

Pulmonary embolism

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Pulmonary embolism (PE) and deep venous thrombosis (DVT) are often regarded as two aspects of the same disease. Indeed, 50–70% of patients with DVT have evidence of silent PE and DVT is present in 70–90% of patients presenting with PE.^{1–5} This analysis may therefore be largely true, but there may also be important reasons—some of them genetic—why some patients present with DVT, some with PE, and others with signs of both. This problem will be discussed at the end of this review. In any event, most studies investigating possible genetic predispositions to venous thrombosis have either concentrated on DVT—for example, the Leiden thrombophilia study⁶—or have not made any distinction between DVT and PE.^{7–9} We therefore have to rely on these data to indicate the role of genetic variation in pulmonary embolism.

A genetic tendency to thromboembolism is also referred to as inherited thrombophilia and, although traditionally regarded as monogenic, it is undoubtedly a polygenic abnormality.^{10 11} The basis of our understanding of the thrombophilic state is that it arises from an imbalance between the procoagulant and anticoagulant components of the coagulation system, resulting in an increased tendency to thrombosis.

This tendency usually becomes manifest as thrombosis as a result of its interaction with numerous environmental and acquired factors.

The coagulation cascade and classical thrombophilia

The coagulation system is activated at the site of injury by the exposure of tissue factor which is ubiquitously expressed in extravascular tissues. Tissue factor binds factor VIIa and triggers a network of serine proteinases and their cofactors resulting in the cleavage of fibrinogen by thrombin to form fibrin, the basis of the blood clot. Regulatory (or inhibitory) systems have evolved in parallel to inhibit this network and prevent inappropriate propagation of this activity (fig 1). The principal inhibitor of the coagulation proteinases—and, in particular, of thrombin—is antithrombin, a member of the serpin group of inhibitors.¹² Factor V and factor VIII are the two principal cofactors of the system and act to augment the serine proteases factors Xa and IXa, respectively. Whilst the enzymes of the coagulation system are inactivated by antithrombin, these cofactors are degraded by protein C, another serine protease, in conjunction with its cofactor protein S. In a sophisticated regulatory mechanism protein C is itself activated by thrombin, but only when it has bound to thrombomodulin which is present on the surface of undamaged endothelium (fig 2). This process is enhanced by the recently described endothelial protein C receptor (EPCR).¹³ Binding of thrombin to thrombomodulin results in a dramatic change in its substrate specificity in which it becomes an anticoagulant protein by preferentially activating protein C.

Many mutations of the genes encoding antithrombin, protein C, and protein S have been described, resulting in reduced levels of protein or production of an abnormal molecule and an associated thrombophilic state. Although in many cases a simple deficiency of the anticoagulant protein results, there is considerable heterogeneity arising from missense mutations.¹⁰ Partial deficiency of antithrombin was the first inherited thrombophilic disorder to be described in 1965 in a Norwegian family.

Identification of protein C, protein S, or antithrombin deficiency is usually straightforward on the basis of antigenic and functional assays. All three are inherited as autosomal dominant conditions with plasma levels approximately 50% of normal. Curiously, 60% of

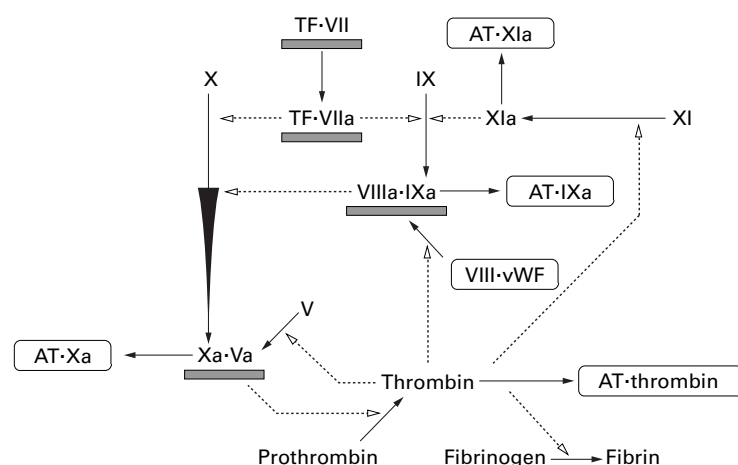


Figure 1 The coagulation network showing the neutralization of the proteases by antithrombin (AT). Coagulation *in vivo* is initiated by the exposure of tissue factor (TF) which binds factor VIIa and greatly increases its activity against factor X and factor IX, thus activating small amounts of each. The small amount of thrombin generated by Xa greatly amplifies further thrombin production by activating the cofactors factor V and factor VIII. When activated, factor VIII is released from its carrier protein, von Willebrand's factor (vWF). Additional activation arises from thrombin activation of factor XI which generates more IXa. Thrombin then cleaves fibrinogen to form fibrin which self-assembles into the clot. The hatched boxes indicate that the complex is formed on a phospholipid surface which is provided by the cell membrane for VIIa-TF and by platelets for VIIIa-IXa and Va-Xa. (Courtesy of Professor EGD Tuddenham).

