

# Role of the geneticist in testing and counseling for inherited thrombophilia

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Within the past decade, the identification of two mutations that are relatively prevalent among the white population (the factor V Leiden and prothrombin G20210A gene mutations) has paved the way for a number of large cohort studies that have greatly advanced our understanding of the pathogenesis of venous thromboembolism (VTE). VTE is clearly a multigenic disorder, with well-characterized examples of gene-gene and gene-environment interactions underlying its pathogenesis. Increasing numbers of patients are being referred for testing, and many more diagnoses of inherited thrombophilia are being made. The purpose of this article is to discuss the practical applications of both diagnostic testing and genetic counseling for the major inherited thrombophilias: inherited resistance to activated protein C/factor V Leiden, prothrombin G20210A mutation, protein C deficiency, protein S deficiency, and antithrombin deficiency. A description of each entity is included along with a discussion of the indications for testing, selection of the most appropriate screening test, and proper interpretation of test results. Informed consent for testing, screening of asymptomatic individuals in special circumstances (such as during pregnancy or before initiation of estrogen therapy), screening of family members, and posttest education are also addressed. This article emphasizes that these polymorphisms should be regarded as risk factors for thrombosis whose clinical expression generally depends on the coexistence of additional thrombophilic mutations or environmental conditions that provoke the development of VTE. *Genet Med* 2003;5(3):133-143.

The practice of medical genetics has evolved from focusing on rare, single-gene disorders to including more prevalent, multigenic disorders. The geneticist may be called upon to participate in the diagnosis and management of individuals with relatively common multigenic disorders or to advise on testing or counseling of at-risk family members. Within the realm of vascular medicine, venous thromboembolism (VTE) is one such multigenic disorder that often triggers a consultation for both laboratory evaluation and interpretation as well as genetic counseling services.

The term venous thromboembolism is used to include both deep venous thrombosis and pulmonary embolism because, from a pathophysiological standpoint, these can be viewed as one and the same disorder. Venous thrombi are composed of fibrin with relatively few platelets and are most often formed in areas of venostasis, such as adjacent to valves. In contrast, the pathogenesis of arterial thrombotic events, including myocardial infarction and stroke, is quite distinct. Testing for inher-

ited thrombophilia is rarely indicated or useful in arterial thrombosis, and relatively little attention will be focused on these disorders in this review.

It is now well accepted that the interplay of inherited or acquired hematologic risk factors or thrombophilias (also referred to as “hypercoagulable states” or “prothrombotic disorders”) with environmental risk factors and life events often underlies the pathogenesis of VTE. Within the past decade, dramatic progress has been made in the ability to identify one or more inherited thrombophilic disorders in probands presenting with VTE, thanks largely to the discovery of two relatively common polymorphisms, namely inherited resistance to activated protein C (APC) (caused in 90–95% of cases by the factor V Leiden allele) and the G20210A mutation in the prothrombin gene. Because of their relatively high prevalence, population-based studies that were not previously feasible have now defined the magnitude of the “baseline” VTE risk for affected heterozygotes and homozygotes as well as the magnitude of the interaction with other inherited thrombophilias and environmental risk factors. However, the appropriate application of laboratory testing, including the reasons for testing, the impact (both positive and negative) on the patient and his or her family members, and the optimal approach to genetic counseling that should ensue after identification of a thrombophilic disorder remain controversial.

The purpose of this review is not primarily to focus on the epidemiology or pathogenesis of VTE, topics which have been extensively reviewed elsewhere<sup>1-3</sup>, but rather to review some of

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the more practical aspects of testing and counseling for thrombophilic disorders, with an emphasis on the heritable forms. The reader is also referred to the recently published proceedings of the *College of American Pathologists Consensus Conference XXXVI: Diagnostic Issues in Thrombophilia*,<sup>4</sup> in which a panel of clinical and laboratory experts systematically reviewed the evidence for and against testing for both inherited and acquired thrombophilias. In this publication, consensus recommendations were made regarding the target populations that should be tested, which tests should be performed, and the overall rationale for testing. Details concerning technical aspects of the assays and a critical comparison of the available laboratory methodologies are also included in this comprehensive document. In addition, a more focused consensus statement on testing for the factor V Leiden mutation (with slightly differing conclusions) was published on behalf of the American College of Medical Genetics (ACMG) in 2001.<sup>5</sup>

## EPIDEMIOLOGY OF VTE

The annual incidence of VTE is approximately 0.1%, or 117 per 100,000 persons, similar to the annual incidence of stroke.<sup>6</sup> However, there is significant age dependency, with the burden of disease occurring in the older age group. Thus, the risk of thrombosis before age 40 is approximately 1 in 10,000 persons per year, rising to 1 in 100 persons per year after age 75.<sup>7</sup>

Not only is VTE a relatively prevalent disease, it is also associated with significant morbidity and mortality. For example, of the estimated 600,000 cases of pulmonary emboli in the United States per annum, about 100,000 will result in death.<sup>6,8</sup> In about one half of individuals in whom pulmonary embolism is a direct or contributing cause of death, the diagnosis was not suspected antemortem<sup>9</sup> because of an under-appreciation of the magnitude of this problem combined with its frequently subtle clinical manifestations. Many hospitalized patients on surgical and medical floors are probably at increased risk of VTE. This risk is further magnified in the presence of inherited thrombophilic disorders. Increasingly, the benefit of prophylactic low-dose anticoagulation is being demonstrated in many of these patient populations.<sup>10</sup>

Standard therapy for established VTE is several days of anticoagulation with rapid-acting heparin or low-molecular-weight heparin, followed by more prolonged anticoagulation with the oral anticoagulant warfarin. It should be noted that the goal of this therapy is to prevent extension and embolization of further clot rather than to achieve lysis of the existing thrombus. Unfortunately, up to 60% of individuals with deep venous thrombosis of the lower extremities are destined to suffer the long-term, and frequently underestimated, effects of the postphlebotic syndrome.<sup>11</sup> This syndrome is caused by venous hypertension and valvular insufficiency, which (respectively) result from chronic vessel obstruction and valve destruction by organized thrombus. These patients experience chronic lower extremity edema and pain and less commonly skin breakdown and may develop infected, nonhealing ulcers.<sup>2</sup> Whether the dissolution of freshly formed deep vein thrombus

by the use of early thrombolytic agents is capable of reducing the long-term development of the postthrombotic syndrome is an intriguing, although so far unproven, hypothesis.

