboII</i> | 79, 37,29 | 108, 79, 37, 29 |
| <i>MS</i> c.2756A>G [17] | F:5'-TGTTCCAGACAGTTAGATGAAAATC-3' R:5'-GATCCAAAGCCTTTTACACTCCTC-3' | <i>HaeIII</i> | 211 | 211, 131, 80 |

Note: F, Forward; R, Reverse; WTA, Wild Type Allele; PA, Polymorphic Allele; bp, base pair.

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Table III. Genotype frequencies.

| Polymorphism | <i>PROS1</i> g.66847T>C | | | <i>EPCR</i> c.4678G>C | | | <i>EPCR</i> c.6936A>G | | | <i>PROC</i> c.565 C>T | | |
|---------------------|-------------------------|--------|-------|------------------------|--------|--------|-----------------------|--------|--------|-----------------------|--------|--------|
| Genotype | TT | TC | CC | GG | GC | CC | AA | AG | GG | CC | CT | TT |
| n | 67 | 21 | 0 | 31 | 43 | 14 | 13 | 60 | 15 | 44 | 25 | 19 |
| (%) | (76.1) | (23.9) | 0 | (35.2) | (48.8) | (16.0) | (14.8) | (68.2) | (17.0) | (50.0) | (28.4) | (21.6) |
| Polymorphism | <i>MTHFR</i> c.677C>T | | | <i>MTHFR</i> c.1298A>C | | | <i>MS</i> c.2756A>G | | | | | |
| Genotype | CC | CT | TT | AA | AC | CC | AA | AG | GG | | | |
| n | 45 | 37 | 6 | 37 | 34 | 17 | 54 | 30 | 4 | | | |
| (%) | (51.1) | (42.0) | (6.9) | (42.0) | (38.6) | (19.4) | (61.4) | (34.1) | (4.5) | | | |

Table IV. *PROS1* g.66847T>C genotype distribution among clinical characteristics.

| Characteristics | <i>PROS1</i> g.66847T>C | | | <i>p</i> value |
|--------------------------------|-------------------------|-----------|-------|----------------|
| | TT | TC | CC | |
| | N (%) | N (%) | N (%) | |
| Venous thrombosis | | | | |
| - | 46 (74.2) | 16 (25.8) | 0 | 0.434 |
| + | 9 (90.0) | 1 (10.0) | 0 | |
| Arterial thrombosis | | | | |
| - | 34 (72.3) | 13 (27.7) | 0 | 0.591 |
| + | 23 (79.3) | 6 (20.7) | 0 | |
| Incidence of thrombosis | | | | |
| - | 38 (73.1) | 14 (26.9) | 0 | 0.458 |
| + | 29 (80.6) | 7 (19.4) | 0 | |

| | | | | |
|--------------------------------|-----------|-----------|---|-------|
| Mean platelet volume | | | | |
| Normal | 15 (68.2) | 7 (31.8) | 0 | 0.605 |
| Decreased | 16 (72.7) | 6 (27.3) | 0 | |
| Increased | 1 (100.0) | 0 | 0 | |
| Mean corpuscular volume | | | | |
| Normal | 44 (81.5) | 10 (18.5) | 0 | 0.419 |
| Decreased | 15 (68.2) | 7 (31.8) | 0 | |
| Increased | 2 (66.7) | 1 (33.3) | 0 | |

Table V. Binary logistic regression analysis.

| Polymorphism | Genotype | Clinical characteristic | B | OR | p value | 95% CI-OR |
|----------------------------|-----------------|--------------------------------|------------|-----------|----------------|------------------|
| <i>PROS1</i> g.66847T>C | TC | Venous thrombosis | - 2.773 | 0.063 | 0.007 | 0.008 – 0.471 |
| <i>EPCR</i> c.4678G>C | GC | Venous thrombosis | - 1.488 | 0.226 | <0.001 | 0.099 – 0.513 |
| <i>EPCR</i> c.6936A>G | AG | Venous thrombosis | - 1.768 | 0.171 | <0.001 | 0.077 – 0.381 |
| | GG | | - 2.485 | 0.083 | 0.017 | 0.011 – 0.641 |
| | AG | Arterial thrombosis | - 0.663 | 0.515 | 0.026 | 0.287 – 0.925 |
| <i>PROC</i> c.565C>T | CT | Venous thrombosis | - 1.846 | 0.158 | 0.003 | 0.047 – 0.534 |
| | TT | | - 2.078 | 0.067 | 0.009 | 0.009 – 0.505 |