with raised plasma prothrombin levels and venous thrombosis.²⁷ This appears to be present in 1–2% of the populations so far studied (Caucasian but not African) and confers an estimated relative thrombotic risk of approximately 3.

Homocystinuria is an inborn error of methionine metabolism resulting in very high plasma levels of homocysteine and a strong predisposition to thrombosis. Homocysteine is thought to produce this effect by damaging endothelium. There is now some evidence that milder degrees of hyperhomocysteinaemia are also associated with an increased risk of thrombosis and are present in approximately 20% of cases.²⁸ The milder disorder is difficult to diagnose but might prove to be important because it is potentially remediable by dietary manipulation. It has a number of genetic causes including abnormalities of β -cystathionine synthase and methylene tetrahydrofolate reductase (MTHFR).²⁹ Homozygotes for the thermolabile variant of MTHFR have higher levels of homocysteine and may, in combination with other factors, have an increased risk of thrombosis.

By analogy with the effects of protein S and protein C deficiency, another possible cause of thrombophilia would be an abnormality or deficiency of thrombomodulin. This has been difficult to investigate because it is a transmembrane protein found on the surface of endothelium. Investigation has thus been directed at detecting genetic variation within the gene and the first mutation in association with thrombosis was reported by Ohlin in 1995.³⁰ A number of other mutations have been described subsequently and some of these do appear to have a relationship with arterial disease.^{31–32} However, a role for thrombomodulin mutations in venous thromboembolic disease is not yet established. Plasma estimation of soluble thrombomodulin fragments is not useful.

There are many other factors with the potential to influence coagulation including minor changes in the levels of procoagulant and anticoagulant molecules, platelets, α_2 -macroglobulin, heparin cofactor II, and possibly also vascular abnormalities. The difficulty in identifying these as prothrombotic factors might be that their influence is not sufficient for them to emerge as monogenic traits; nonetheless, this does not preclude their contributing significantly to the phenotype. Finally, the Nurse's health study found body mass index (BMI) to be an additional predictor of PE which adds another genetic element to the problem.³³ It is possible that a marked prothrombotic state might be produced by a multitude of minor factors in the absence of any single powerful abnormality. As described below, modulation of classic thrombophilic traits by such factors suggests that such combinations might be extremely important.

Fibrinolysis

Defects of the fibrinolytic mechanisms have proved elusive. Low levels of fibrinolytic activity are found in as many as 30% of patients with thrombosis, but it is not clear whether this is

cause or effect.³⁴ A major contributor to low fibrinolytic activity is plasminogen activator inhibitor 1 (PAI-1), the levels of which are quite variable and determined partly by a number of promoter polymorphisms as well as by acquired factors.³⁵ However, levels of fibrinolytic factors do not predict venous thrombosis.³⁶ Even when single gene defects of fibrinolysis have been identified (as in some families with plasminogen deficiency), the association with thrombosis has not been consistent.³⁷

Polygenic thrombophilia

For the purposes of investigation and management, thrombophilia and the factor deficiencies described above have been treated as monogenic conditions. However, several lines of evidence indicate that we now have enough information to regard the risk of thrombosis and the concept of thrombophilia as polygenic.

The first clue to this was the observation that, in some kindreds with thrombophilia, there was a marked difference in phenotype associated with the same genetic lesion. This problem also arises in large groups of patients. For example, the prevalence of protein C deficiency estimated from studies of thrombophilic patients is as low as 1 in 16000–36000. However, when population studies are performed the prevalence of levels consistent with heterozygous deficiency is as high as 1 in 200–300. Moreover, the protein C deficient individuals identified in the population based studies almost never have a personal or family history of thrombosis. Because protein C is so clearly associated with thrombosis in thrombophilic kindreds, it is evident that some other genetic factor is contributing to the phenotype. This has been strikingly demonstrated by a study of the effect of co-inheriting protein C deficiency and FVL. In one study 78% of those with both abnormalities were symptomatic compared with 31% of those with protein C deficiency alone.³⁸ Similar data showing the same effect are now available for antithrombin deficiency combined with FVL and protein S deficiency combined with FVL.^{39–40} Moreover, in the latter study Zoller and colleagues found that the frequency of thrombosis in family members without protein S deficiency or FVL was still higher than expected in the population at large. This suggests that further genetic factors must be present. A recent report suggested that FVL was also important in determining which patients with homocystinuria developed thrombotic complications.⁴¹ The risk associated with the combination was found to be greater than the sum of the individual conditions.⁴²