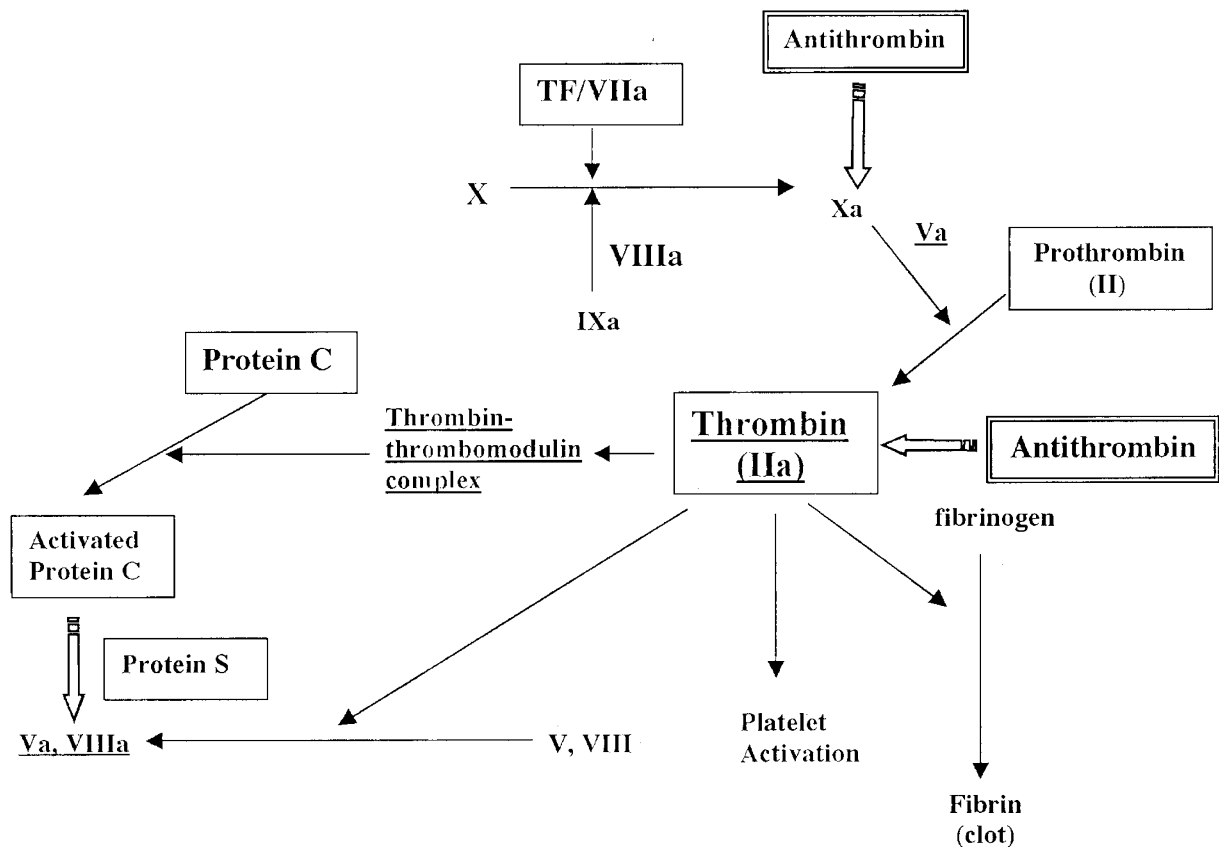
## HOW DO INHERITED THROMBOPHILIAS PREDISPOSE TO VTE?

Although the concept of separate “intrinsic” and “extrinsic” pathways to initiate coagulation helps to explain the prothrombin time (PT) and activated partial thromboplastin time (APTT) clotting tests *in vitro*, there exists only a single pathway to initiate coagulation *in vivo*. Specifically, the interaction of factor VII(a) with tissue factor (corresponding to the old extrinsic pathway) leads to the activation of factors IX and X and ultimately to the generation of thrombin. Not only does thrombin cleave fibrinogen to form insoluble fibrin strands in the final steps of the coagulation pathway, it also activates platelets and amplifies coagulation by proteolytically activating factors V and VIII. Regulatory proteins, such as proteins C and S and antithrombin, help to check this feedback loop through inactivation of procoagulant factors. For a schematic of the coagulation pathway, see Figure 1.

Clinical outcomes, such as thrombus formation or disseminated intravascular coagulation (DIC), are dependent on the magnitude and rate of thrombin generation. This, in turn, is a function of the magnitude and characteristics of the inciting stimulus, the relative balance of pro- and anticoagulant activities, the degree of inherent thrombolytic activity, and the presence of vascular stasis. Although thrombin generation occurs on a continual on-going basis *in vivo*, it usually does not result in thrombus formation. Inherited thrombophilias may, however, tip the balance in favor of thrombosis, either because of a partial deficiency of a regulatory anticoagulant protein (antithrombin, protein C, or protein S), dysfunction of an existing anticoagulant mechanism (APC resistance), or “gain of function” mutations that lead to elevated levels of procoagulants (elevated prothrombin levels).

The first thrombophilic disorder to be recognized was antithrombin (III) [AT(III)] deficiency, described by Egeberg<sup>12</sup> in a large Norwegian kindred in 1965. Antithrombin is a 58 kD member of the serine protease inhibitor (or SERPIN) family that is synthesized in the liver as a 432 amino acid glycoprotein. The antithrombin gene is located on chromosome 1. The physiologic function of antithrombin, namely irreversible inactivation of thrombin and several other serine proteases (most notably factor Xa) (Fig. 2), is enhanced approximately 1000-fold by pharmacologic doses of its cofactor heparin or the endogenous counterpart, endothelial-bound heparan-sulfate moieties.