| | | | | | | |
|--------------------------|----|-----------------------------------|------------|-------|-------|---------------|
| | TT | Incidence of thrombosis | - 1.030 | 0.357 | 0.048 | 0.129 – 0.992 |
| | CT | Decreased mean platelet volume | - 0.944 | 0.389 | 0.034 | 0.162 – 0.931 |
| | TT | | - 1.322 | 0.267 | 0.019 | 0.089 – 0.803 |
| <i>MTHFR</i> c.677C>T | CT | Venous thrombosis | - 1.540 | 0.214 | 0.001 | 0.089 – 0.518 |
| <i>MS</i> c.2756A>G | AG | Venous thrombosis | - 2.398 | 0.091 | 0.001 | 0.021 – 0.387 |

Note: B, regression coefficient; OR, odds ratio; 95% CI, confidence interval.

| | | | | | | | | | | |
|--------------------------------|-----------|-----------|----------|-------|-----------|----------|-------|-----------|-----------|-------|
| Normal | 8 (36.4) | 12 (54.5) | 2 (9.1) | 1 | 20 (90.9) | 2 (9.1) | 0.315 | 14 (63.6) | 8 (36.4) | 0.872 |
| Decreased | 9 (40.9) | 11 (50.0) | 2 (9.1) | | 20 (90.9) | 2 (9.1) | | 13 (59.1) | 9 (40.9) | |
| Increased | 0 | 1 (100.0) | 0 | | 1 (100.0) | 0 | | 1 (100.0) | 0 | |
| Mean corpuscular volume | | | | | | | | | | |
| Normal | 21 (38.9) | 28 (51.9) | 5 (9.3) | 0.689 | 49 (90.7) | 5 (9.3) | 0.108 | 33 (61.1) | 21 (38.9) | 0.074 |
| Decreased | 4 (18.2) | 12 (54.5) | 6 (27.3) | | 16 (72.7) | 6 (27.3) | | 18 (81.8) | 4 (18.2) | |
| Increased | 3 (100.0) | 0 | 0 | | 3 (100.0) | 0 | | 0 | 3 (100.0) | |

Table VII. *EPCR* c.6936A>G genotype distribution among clinical characteristics.

| Characteristics | <i>EPCR</i> c.6936A>G | | | <i>p</i> value | Recessive model | | <i>p</i> value | Dominant model | | <i>p</i> value |
|--------------------------------|-----------------------|-----------|-----------|----------------|-----------------|-----------|----------------|----------------|----------|----------------|
| | AA | AG | GG | | AG+AA | GG | | AG+GG | AA | |
| | N (%) | N (%) | N (%) | | N (%) | N (%) | | N (%) | N (%) | |
| Venous thrombosis | | | | | | | | | | |
| - | 9 (14.5) | 41 (66.1) | 12 (19.4) | 0.780 | 50 (80.6) | 12 (19.4) | 0.475 | 53 (85.5) | 9 (14.5) | 0.655 |
| + | 2 (20.0) | 7 (70.0) | 1 (10.0) | | 9 (90.0) | 1 (10.0) | | 8 (80.0) | 2 (20.0) | |
| Arterial thrombosis | | | | | | | | | | |
| - | 7 (14.9) | 33 (70.2) | 7 (14.9) | 0.604 | 40 (85.1) | 7 (14.9) | 0.515 | 40 (85.1) | 7 (14.9) | 0.515 |
| + | 6 (20.7) | 17 (58.6) | 6 (20.7) | | 23 (79.3) | 6 (20.7) | | 23 (79.3) | 6 (20.7) | |
| Incidence of thrombosis | | | | | | | | | | |
| - | 7 (13.5) | 37 (71.2) | 8 (15.4) | 0.772 | 44 (84.6) | 8 (15.4) | 0.619 | 45 (86.5) | 7 (13.5) | 0.677 |
| + | 6 (16.7) | 23 (63.9) | 7 (19.4) | | 7 (19.4) | 29 (80.6) | | 30 (83.3) | 6 (16.7) | |