Looking for thrombophilia

Identifying a prothrombotic trait has important implications for the patient and also for the family, helping to guide management and avoid further thromboses. Amongst a group of patients suffering their first venous thrombosis, deficiency of antithrombin, protein S or protein C will together only account for approximately 10% of cases. In the past this made the investi-

gation of patients for thrombophilia a relatively unrewarding exercise, thought by some to be unwarranted. Restricting investigation to those patients who were young (<45 years) with family histories or with recurrent problems failed to select those patients with deficiency.⁷

The situation was greatly improved by the discovery of APCR and raised factor VIII levels, such that we can now identify a relevant prothrombotic factor in more than 50% of patients. This is the case even if we assume that some patients will have more than one abnormality. Is there any prospect of increasing this figure to 100%? It is unlikely that we will find an abnormality in all such cases. Some are likely to be explained by acquired abnormalities such as the lupus anticoagulant, malignancy, paroxysmal nocturnal haemoglobinuria, or Behçet's syndrome. Others will be almost entirely the result of factors such as trauma, fractures, immobility, or obesity. Nonetheless, testing for thrombophilia is now an important and valuable part of clinical management.

It should be routine practice when a genetic abnormality is detected to offer testing to other family members. The decision to take up this offer is not straightforward; as with other genetic disorders the patient may be concerned about confidentiality, disclosure to insurance companies, and whether they wish to be burdened with this information. The pros and cons must be carefully explained beforehand but the matter is often brought to a head by the wish for oestrogen therapy or by impending surgery.

Differences between DVT and PE

A number of recent studies have found that the incidence of FVL in patients with isolated PE is much lower than in those with DVT or DVT + PE. In the study by Martinelli *et al*⁴³ 252 patients with a first episode of DVT and/or PE were studied for the presence of the FVL mutation. It was found that FVL was significantly associated with isolated DVT or DVT + PE but not with isolated PE (odds ratios (95%CI) 10.0 (4.0 to 22.5), 5.5 (2.2 to 15.8), and 1.8 (0.3 to 9.6), respectively). These results are similar to those of another study based on an anticoagulation clinic which found odds ratios of 3.3 (1.0 to 10.6) for PE and 6.9 (3.6 to 12.6) for DVT.⁴⁴ These results tend to suggest that DVT and DVT + PE are indeed the same disease and this fits with the high proportion of patients with DVT who also have a PE on formal scanning. At the same time it suggests that primary PE is not quite the same disease and this may be due to a number of reasons. The simplest explanation would be that these cases arise from emboli which have formed at sites other than the deep veins of the leg (as the literature frequently reports them to have done²³) and that FVL does not play such an important role in clot formation at these sites. The presence of local lesions at these sites may be predominantly responsible for the thrombosis that subsequently embolises and thus less dependent on abnormalities such as FVL. The authors also suggest that clots arising in the presence of FVL may be less likely to embolise

in toto and the stepwise increase in risk across the three categories is in keeping with this. Malignancy seems to have a similar relationship with both DVT and PE but some cases of apparently isolated PE may in fact be tumour emboli.⁴⁵ It is interesting, given the strong synergism between FVL and the OCP in increasing the risk of thromboembolic disease,⁴⁶ that the PIOPED study did not find oestrogen use to be associated with an increased risk of PE⁴⁷ and in other studies the relationship was found to be relatively weak.^{48 49}

Conclusion

The understanding of the coagulation mechanism has led to a greater understanding of those genetic variations which result in an increased tendency to thrombosis. There is also now evidence that these same factors result in an increased risk of recurrence.⁵⁰ Despite this increase in understanding, the factors underlying many cases of thrombosis remain unidentified. There are clearly several areas such as thrombomodulin that are not yet adequately explored. However, recent evidence suggests that we will only fully understand these cases when we can combine several, some possibly minor, genetic factors with continuing and transient environmental factors in our assessment.

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