The second major regulatory pathway for containment of thrombin generation is the protein C/protein S pathway (Fig. 3). Protein C is proteolytically activated to the serine protease APC by thrombin bound to its endothelial cofactor protein, thrombomodulin. In the presence of its cofactor, protein S, APC down-regulates further thrombin generation by proteolytic inactivation of clotting factors Va (primarily at Arg 506



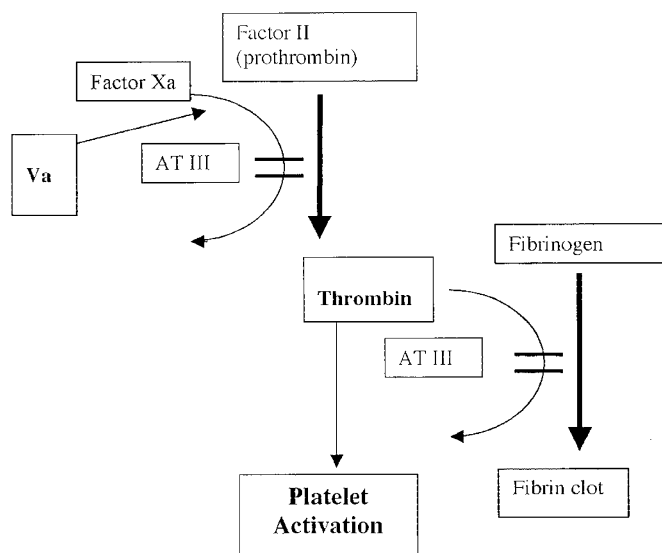
**Fig. 1** Overview Of the coagulation system. Activated forms of the coagulation proteins are designated by the suffix a. Coagulation is initiated by the contact of factor VIIa with tissue factor (TF). The TF/VIIa complex activates factor X both directly and indirectly by way of activation of factor IX (not shown). Ultimately, thrombin is formed. Thrombin possesses several procoagulant activities, but it can be inactivated directly by antithrombin or by binding to endothelial-bound thrombomodulin. The latter complex is responsible for activating the natural anticoagulant, protein C. Specific details of the two major anticoagulant pathways (the antithrombin and protein C/protein S pathways) are dealt with in greater detail in Figures 2 and 3.

and to a lesser extent Arg 306 and Arg 679) and VIIIa. Both protein C and protein S are synthesized by hepatocytes and require a specific vitamin-K-dependent posttranslational modification step for full functional activity ( $\gamma$ -carboxylation of multiple glutamic acid residues in the membrane-binding Gla domain). The gene for the 62 kD zymogen protein C is located on chromosome 2, whereas in the case of protein S, an active gene and a pseudogene are located on chromosome 3. Heterozygous deficiency states for proteins C and S associated with familial VTE were first described in 1981 and 1984, respectively.<sup>13,14</sup> It was subsequently recognized that homozygous deficiency of either protein is associated with severe thrombosis in the form of neonatal purpura fulminans.

Thus, until the discovery of a novel inherited thrombophilia in 1993, all described inherited thrombophilias were caused by the loss of function of regulatory proteins. In a landmark publication that year, Dahlback et al.<sup>15</sup> described a Swedish kindred with inherited resistance to APC, as defined by the inability of exogenously added APC to produce the expected prolongation of the APTT of affected family members. It was quickly realized that inherited resistance to APC is a prevalent disorder, affecting 5 to 6% of the Northern European population, up to 20% of "all comers" with VTE, and up to 50% of a more selected population of "thrombophilic" individuals with

early-onset VTE or a positive family history of VTE.<sup>16</sup> Bertina and colleagues<sup>17</sup> at the University of Leiden demonstrated that 90 to 95% of individuals with the APC resistance phenotype have a polymorphism as a consequence of a G1691A nucleotide change in the factor V gene, leading to an Arg506Gln substitution in the factor V molecule, also known as factor V Leiden. As expected, this critical amino acid substitution retards the inactivation of factor Va by APC, thus leading to a net increase in thrombin generation and providing a plausible biological mechanism to explain the thrombotic propensity of affected individuals.

Gain of function polymorphisms in procoagulant proteins are probably relatively common, although the only one to be well characterized thus far is the G to A nucleotide substitution at base pair 20210 in the 3' untranslated region of the prothrombin gene. This mutation results in increased recognition of the 3' end-cleavage signal and increased 3' end processing, with resultant accumulation of mRNA<sup>18</sup> and plasma prothrombin levels that are approximately 30% higher than mean normal levels in affected heterozygotes and about 70% higher in affected homozygotes.<sup>19</sup> The Leiden Thrombophilia Study has demonstrated that elevated plasma levels of factors VIII, IX, XI, and thrombin-activatable fibrinolysis inhibitor (TAFI) are also risk factors for VTE.<sup>20</sup> The longitudinal investigation



**Fig. 2** Anticoagulant activities of antithrombin. Activated factor X (Xa), in the presence of its cofactor, factor Va, catalyzes the conversion of prothrombin to thrombin. Thrombin then enzymatically converts fibrinogen to fibrin. Antithrombin covalently binds to and inactivates both factor Xa and thrombin.

