Classic thrombophilic gene variants

Pier Mannuccio Mannucci1; Massimo Franchini2

1A. Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Ca’ Granda Maggiore Policlinico Hospital Foundation, Milan, Italy; 2Department of Transfusion Medicine and Hematology, Carlo Poma Hospital, Mantova, Italy

Summary
Thrombophilia is defined as a condition predisposing to the development of venous thromboembolism (VTE) on the basis of a hypercoagulable state. Over the past decades, great advances in the pathogenesis of VTE have been made and nowadays it is well established that a thrombophilic state may be associated with acquired and/or inherited factors. The rare loss-of-function mutations of the genes encoding natural anticoagulant proteins (i.e. protein C, protein S and antithrombin) and the more common gain-of-function polymorphisms factor V Leiden and prothrombin G20210A are the main genetic determinants of thrombophilia. In addition, non-O blood group has been consistently demonstrated to be the most frequent inherited marker of an increased risk of VTE. The mechanism role of these inherited thrombophilia markers will be discussed in this narrative review.

Keywords
Inherited thrombophilia, natural anticoagulants, factor V Leiden, prothrombin mutation, ABO blood group

Introduction
Thrombophilia is defined as a hypercoagulable state leading to a thrombotic tendency (1–3). In 1856, Rudolf Virchow conceived the theory of the triad, i.e. vessel wall damage, blood stasis and hypercoagulability, in order to explain the etiology of thrombosis (4). This concept was prophetic, because it is now well established that all the components of the triad play important mechanistic roles in the development of the clinical manifestations of thrombosis. During the past two to three decades, much progress has been made in the identification and characterisation of the cellular and molecular mechanisms that influence the Virchow’s triad. It is now accepted that the combination of stasis and hypercoagulability, much more than vascular endothelial damage, is crucial for the occurrence of venous thromboembolism (VTE), venous thrombi being mainly constituted by fibrin and red blood cells and less by blood platelets. In contrast, platelets are essential for primary haemostasis and play a pivotal role in the development of arterial thrombosis (5).

With this background, the term thrombophilia is now employed to describe a tendency to develop VTE (not arterial thrombosis), owing to abnormalities of blood coagulation that can be inherited, acquired or mixed (both congenital and acquired). Inherited thrombophilias include loss-of-function mutations in the genes that encode the natural anticoagulant proteins antithrombin, protein C and protein S (Figure 1), as well as gain-of-function mutations in the genes encoding factor V (factor V Leiden) and prothrombin (prothrombin G20210A) (6). In addition, consistent data have been accumulated on the association between a very frequent biomarker such as the non-O blood types (i.e. A, B and AB) and VTE (7, 8). The main risk factors of VTE will be discussed in this narrative review, focusing only on established thrombophilic gene variants.

Loss-of-function mechanisms
Antithrombin deficiency
Antithrombin, a single chain glycoprotein member of the serine protease inhibitor (serpin) superfamily synthesised by the liver, is the major inhibitor of coagulation serine proteases, in particular thrombin and activated factor X (FXa) (9, 10). The result of this anticoagulant activity is a reduction in both the generation and half-life of thrombin, the final enzyme of blood coagulation. In addition to the active site responsible for coagulation factor inactivation, the antithrombin molecule contains a heparin-binding site (10). When exogenous heparin or endogenous heparan sulphate bind to this site, the ability of antithrombin to inactivate activated coagulation factors is greatly enhanced. Currently, more than 250 loss-of-function mutations have been identified in the antithrombin gene (SERPIN1) located at chromosome 1q 23–25, including missense and nonsense mutations, insertions and deletions (11, 12). Most mutations, that lead to a reduction of plasma antithrombin levels or to a decreased ability of this anticoagulant protein to interact with the activated coagulation factors or heparin, lead to an increased risk of VTE (13).

Antithrombin deficiency is transmitted as an autosomal dominant trait and the penetrance of this disease is very high, since most affected family members experience a thrombotic event by the age of 45–50 years (14). In the general population, the
estimated prevalence of antithrombin deficiency is extremely low, ranging between 0.02 and 0.2%, and in unselected patients with VTE is around 1% (15–17) (Table 1). This deficiency is the most severe among the inherited thrombophilias, causing more than 50-fold increased risk for VTE compared to that of individuals not carrying this defect (12).