| | | | | | | | | | | |
|--------------------------------|----------|-----------|-----------|-------|-----------|-----------|-------|-----------|----------|-------|
| Mean platelet volume | | | | | | | | | | |
| Normal | 3 (13.6) | 18 (81.8) | 1 (4.5) | 0.529 | 21 (95.5) | 1 (4.5) | 0.299 | 19 (86.4) | 3 (13.6) | 0.934 |
| Decreased | 4 (18.2) | 12 (54.5) | 6 (27.3) | | 16 (72.7) | 6 (27.3) | | 18 (81.8) | 4 (18.2) | |
| Increased | 0 | 1 (100.0) | 0 | | 1 (100.0) | 0 | | 1 (100.0) | 0 | |
| Mean corpuscular volume | | | | | | | | | | |
| Normal | 5 (9.3) | 39 (72.2) | 10 (18.5) | 0.127 | 44 (81.5) | 10 (18.5) | 0.301 | 49 (90.7) | 5 (9.3) | 0.096 |
| Decreased | 7 (31.8) | 13 (59.1) | 2 (9.1) | | 20 (90.9) | 2 (9.1) | | 15 (68.2) | 7 (31.8) | |
| Increased | 0 | 3 (100.0) | 0 | | 3 (100.0) | 0 | | 3 (100.0) | 0 | |

Table VIII. *PROC* c.565C>T genotype distribution among clinical characteristics.

| Characteristics | <i>PROC</i> c.565C>T | | | <i>p</i> value | Recessive model | | <i>p</i> value | Dominant model | | <i>p</i> value |
|--------------------------------|----------------------|-----------|-----------|----------------|-----------------|-----------|----------------|----------------|-----------|----------------|
| | CC | CT | TT | | CT+CC | TT | | CT+TT | CC | |
| | N (%) | N (%) | N (%) | | N (%) | N (%) | | N (%) | N (%) | |
| Venous thrombosis | | | | | | | | | | |
| - | 28 (45.2) | 19 (30.6) | 15 (24.2) | 0.555 | 47 (75.8) | 15 (24.2) | 0.316 | 34 (54.8) | 28 (45.2) | 0.383 |
| + | 6 (60.0) | 3 (30.0) | 1 (10.0) | | 9 (90.0) | 1 (10.0) | | 4 (40.0) | 6 (60.0) | |
| Arterial thrombosis | | | | | | | | | | |
| - | 21 (44.7) | 14 (29.8) | 12 (25.5) | 0.387 | 35 (74.5) | 12 (25.5) | 0.223 | 26 (55.3) | 21 (44.7) | 0.238 |
| + | 17 (58.6) | 8 (27.6) | 4 (13.8) | | 25 (86.2) | 4 (13.8) | | 12 (41.4) | 17 (58.6) | |
| Incidence of thrombosis | | | | | | | | | | |
| - | 23 (44.2) | 15 (28.8) | 14 (26.9) | 0.282 | 38 (73.1) | 14 (26.9) | 0.144 | 29 (55.8) | 23 (44.2) | 0.193 |
| + | 21 (58.3) | 10 (27.8) | 5 (13.9) | | 31 (86.1) | 5 (13.9) | | 15 (41.7) | 21 (58.3) | |

| | | | | | | | | | | |
|--------------------------------|-----------|-----------|-----------|-------|-----------|-----------|-------|-----------|-----------|-------|
| Mean platelet volume | | | | | | | | | | |
| Normal | 13 (59.1) | 6 (27.3) | 3 (13.6) | 0.834 | 19 (86.4) | 3 (13.6) | 0.552 | 9 (40.9) | 13 (59.1) | 0.555 |
| Decreased | 11 (50.0) | 7 (31.8) | 4 (18.2) | | 18 (81.8) | 4 (18.2) | | 11 (50.0) | 11 (50.0) | |
| Increased | 1 (100.0) | 0 | 0 | | 1 (100.0) | 0 | | 0 | 1 (100.0) | |
| Mean corpuscular volume | | | | | | | | | | |
| Normal | 26 (48.1) | 16 (29.6) | 12 (22.2) | 0.380 | 42 (77.8) | 12 (22.2) | 0.223 | 28 (51.9) | 26 (48.1) | 0.756 |
| Decreased | 11 (50.0) | 8 (36.4) | 3 (13.6) | | 19 (86.4) | 3 (13.6) | | 11 (50.0) | 11 (50.0) | |
| Increased | 1 (33.3) | 0 | 2 (66.7) | | 1 (33.3) | 2 (66.7) | | 2 (66.7) | 1 (33.3) | |

Table IX. *MTHFR* c.677C>T genotype distribution among clinical characteristics.