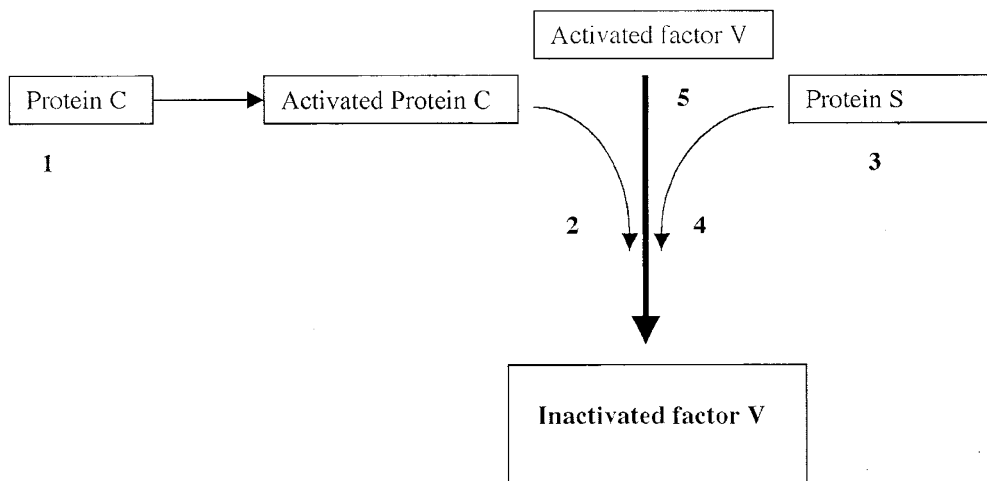
of thromboembolism etiology (LITE) study has similarly provided evidence that elevated plasma factor VII levels may be a risk factor for VTE.<sup>21</sup> However, despite the fact that a familial association has been reasonably well established for some of these (e.g., elevated factor VIII levels), so far there are no candidate polymorphisms to explain the observed phenotypes. For this reason, as well as some additional confounding assay and sample variables, most thrombosis experts do not currently recommend routine assay of these hemostatic factors, with the exception of the prothrombin G20210A polymorphism.

Finally, mild to moderate degrees of hyperhomocyst(e)inemia are postulated to be a risk factor for VTE, although the suggestive evidence from retrospective case control studies is not yet fully supported by adequate data from prospective studies.<sup>22</sup> The phenotype of mild to moderate hyperhomocyst(e)inemia may be explained by dietary vitamin deficiency states, renal failure, certain medications, or genotypic variants. A relatively common C677T base-pair polymorphism in the gene for the enzyme methylene tetrahydrofolate reductase (MTHFR) may predispose to hyperhomocyst(e)inemia in individuals who are mildly deficient in dietary folate, although the body of evidence now suggests that homozygosity for this polymorphism is probably not in itself a true risk factor for arterial or venous thrombosis.<sup>22</sup>

### EPIDEMIOLOGY OF CONGENITAL THROMBOPHILIAS

Crowther and Kelton<sup>23</sup> have recently proposed that the congenital thrombophilias might be appropriately classified into two separate categories. Group 1 disorders include deficiency states of regulatory proteins (protein C, protein S, and antithrombin). Although group 1 disorders are relatively less common, they are associated with relatively higher risks for thrombosis than the more common but lower penetrant “gain of function” (group 2) disorders, such as factor V Leiden, prothrombin G20210A mutation, and elevated levels of factors VIII, IX, and XI (Table 1). Therefore, in considering the epidemiology of VTE in the population as a whole, the attributable risk (a function of both relative risk and prevalence) is much greater for the group 2 disorders, whereas, for a given individual, the risks associated with the group 1 disorders would be more significant.

Although some publications claim a positive association between several other coagulation protein deficiency or over-



**Fig. 3** The protein C/protein S pathway. The prothrombotic activity of factor Va is down-regulated through an activated protein C-mediated cleavage reaction. This reaction is enhanced in the presence of the cofactor for activated protein C, protein S. A quantitative deficiency (1) or functional deficiency (2) of protein C impairs one pathway for the control of thrombin generation, namely the cleavage and inactivation of activated factor V. Similarly, quantitative (3) or functional (4) deficiencies of protein S can have a similar effect. Finally, resistance of the activated factor V molecule to activated protein C-mediated cleavage (5), most often caused by the factor V Leiden mutation, prevents efficient cleavage and inactivation of activated factor V.

**Table 1**  
Common inherited risk factors for thrombosis

Risk factor	Prevalence in general population (white) (%)	Prevalence in patients with VTE (%)	Relative Risk for VTE
Factor V Leiden heterozygote	5.0 <sup>35</sup>	20 <sup>29</sup>	4.0–7.0 <sup>32,52</sup>
Factor V Leiden homozygote	0.02 <sup>36</sup>	1.5 <sup>36</sup>	80.0 <sup>36</sup>
Prothrombin G20210A heterozygote	0.7–4.0 <sup>37</sup>	6 <sup>19</sup>	2.8 <sup>19</sup>
Factor V Leiden/prothrombin compound heterozygote	0.1 <sup>26</sup>	2.2 <sup>26</sup>	20.0 <sup>26</sup>
Protein C deficiency <sup>24,28,29,33</sup>	0.2–0.4 <sup>39</sup>	2.5–3 <sup>28,31</sup>	3.4–7.8
Heterozygote			
Protein S deficiency heterozygote	0.03–0.13 <sup>25</sup>	1–3 <sup>27</sup>	2.4–20.0 <sup>27,30</sup>
Antithrombin deficiency heterozygote	0.07–0.2 <sup>38,40</sup>	0.5–3 <sup>31,34</sup>	5.0–50.0 <sup>30,40</sup>

VTE, venous thromboembolism.

expression states [e.g., elevated levels of plasminogen activator inhibitor (PAI)-1 and deficiency of tissue plasminogen activator (tPA) or heparin cofactor 1], proof of causality in VTE is incomplete at the current time. Therefore, screening for these disorders cannot be recommended.

In addition, it is worth noting that most of our understanding of inherited risk factors for thrombosis is derived from the study of largely white populations. Thus, although factor V Leiden and the prothrombin G20210A mutations are regularly described as common risk factors for thrombosis, this description may apply only to European populations.<sup>37,41,42</sup> Although some attribute the presence of factor V Leiden and prothrombin mutation in some African and Asian populations to migration or colonization, others have asserted that the presence of these mutations in small genetic isolates may argue for multiple origins for at least the factor V Leiden mutation.<sup>43</sup> For other hypercoagulable states, such as protein S deficiency, it has been pointed out that no studies have been performed to date to determine the prevalence of these conditions in African and Asian populations.<sup>44</sup> Thus, although studies have begun to elucidate the basis of familial thrombophilia in white populations, a great deal remains to be learned in other populations.