On the basis of functional and immunological assays, two types of antithrombin deficiency can be distinguished. Type I, due to a wide variety of DNA mutations, is a quantitative defect characterised by reduced functional and antigen levels; type II, due to missense mutations, is a qualitative defect characterised by normal antithrombin antigen with an impaired inhibitory activity due to the production of a variant protein (18). Type I is associated clinically with premature recurrent VTE, whereas in type II the risk of thrombosis is closely related to the position of the amino acid substitution within the protein. Thus, carriers of heterozygous mutations within the heparin binding domain of antithrombin have a low or absent risk of thrombosis, in variance with those with mutations at or close to the reactive site of the protein (3, 5). The only individuals homozygous for antithrombin deficiency described so far carry heparin-binding site defects, suggesting that the other subtypes are associated with embryonic lethality (19).

**Protein C deficiency**

Protein C, described for the first time in 1979 (20), is a vitamin-K–dependent glycoprotein synthesised in the liver in an inactive form. Protein C is activated by thrombin and this process is accelerated by the complex formed by thrombin with the endothelial protein C receptor (EPCR) and thrombomodulin. Together with protein S, activated protein C (APC) quenches thrombin generation by inactivating activated coagulation factors V (FVa) and VIII (FVIIIa).

Inherited protein C deficiency is transmitted as a dominant autosomal trait (21, 22) and more than 200 loss-of-function mutations in the protein C gene (PROC), located at chromosome 2q13-q14, have been reported (23). Protein C deficiency is very rare in the general population (around 0.2%) and its frequency in unselected patients with VTE is 3% (17) (Table 1). The penetrance of the disease is lower than that of antithrombin deficiency, and heterozygotes have a 15-fold increased risk for premature VTE compared to the general population (12, 21). Homozygotes have a more severe clinical picture, not infrequently leading to neonatal purpura fulminans, a potentially fatal condition characterised by microvascular thrombosis and skin necrosis (17).

Protein C deficiency is classified on the basis of the plasma levels of the enzymatic activity and antigen and, similarly to antithrombin deficiency, can be divided in two subtypes. Type I, the most common, is characterised by a parallel reduction in plasma antigen and activity, reflecting a reduced synthesis of a functional protein. The rarer type II is characterised by normal antigen reduced functional activity, reflecting normal synthesis of a dysfunctional protein. In type I, the majority of mutations are of the missense variety, leading to premature termination of synthesis or disruption of protein folding, deletions and insertions occurring with a much lower frequency (~10%). In type II deficiency, missense

![Figure 1: Simplified scheme of the coagulation and anticoagulation system. TF, tissue factor; AT, antithrombin; PC, protein C; APC, activated protein C; PS, protein S.](image-url)
mutations are located mainly in the γ-carboxyglutamic acid and protease domains (23, 24).

Protein S deficiency

Protein S, which derives its name from the city of Seattle where it was discovered (25), is a vitamin-K-dependent protein of liver synthesis circulating in plasma in a free functionally active form (approximately 40%) and an inactive form bound to the C4b-binding complement protein (approximately 60%) (26). Protein S functions as a cofactor of APC for the degradation of activated factors Va and VIIIa and as a cofactor of tissue factor pathway inhibitor in the inhibition of factor Xa (26).

Inherited protein S deficiency is transmitted as an autosomal dominant trait and its gene (PROS1) is located at 3q11.2 chromosome. Almost 200 loss-of-function mutations have been identified in the PROS1 gene so far, the majority being missense mutations or short deletions or insertions (27), although large deletions were relatively common in recent studies (28–30). The prevalence of protein S deficiency in the general population is estimated at 0.03–0.1%, while its prevalence in patients with VTE is around 2%, similar to that of protein C deficiency (Table 1) (17). Inherited protein S deficiency has a clinical presentation very similar to that observed for protein C deficiency (25). Thus, heterozygotes experience early and recurrent episodes of VTE and sometimes skin necrosis, while the very rare homozygotes exhibit a more severe clinical picture with neonatal purpura fulminans. The risk of VTE is 10-fold higher in carriers than in non-carriers. Three types of protein S defects have been described: type I is a qualitative deficiency with decreased plasma levels of functional and immunoreactive total and free protein S; type II is a qualitative deficiency with decreased cofactor activity but normal total and free protein S levels; type III is a quantitative deficiency, with reduced functional activity and free protein S antigen levels but normal total protein S levels (12).

Gain-of-function mechanisms

Factor V Leiden

In 1993, a poor anticoagulant response to APC was associated with an increased risk of VTE (31, 32). The following year, a gain-of-function mutation in the F5 gene (chromosome 1q23) was first described in the city of Leiden (33, 34) as being responsible for the majority of cases of APC resistance. The factor V Leiden mutation, which results from a substitution of adenine to guanine at the 1691 position (G1691A) in exon 10 of the F5 gene, is located in the part of the gene encoding one of the cleavage sites of the factor (Arg506) involved in the inactivation of activated factor V (6). As a consequence, the mutant activated factor V molecule (factor V Leiden) is resistant to proteolytic inactivation by APC and retains full procoagulant activity (33). Another point mutation in the F5 gene occurs at Arg306, another APC cleavage site, and the resulting factor V variants (factor V Hong Kong and factor V Cambridge) cause varying degrees of APC resistance, intermediate between wild-type factor V and factor V Leiden, and are usually associated with a lower VTE risk than that of factor V Leiden (35).