| Characteristics | <i>MTHFR</i> c.677C>T | | | <i>p</i> value | Recessive model | | <i>p</i> value | Dominant model | | <i>p</i> value |
|--------------------------------|-----------------------|-----------|----------|----------------|-----------------|----------|----------------|----------------|-----------|----------------|
| | CC | CT | TT | | CT+CC | TT | | CT+TT | CC | |
| | N (%) | N (%) | N (%) | | N (%) | N (%) | | N (%) | N (%) | |
| Venous thrombosis | | | | | | | | | | |
| - | 30 (48.4) | 28 (45.2) | 4 (6.5) | 0.675 | 58 (93.5) | 4 (6.5) | 0.682 | 32 (51.6) | 30 (48.4) | 0.279 |
| + | 3 (30.0) | 6 (60.0) | 1 (10.0) | | 9 (90.0) | 1 (10.0) | | 7 (70.0) | 3 (30.0) | |
| Arterial thrombosis | | | | | | | | | | |
| - | 26 (55.3) | 19 (40.4) | 2 (4.3) | 0.629 | 45 (95.7) | 2 (4.3) | 0.616 | 21 (44.7) | 26 (55.3) | 0.374 |
| + | 13 (44.8) | 14 (48.3) | 2 (6.9) | | 27 (93.1) | 2 (6.9) | | 16 (55.2) | 13 (44.8) | |
| Incidence of thrombosis | | | | | | | | | | |
| - | 31 (59.6) | 18 (34.6) | 3 (5.8) | 0.170 | 49 (94.2) | 3 (5.8) | 0.639 | 21 (40.4) | 31 (59.6) | 0.056 |
| + | 14 (38.9) | 19 (52.8) | 3 (8.3) | | 33 (91.7) | 3 (8.3) | | 22 (61.1) | 25 (38.9) | |

| | | | | | | | | | | |
|--------------------------------|-----------|-----------|---------|-------|-----------|---------|-------|-----------|-----------|-------|
| Mean platelet volume | | | | | | | | | | |
| Normal | 8 (36.4) | 13 (59.1) | 1 (4.5) | 0.427 | 21 (95.5) | 1 (4.5) | 1 | 14 (63.6) | 8 (36.4) | 0.272 |
| Decreased | 13 (59.1) | 7 (31.8) | 2 (9.1) | | 20 (90.9) | 2 (9.1) | | 9 (40.9) | 13 (59.1) | |
| Increased | 0 | 1 (100.0) | 0 | | 1 (100.0) | 0 | | 1 (100.0) | 0 | |
| Mean corpuscular volume | | | | | | | | | | |
| Normal | 26 (48.1) | 23 (42.6) | 5 (9.3) | 0.872 | 49 (90.7) | 5 (9.3) | 0.656 | 28 (51.9) | 26 (48.1) | 0.714 |
| Decreased | 11 (50.0) | 10 (45.5) | 1 (4.5) | | 21 (95.5) | 1 (4.5) | | 11 (50.0) | 11 (50.0) | |
| Increased | 2 (66.7) | 1 (33.3) | 0 | | 3 (100.0) | 0 | | 1 (33.3) | 2 (66.7) | |

Table X. *MTHFR* c.1298A>C genotype distribution among clinical characteristics.

| Characteristics | <i>MTHFR</i> c.1298A>C | | | <i>p</i> value | Recessive model | | <i>p</i> value | Dominant model | | <i>p</i> value |
|--------------------------------|------------------------|-----------|-----------|----------------|-----------------|-----------|----------------|----------------|-----------|----------------|
| | AA | AC | CC | | AC+AA | CC | | AC+CC | AA | |
| | N (%) | N (%) | N (%) | | N (%) | N (%) | | N (%) | N (%) | |
| Venous thrombosis | | | | | | | | | | |
| - | 27 (43.5) | 27 (43.5) | 8 (12.9) | 0.019 | 54 (87.1) | 8 (12.9) | 0.163 | 35 (56.5) | 27 (43.5) | 0.120 |
| + | 7 (70.0) | 0 | 3 (30.0) | | 7 (70.0) | 3 (30.0) | | 3 (30.0) | 7 (70.0) | |
| Arterial thrombosis | | | | | | | | | | |
| - | 20 (42.6) | 19 (40.4) | 8 (17.0) | 0.995 | 39 (83.0) | 8 (17.0) | 0.980 | 27 (57.4) | 20 (42.6) | 0.920 |
| + | 12 (41.4) | 12 (41.4) | 5 (17.2) | | 24 (82.8) | 5 (17.2) | | 17 (58.6) | 12 (41.4) | |
| Incidence of thrombosis | | | | | | | | | | |
| - | 19 (36.5) | 22 (42.3) | 11 (21.2) | 0.453 | 41 (78.8) | 11 (21.2) | 0.600 | 33 (63.5) | 19 (36.5) | 0.208 |
| + | 18 (50.0) | 12 (33.3) | 6 (16.7) | | 30 (83.3) | 6 (16.7) | | 18 (50.0) | 18 (50.0) | |