Notwithstanding this proviso, extensive study of the impact of prototypic thrombophilic disorders such as factor V Leiden has been accomplished during the past decade. Studies have consistently demonstrated that the risk of first VTE is increased 4- to 7-fold in heterozygous subjects, whereas the estimated risk for VTE in individuals with two alleles is increased 50- to 80-fold.<sup>36</sup> The annual incidence of a first thrombotic event in heterozygous factor V Leiden carriers has been estimated at 0.3 to 0.5%.<sup>45</sup> The lifetime risk for developing VTE in this group of individuals has been independently estimated at approximately 10%.<sup>46</sup> In factor V Leiden homozygotes, the overall incidence of VTE has been estimated at 1% per year, rising to 2% per year in individuals aged greater than 50 years.<sup>36</sup>

The prothrombin G20210A mutation is the second most commonly identified inherited thrombophilia. Like factor V Leiden, the prothrombin mutation is essentially absent in per-

sons of Asian or African origin.<sup>47</sup> However, in contrast with factor V Leiden, this mutation is found more frequently in southern European than in northern European populations (3–4% vs. 1.7%, respectively).<sup>37</sup> The annual incidence of VTE in persons heterozygous for prothrombin G20210A is approximately 0.46%.<sup>30</sup>

Inherited thrombophilias caused by protein C/S and antithrombin deficiencies tend to be far less prevalent in the general population (Table 1). Estimates of the relative risk of VTE associated with these conditions vary widely. Such variation may be caused by differences in the definition of deficiency or in the methodology used to assess the deficiency. Although more recent population-based studies have suggested that these conditions may be more prevalent than previously thought,<sup>38,39</sup> other prospective studies have suggested that the relative risk associated with these deficiencies is not so significant as previously believed.<sup>33</sup> Previous estimates of the relative risk associated with these deficiencies may have been overestimated because of unappreciated gene-gene interactions between these deficiencies and more common risk factors, such as factor V Leiden.<sup>48,49</sup>

## GENE-GENE INTERACTIONS IN THE PATHOGENESIS OF VTE

With the discovery of the factor V Leiden mutation, family studies in well-characterized kindreds with deficiencies of regulatory proteins such as protein C<sup>48</sup> or antithrombin<sup>49</sup> demonstrated that the apparently higher penetrance of VTE in symptomatic individuals (compared with affected asymptomatic family members) could be explained by coinheritance of a factor V Leiden allele. VTE can now be considered a classic multigenic disorder so that the workup of probands found to carry the factor V Leiden allele, for example, should be extended to include testing for other thrombophilic disorders. The prevalence of double heterozygosity for factor V Leiden and prothrombin 20210A mutations is about 1 in 1000 of the population.<sup>26</sup> Although this combination clearly increases the risk of

first VTE more than the presence of either mutation alone, the real importance in identifying more than one disorder is that it may change the duration of oral anticoagulant treatment in an affected person as well as alter the screening strategy for asymptomatic family members.

## GENE-ENVIRONMENT INTERACTIONS IN THE PATHOGENESIS OF VTE

Almost 150 years ago, Rudolf Virchow described three interdependent factors that promote clot formation: stasis of blood, vessel-wall damage, and hypercoagulable blood components. Indeed, VTE is usually a multifactorial event that reflects the interaction of hematologic risk factors with environmental exposures and predisposing life events. Recognized risk factors include pregnancy and the postpartum period, estrogen therapy in the form of oral contraceptives (OCP) or hormone replacement therapy, surgery or trauma, underlying cancer, prolonged immobility (usually defined as  $\geq 3$  days in bed), and indwelling venous catheters. The risk associated with surgery may be caused by several factors, including immobilization, duration of general anesthesia, and vascular endothelial damage. Even surgeries traditionally considered to be relatively minor, such as arthroscopic knee surgery, have been shown recently to be associated with an increased risk of VTE in thrombophilic individuals.<sup>50</sup> Although still controversial, evidence is increasing that prolonged air travel ( $>4$ – $5$  hours) is also a risk factor for VTE.<sup>51</sup>

In addition, the increased risk of VTE because of the interaction between factor V Leiden and oral estrogen therapies has been the topic of much investigation. These data have demonstrated a multiplicative rather than an additive escalation in risk between factor V Leiden mutation and OCP or hormone replacement therapy. More specifically, women taking OCP containing at least 50  $\mu\text{g}$  of ethynylestradiol have a 4-fold increased risk of VTE compared with a 35-fold increased risk of VTE in women using OCP who are heterozygous for factor V Leiden.<sup>52</sup> The incidence of fatal VTE while taking OCP in heterozygous factor V Leiden carriers is about 5 in 100,000, compared with an incidence of 0.7 in 100,000 in normal women.<sup>53</sup>

## ASSOCIATION OF INHERITED THROMBOPHILIA WITH OTHER ADVERSE CLINICAL OUTCOMES

The association between thrombophilia and clinical disorders other than VTE is more controversial. Complications of pregnancy, such as recurrent miscarriage and second or third trimester fetal loss, preeclampsia, and intrauterine growth retardation, have received significant attention.<sup>54,55</sup> By analogy, antiphospholipid syndrome, an acquired hypercoagulable state, is an established risk factor for recurrent miscarriage. To date, there are no large-scale randomized studies examining the role of anticoagulation in preventing adverse events during pregnancy in affected women. However, a recent large Scottish study has cast doubt on the role of inherited thrombophilia in certain of these adverse outcomes, such as preeclampsia.<sup>56</sup>

Therefore, at the present time, the association of complications during pregnancy with inherited thrombophilia must be viewed as controversial.