The factor V Leiden mutation has a dominant autosomal transmission and in heterozygosis is the most common prothrombotic gene mutation in the Caucasian population, with a prevalence of about 5%, ranging from 2-10% with a positive gradient from Southern to Northern Europe (36). This prevalence rises up to 20% in non-selected VTE patients. The risk of VTE is increased approximately seven-fold in heterozygous and 80-fold in homozygous carriers of the mutation compared to non-carriers (Table 1) (37, 38). Homozygosity for factor V Leiden mutation occurs in approximately in one in 1,000 individuals in the general population and in 1% of non-selected patients with VTE.

Prothrombin G20210A

This mutation in the prothrombin gene (located on chromosome 11p11-q12), first described in 1996 by the Leiden group (39), consists of a guanine to adenine substitution at the nucleotide 20210 within the 3’ untranslated region of the gene that results in an approximately 30% increase of prothrombin plasma levels (39). Like factor V Leiden, prothrombin G20210A has a dominant autosomal transmission and is the second most common thrombophilia abnormality, with a prevalence of heterozygotes in populations of Caucasian origin of 2–3%, that increases to 6% in patients with VTE (Table 1) (40). The mutation is more common in Southern than in Northern Europe, with a gradient opposite to that of factor V Leiden. Heterozygosity for prothrombin G20210A mutation confers an approximately three- to four-fold increased risk of developing VTE. Thus these individuals exhibit a relatively low thrombotic risk, and most of them will not develop a premature thrombotic episode. In contrast, homozygosity for the prothrombin gene mutation which is much rarer and occurs in four out of

Table 1: Prevalence of thrombophilia abnormalities and relative risk of venous thromboembolism (VTE).

<table>
<thead>
<tr>
<th>Thrombophilia abnormality</th>
<th>Prevalence (%)</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General population</td>
<td>Patients with VTE</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>0.02–0.2</td>
<td>1</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>0.2–0.4</td>
<td>3</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>0.03–0.1</td>
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<tr>
<td>Factor V Leiden (heterozygous)</td>
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<td>20</td>
</tr>
<tr>
<td>Factor V Leiden (homozygous)</td>
<td>0.02</td>
<td>1.5</td>
</tr>
<tr>
<td>Prothrombin G20210A (heterozygous)</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Prothrombin G20210A (homozygous)</td>
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<td>&lt;1</td>
</tr>
<tr>
<td>Non-O blood group</td>
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</tbody>
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10,000 people, causes an approximately 30-fold increased VTE risk. The risk of VTE recurrence is similar to that of factor V Leiden (1.4-fold) (12).

Conclusions

Our knowledge on the genetic basis of thrombophilia has dramatically improved in the last few decades, leading to a much improved understanding of the mechanisms of VTE. All in all, inherited thrombophilia helps to explain the pathogenesis in approximately 40% of VTE episodes, and the contribution of each different abnormality varies greatly according to their different frequency and clinical penetrance (57). Thus, while the rarest abnormalities (natural anticoagulant deficiencies, homozygous factor V Leiden and prothrombin G20210A, combined defects) result in a severe thrombophilia phenotype, other more common genetic variants (non-O blood type, heterozygous factor V Leiden and prothrombin G20210A) are associated with a smaller risk of VTE, that often develops only in association with triggering factors. Considering that thrombophilic abnormalities are stronger risk factors for primary than for secondary (recurrent) thrombosis (Table 1), screening of asymptomatic relatives of patients with severe thrombophilia may be useful. Furthermore, because of the multifactorial pathogenesis of VTE, the assessment of the thrombotic risk should be individualised, being the result of the additive (or supra-additive) effect of inherited and acquired risk factors (i.e. pregnancy, oral contraceptive, confinement to bed, trauma, surgery, cancer, inflammatory states). Most importantly, patients’ age should be taken into strong account, as the thromboembolic risk dramatically increases during the decades of life. Finally, some believe that gain-of-function polymorphisms (i.e. A and B antigens, factor V Leiden and prothrombin G20210A), by inducing a greater propensity toward blood clot formation and a lower risk of bleeding, may have conferred a survival advantage to early humans (58).

Conflicts of interest

None declared.

References