| | | | | | | | | | | |
|--------------------------------|-----------|-----------|-----------|-------|-----------|-----------|-------|-----------|-----------|-------|
| Mean platelet volume | | | | | | | | | | |
| Normal | 11 (50.0) | 9 (40.9) | 2 (9.1) | 0.157 | 20 (90.9) | 2 (9.1) | 0.116 | 11 (50.0) | 11 (50.0) | 0.533 |
| Decreased | 9 (40.9) | 5 (22.7) | 8 (36.4) | | 14 (63.6) | 8 (36.4) | | 13 (59.1) | 9 (40.9) | |
| Increased | 1 (100.0) | 0 | 0 | | 1 (100.0) | 0 | | 0 | 1 (100.0) | |
| Mean corpuscular volume | | | | | | | | | | |
| Normal | 24 (44.4) | 19 (35.2) | 11 (20.4) | 0.813 | 43 (79.6) | 11 (20.4) | 0.966 | 30 (55.6) | 24 (44.4) | 0.674 |
| Decreased | 10 (45.5) | 8 (36.4) | 4 (18.2) | | 18 (81.8) | 4 (18.2) | | 12 (54.5) | 10 (45.5) | |
| Increased | 1 (33.3) | 2 (66.7) | 0 | | 3 (100.0) | 0 | | 2 (66.7) | 1 (33.3) | |

Table XI. *MS c.2756A>G* genotype distribution among clinical characteristics.

| Characteristics | <i>MS c.2756A>G</i> | | | <i>p</i> value | Recessive model | | <i>p</i> value | Dominant model | | <i>p</i> value |
|--------------------------------|------------------------|-----------|---------|----------------|-----------------|---------|----------------|----------------|-----------|----------------|
| | AA | AG | GG | | AG+AA | GG | | AG+CC | GG | |
| | N (%) | N (%) | N (%) | | N (%) | N (%) | | N (%) | N (%) | |
| Venous thrombosis | | | | | | | | | | |
| - | 37 (59.7) | 22 (35.5) | 3 (4.8) | 0.505 | 59 (95.2) | 3 (4.8) | 0.477 | 25 (40.3) | 37 (59.7) | 0.218 |
| + | 8 (80.0) | 2 (20.0) | 0 | | 10 (100.0) | 0 | | 2 (20.0) | 8 (80.0) | |
| Arterial thrombosis | | | | | | | | | | |
| - | 27 (57.4) | 18 (38.3) | 2 (4.3) | 0.833 | 45 (95.7) | 2 (4.3) | 0.861 | 20 (42.6) | 27 (57.4) | 0.484 |
| + | 19 (65.5) | 9 (31.0) | 1 (3.4) | | 28 (96.6) | 1 (3.4) | | 10 (34.5) | 19 (65.5) | |
| Incidence of thrombosis | | | | | | | | | | |
| - | 30 (57.7) | 19 (36.5) | 2 (5.8) | 0.651 | 49 (94.2) | 3 (5.8) | 0.508 | 22 (42.3) | 30 (57.7) | 0.395 |
| + | 24 (66.7) | 11 (30.6) | 1 (2.8) | | 35 (97.2) | 1 (2.8) | | 12 (33.3) | 24 (66.7) | |

| | | | | | | | | | | |
|--------------------------------|-----------|-----------|----------|-------|------------|----------|-------|-----------|-----------|-------|
| Mean platelet volume | | | | | | | | | | |
| Normal | 14 (63.6) | 8 (36.4) | 0 | 0.707 | 22 (100.0) | 0 | 0.622 | 8 (36.4) | 14 (63.6) | 0.728 |
| Decreased | 14 (63.6) | 7 (31.8) | 1 (4.5) | | 21 (95.5) | 1 (4.5) | | 8 (36.4) | 14 (63.6) | |
| Increased | 0 | 1 (100.0) | 0 | | 1 (100.0) | 0 | | 1 (100.0) | 0 | |
| Mean corpuscular volume | | | | | | | | | | |
| Normal | 34 (63.0) | 18 (33.3) | 3 (3.7) | 0.301 | 52 (96.3) | 2 (3.7) | 0.187 | 20 (37.0) | 34 (63.0) | 0.910 |
| Decreased | 12 (54.5) | 9 (40.9) | 1 (4.5) | | 21 (95.5) | 1 (4.5) | | 10 (45.5) | 12 (54.5) | |
| Increased | 2 (66.7) | 0 | 1 (33.3) | | 2 (66.7) | 1 (33.3) | | 1 (33.3) | 2 (66.7) | |