Studies investigating the role of prothrombin G20210A and factor V Leiden in the pathogenesis of arterial thrombosis have also been conflicting.<sup>57</sup> Clearly, routine investigation for these mutations is not indicated in patients with arterial thrombosis. However, it is possible that in the presence of other atherosclerotic risk factors (such as smoking, hypertension, or elevated cholesterol), these mutations may increase the risk for arterial thrombosis, particularly in certain populations in which these events normally occur with low frequency, such as with young women or children.<sup>57</sup>

## THE GOALS OF THROMBOPHILIA TESTING

Genetic testing in VTE patients should be performed to address specific issues of patient management. Testing may be used to guide the duration of anticoagulant therapy, quantify the need for primary or secondary thrombosis prophylaxis, or determine whether family members also need evaluation. Specifically, patients identified as compound heterozygotes (such as factor V Leiden and antithrombin deficiency) may have improved outcomes from continuing their anticoagulation indefinitely rather than the standard 3 to 6 months of treatment following a first event, whereas it is controversial whether more than 3 to 6 months of therapy is warranted for individuals shown to be heterozygous for only one mutation.<sup>26</sup> In addition, there is no evidence that high-intensity anticoagulation [international normalized ratio (INR)  $>3.0$ ] is of any benefit to patients with one or more inherited thrombophilias. Genetic testing for inherited thrombophilia may also be used to identify inherited disorders that would necessitate genetic counseling because of risks to other family members.

## WHICH PATIENTS ARE APPROPRIATE FOR TESTING?

There are recognized circumstances for which the workup for inherited thrombophilia is relatively undisputed (Table 2). Conversely, situations for which testing is not recommended include an initial venous thrombosis of known etiology in patients greater than age 50, including thrombosis in the setting of surgery or active malignancy, thrombosis caused by trauma or intravascular cannulation, and thrombosis occurring while taking selective estrogen receptor modulators (such as tamoxifen). For a more in-depth discussion of which individuals may require inherited thrombophilia testing, see the previously referenced *College of American Pathologists Consensus Conference XXXVI: Diagnostic Issues in Thrombophilia*.<sup>4</sup>

## LABORATORY EVALUATION OF THROMBOPHILIAS

Many plasma-based assays for coagulation factors suffer from imprecision and inaccuracy that may begin with preanalytical sample mishandling. Careful sample collection and timing may thus be as critical as the selection of the laboratory.

**Table 2**  
Situations in which thrombophilic testing is recommended

1. Venous thromboembolism (VTE) at an unusual site (cerebral, mesenteric, portal, hepatic)
2. Recurrent VTE
3. Idiopathic VTE (unrelated to surgery, trauma)
4. VTE during pregnancy or the postpartum period
5. Strong family history of VTE in first degree relatives
6. Recurrent fetal loss, especially in the 2 <sup>nd</sup> or 3 <sup>rd</sup> trimester
7. VTE while on oral contraceptive, hormone replacement, or methotrexate
8. Life-threatening venous thrombosis (pulmonary embolus, cerebral vein thrombosis, etc.)
9. First VTE at a young age (<45–50 years of age)
10. Asymptomatic, first-degree relatives of a proband with a known inherited thrombophilia

Because of significant sex differences in plasma levels of many variables (e.g., protein S levels), gender-specific reference ranges are preferable but are frequently not provided.

In common with deficiencies of other coagulation regulatory proteins, heterozygous antithrombin deficiency may be caused by multiple genetic mutations. Homozygous antithrombin deficiency is a very rare disorder associated with a severe early-onset thrombotic diathesis. Deficiency states may be broadly classified as type I (approximately 50% reduction in both plasma antigen and functional activity) or type II (normal antigenic level with reduced functional activity). Therefore, it is important that, at a minimum, a functional assay for antithrombin be performed when considering this diagnosis. In practice, this is usually an amidolytic (chromogenic) assay. A functional assay will detect antithrombin deficiency caused by both quantitative and functional deficiencies of antithrombin. The antigenic antithrombin assay can be used to further distinguish type I deficiencies from type II.

As with antithrombin deficiency, protein C deficiency is conveniently classified as type I or type II. As such, the labora-

tory evaluation of protein C is analogous to the evaluation of antithrombin and should include at least an amidolytic assay for protein C.

Protein S deficiency states are somewhat more complex because only about 40% of the protein exists in the free, functionally active form. Therefore, either a functional or immunoassay for free protein S is required for diagnosis.<sup>44</sup>

First-generation functional assays for APC resistance are based on the original assay proposed by Dahlback et al.,<sup>15</sup> namely the failure of exogenously added APC to produce the expected prolongation of the patient's APTT. This assay is prone to interference from oral anticoagulants and other circulating inhibitors of the APTT such as lupus anticoagulants. A more recent "second generation" assay in which patient plasma is mixed with factor V deficient plasma<sup>58</sup> is a valid screen for factor V Leiden under many of these circumstances. In any case, many laboratories prefer to proceed directly to definitive DNA-based assay for the factor V Leiden mutation in individuals suspected or shown to have APC resistance by either assay. This polymerase chain reaction (PCR) assay also has the advantage that it can be combined with screening tests for other prothrombotic target gene mutations (such as the prothrombin G20210A mutation) on multigene platforms. For a summary of testing for each disorder, see Table 3.

### IMPORTANCE OF FAMILY HISTORY

Because VTE can have both genetic and acquired etiologies, a thorough pedigree can serve an important function in the evaluation of individuals with thrombosis. A positive family history may suggest one or more inherited thrombotic risk factors. At least one study has identified positive family history as an independent risk factor for thrombosis.<sup>59</sup> A pedigree may also aid in the genetic counseling process by identifying at-risk family members. If possible, a three to four generation pedigree should be obtained. Special attention should be paid to individuals with thrombosis, bleeding, and pregnancy loss. Arterial thrombotic events, including stroke or myocardial infarction, may be relevant if they were associated with a rela-

**Table 3**  
Recommended testing for inherited thrombophilias

Disorder	Assay
Antithrombin deficiency	Chromogenic assay for antithrombin function Antigenic assay to distinguish type I from type II deficiency
Protein C deficiency	Chromogenic assay for protein C function Antigenic assay to distinguish type I from type II deficiency
Protein S deficiency	Functional (clotting) or immunoassay for free protein S
Activated protein C resistance	Functional clotting assay in which APC added to plasma fails to cause expected APTT prolongation
Factor V Leiden mutation	DNA-based assay (PCR)
Prothrombin G20210A mutation	DNA-based assay (PCR)

APC, activated protein C; APTT, activated partial thromboplastin time; PCR, polymerase chain reaction.

tively early onset (before age 45–50). In addition to questions about thrombotic events, specific questions about warfarin or heparin use may aid in patient recall, as may the use of outmoded terminology, such as the term “milk leg,” which was formerly used to refer to the clinical manifestations of postpartum deep vein thrombosis. Statistically validated questionnaires have been published to aid in identifying individuals with a personal or family history of thrombosis.<sup>60</sup>

Diagnostic evaluations of other family members should be verified by obtaining medical records. This is especially important when physicians are being asked by asymptomatic family members to rule out a thrombotic risk factor previously identified in the family. Laboratory evaluations of symptomatic family members should be reviewed for completeness. Individuals evaluated before the mid 1990s, for example, may not have had testing for factor V Leiden or the prothrombin G20210A mutation. Physicians should also verify that available laboratory workups were appropriately performed and interpreted. For example, individuals who are evaluated while taking warfarin may be inadvertently diagnosed with a deficiency of one or more of the vitamin-K-dependent regulatory factors, protein S or protein C. Similarly, a spurious diagnosis of antithrombin deficiency may occur when antigenic or functional levels are performed during heparin administration.

Because of the difficulties that may be encountered in establishing a diagnosis of deep vein thrombosis or pulmonary embolism, it is important to review previous medical records on affected individuals and family members for the diagnostic radiology strategy and study reports. It is not uncommon to find these diagnoses were made on purely clinical grounds in previous years, in which case the diagnosis should be viewed as being in doubt given the notorious inaccuracies in establishing a diagnosis of VTE by clinical assessment alone.

## IS INFORMED CONSENT REQUIRED BEFORE THROMBOPHILIA TESTING?

Both the College of American Pathologists (CAP) and the ACMG agree that formal written consent should not be required before testing for inherited thrombophilia.<sup>5,61</sup> In symptomatic individuals, in which such testing may have a significant impact on medical management, a discussion of the inherited nature of thrombophilia should be undertaken. This discussion should include implications for family, confidentiality, insurance eligibility, and employment.<sup>5</sup> In asymptomatic individuals requesting testing for inherited thrombophilia, a more in-depth discussion of the above issues is warranted.

In addition, asymptomatic patients should understand that many forms of familial thrombophilia are low-penetrance risk factors for venous thrombosis. For the more common inherited thrombophilias (e.g., factor V Leiden), the majority of heterozygous individuals never experience a thrombotic event,<sup>62</sup> and the impact on quality of life and life expectancy for affected individuals may not be so great as often perceived. In the case of factor V Leiden heterozygotes, national data from The Netherlands have shown that life expectancy is normal.<sup>63</sup>

Furthermore, possible interventions, such as prophylactic anticoagulation, may carry a greater risk to the individual than the risk of a thrombosis. Thus, the identification of a prothrombotic risk factor may not alter a patient’s medical management. With this in mind, asymptomatic patients should clearly understand that they have the option to decline testing for an inherited thrombophilia.

Practitioners should address patient concerns regarding insurance discrimination. Although this phenomenon has not become so prevalent as initially feared,<sup>64</sup> several anecdotal reports have hinted at the possible misuse of genetic tests by both employers and insurers.<sup>65</sup> Clinicians may be approached by asymptomatic family members of VTE patients requesting “anonymous” factor V Leiden testing. Although physicians should be sensitive to these concerns, it is generally very difficult to provide specific recommendations for anticoagulation without documentation of history and laboratory evaluations to justify such recommendations.

An important consideration in testing for inherited thrombophilia is the possibility that such testing may reveal unanticipated information regarding family relationships. Haplotype analysis suggests that the common factor V Leiden and prothrombin G20210A mutations arose as founder mutations between 20,000 and 30,000 years ago.<sup>41,42</sup> Thus, it is expected that at least one parent of a proband will also carry the mutation. The absence of such mutations in both parents of a proband cannot be readily explained by invoking a *de novo* mutation. Thus, genetic testing of parents may inadvertently reveal evidence suggestive of nonpaternity.

## POSTTEST EDUCATION

Following the identification of inherited thrombotic risk factors, patients should be counseled regarding both the medical significance and the inheritance pattern of the risk factor. Despite professional guidelines recommending the inclusion of such information in molecular reports, a recent study found that only half of positive factor V Leiden reports included appropriate genetic counseling recommendations.<sup>66</sup>

Medical management discussions should include an estimate of the risk for thrombosis (or recurrence of thrombosis), an estimate of the risks associated with prophylactic anticoagulation, recommendations regarding avoidance of environmental risk factors such as estrogen-containing OCP, and discussion of strategies for VTE prophylaxis in high-risk situations (pregnancy, trauma, surgery). The risk of a venous thromboembolic event has to be balanced against the risk of unwanted pregnancy when OCP are withheld. In addition, the risk of thrombosis should be quantified against the 1.3% annual incidence of major bleeding (defined as intracranial or retroperitoneal bleeding, bleeding requiring hospitalization or transfusion, and bleeding leading to death) in those patients anticoagulated with warfarin at an INR of 2.0 to 3.0.<sup>67</sup>

Genetic counseling following the identification of familial thrombophilia illustrates the complexity of counseling in multifactorial conditions. A discussion of autosomal dominant in-



heritance may suffice to describe the inheritance of most prothrombotic risk factors. However, patients should be made aware of several additional issues. First, it should be emphasized that these conditions are *common risk factors* for thrombosis, not *rare genetic diseases*.

Second, although some risk factors are relatively mild in the heterozygous state, they may be associated with severe neonatal onset in the homozygous state. For example, as already mentioned, although heterozygous protein C deficiency is associated with only a modest increase in thrombotic risk, homozygous protein C deficiency is associated with neonatal purpura fulminans.<sup>68</sup>

Finally, patients should be aware that exclusion of a particular inherited risk factor may not relieve them of all the familial risk for thrombosis. Studies in thrombophilic families known to harbor one or more prothrombotic mutations have shown that exclusion of these mutations in asymptomatic family members does not necessarily correlate with a reduction in clotting risk to baseline.<sup>69</sup> Thus, current state of the art laboratory evaluations cannot exclude all familial risk, even in families with known risk factors.

Ideally, patients should be provided with written materials summarizing the above information. This may take the form of a dictated patient letter, informational pamphlets, or references to helpful web sites (such as the American Venous Forum). Such information may help patients understand their own personal risk and also help them communicate this information to relevant family members.

## SCREENING REQUESTS FROM ASYMPTOMATIC FAMILY MEMBERS

Consensus guidelines for the most common forms of inherited thrombophilia have not advocated routine testing of asymptomatic family members.<sup>5,61</sup> These guidelines have recommended considering each request on a case-by-case basis.

When considering requests from asymptomatic family members, the following should be taken into consideration. First, it is important to consider whether such testing would significantly alter a patient's medical management. The aforementioned recommendations not to screen asymptomatic family members on a routine basis are largely based on European studies.<sup>32,59,62,70</sup> Such studies noted that routine prophylaxis following surgery and immobilization would have significantly reduced the risk for thrombosis in at-risk family members, thus obviating the need for formal testing. However, routine prophylactic anticoagulation following nonorthopedic surgery is a much more common practice in Europe than in the United States. Thus, identification of asymptomatic prothrombin G20210A heterozygotes or factor V Leiden heterozygotes may be of greater clinical significance in the United States.

Second, it is important to consider the number of risk factors that an individual may potentially carry. Although identifying a single mutation may not significantly alter management, identifying homozygous or doubly heterozygous

individuals may be of more clinical significance. For example, Koeleman et al.<sup>71</sup> showed that individuals in thrombophilic families who carried two defects had a 2- to 3-fold increased risk of thrombosis when compared with family members who carried only one.

Finally, although some inherited risk factors are associated with only a modestly increased risk for thrombosis, others, such as protein S deficiency and antithrombin deficiency, may be associated with a much more significant risk. The cumulative risk of thrombosis may reach 50% by age 50 in these conditions. Therefore, identification of these states in asymptomatic individuals may be of greater medical significance.<sup>70</sup>

## GENERAL SCREENING FOR THROMBOPHILIA AND SPECIAL POPULATIONS

Both the ACMG and the CAP have recommended against general population screening for inherited thrombophilias. For common risk factors, such as factor V Leiden and prothrombin mutation, such screening would identify large numbers of heterozygotes, the majority of whom would never experience an episode of VTE.<sup>62</sup> Other common variants, such as the MTHFR C677T polymorphism, have only a questionable association with thrombosis.<sup>22</sup> More significant risk factors for thrombosis, such as antithrombin deficiency, are much less prevalent in the general population.

A more controversial issue is the value of screening for inherited thrombophilia in asymptomatic individuals who will be exposed to risk factors known to increase the risk for thrombosis, the most frequently cited example being those women requesting OCP. Many, if not all, of the thrombotic risk factors interact with OCP in a synergistic fashion, increasing the risk for thrombosis. As already mentioned, factor V Leiden heterozygotes have been shown to possess a 35-fold increased risk for deep vein thrombosis while using OCP.<sup>54</sup> Individuals with antithrombin deficiency may have up to a 4.3% risk per year for thrombosis while using OCP.<sup>70</sup> Thus, at first glance, it may seem prudent to screen all women requesting OCP for common thrombotic risk factors. However, estimates are that approximately 400,000 women would need to be screened to identify 20,000 factor V Leiden heterozygotes, all of whom would potentially be denied access to OCP. Such a strategy would be extremely costly and deny a large number of women access to safe contraception, whereas this practice is estimated to prevent just one thromboembolic death.<sup>55</sup> Because of this, some have suggested screening only those women with a positive family history of VTE for the common inherited forms of thrombophilia.<sup>55</sup> Although some authors have challenged the efficacy of using family history as a screening tool,<sup>72</sup> a positive family history would certainly warrant a more careful evaluation of risks and benefits.

## THROMBOPHILIA TESTING IN CHILDREN

Because most forms of familial thrombophilia are low penetrance, multifactorial, adult-onset disorders, neither CAP nor

ACMG have recommended prenatal testing or general newborn screening.<sup>5,61</sup> More controversial, however, is screening of asymptomatic children of probands. Because children have a low a priori risk of thrombosis, identification of thrombotic risk factors would not affect medical management during the preadolescent years. Therefore, most guidelines suggest that screening of asymptomatic children may be delayed until puberty.<sup>53</sup> In exceptional families with childhood thrombosis, it may be appropriate to screen siblings of affected individuals.<sup>73</sup> In contrast with adults, it is notable that several studies have identified inherited thrombophilic disorders as a risk factor for stroke in children.<sup>53</sup> Whether this indicates a fundamentally different pathophysiology of stroke in childhood or the clinical expression of risk factors that are uncovered by a higher “signal to noise” ratio in children is at present unclear.

## CONCLUSIONS

With increasing awareness of thrombophilic disorders, there are increasing numbers of individuals in whom one or more of these risk factors for VTE are being discovered. These tests are being requested for persons with a history of VTE and their unaffected family members as well as for women with a history of adverse outcomes during pregnancy and for patients with myocardial infarction and stroke. The appropriateness of testing (in particular, whether the discovery of an underlying thrombophilic disorder is likely to contribute to management) should always be considered, and if possible, discussed with the patient before testing. As a general rule, these tests are requested too frequently and follow-up counseling tends to be inadequate. Moreover, in asymptomatic individuals, discovery of one or more thrombophilic disorders rarely indicates the need for long-term prophylaxis with oral anticoagulants but might indicate the need for short-term prophylaxis in certain high-risk situations, including surgery and trauma, and in some cases of pregnancy or the postpartum period. Discovery of a thrombophilic disorder in an asymptomatic woman might also impact the choice of contraceptive therapy, although this decision should be balanced against any increased chance of an unwanted conception. Although geneticists may not be directly involved in the medical management of these individuals, they will likely be called upon to assess the utility, risks, and benefits of identifying a thrombophilic disorder in family members. Geneticists would benefit from developing a systematic approach to genetics testing in common multifactorial diseases such as VTE.

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