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. 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 study—or have not made any distinction between DVT and PE. We therefore have to rely on these data to indicate the role of genetic variation in pulmonary embolism.

A genetic tendency to thrombosis is also referred to as inherited thrombophilia and, although traditionally regarded as monogenic, it is undoubtedly a polygenic abnormality. 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.

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. 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). 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. 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...
protein S circulates in plasma bound to C4b binding protein (a regulatory factor in the complement system). It is only the free unbound protein S which can function as a cofactor for activated protein C (APC) and measurement of the total antigenic protein S may be misleading. A functional assay for protein S is therefore useful in diagnosis but interpretation may be difficult because it gives a falsely low result in the presence of factor V Leiden (see below). Addition of APC to an in vitro plasma clotting test such as the APTT causes a prolongation of the time to clot formation as a result of the accelerated degradation of FVa and FVIIIa. The field of thrombophilia changed dramatically in 1993 following the observation by Dahlback et al that, in plasma samples from one family prone to thrombosis, this effect of APC was less than normal. This phenomenon of activated protein C resistance (APCR) was later shown in >90% of cases to be due to the replacement of Arg by Gln at residue 506 in the factor V molecule (FVR506Q). This residue is at the APC cleavage site principally responsible for factor Va inactivation and the resultant factor V molecule (now frequently called factor V Leiden; FVL) is thus resistant to degradation by APC. It is easy to imagine how this may result in an increased tendency to thrombosis. The mutation responsible can readily be detected by a PCR based test and segregates both with the APCR and the thrombophilic phenotypes. FVL has a remarkable pattern of prevalence; in most of Europe the gene frequency is 5–10% but it is virtually absent in some other populations such as in the Japanese and in most Africans. Further studies have shown that FVL occurs on only a single haplo-

**Thrombophilia and risk of thrombosis**

Accurate population based data to assess the absolute risk associated with these thrombophilic disorders are lacking because prospective studies have not been done. Many of the data are from cross sectional studies of affected families which thus tend to overestimate the risk. From these studies the thrombotic risk associated with antithrombin, protein S or protein C deficiency is estimated to be approximately 2.0% per annum which is 20–30 times greater than for age matched controls. In cumulative terms there is a 70–100% chance of thrombosis by the age of 50 years. The figure for FVL is smaller at approximately 1% per annum or 5–10 times age matched controls. In addition, the Leiden thrombophilia study, a case matched control study, estimated the relative risk of venous thrombosis from APCR to be approximately 7 for heterozygotes and the increase in risk for homozygotes is probably in the region of 80-fold. It should be noted that many heterozygotes for FVL and even some homozygotes might never suffer any thrombotic complications. Unlike the causes of classical thrombophilia, FVL may have its most marked effect in the elderly.

The rare protein C or protein S homozygous deficiency state is associated with a usually fatal syndrome called purpura fulminans arising at or shortly after birth, although a number of individuals have now survived owing to vigorous intervention and support with replacement therapy. Complete deficiency of antithrombin is probably lethal.

**Newer causes of thrombophilia**

Within the complex coagulation system there are numerous other proteins, abnormality, deficiency or excess of which might be expected to cause thrombophilia. So far few of these have been clearly implicated, but some abnormal fibrinogen molecules, for example, are associated with thrombosis, although they constitute ≤1% of patients with thrombosis. A later finding to emerge from the Leiden thrombophilia study was that high levels of factor VIII (>1.5 IU/ml) were present in approximately 20–25% of patient with venous thrombosis. This study did not establish a causal relationship between factor VIII and thrombosis but it seems likely that this is so, although factor VIII is also an acute phase reactant. It will be interesting to determine whether the increased factor VIII levels in these patients has a genetic basis or not, and there is some evidence of familial clustering of factor VIII levels.

A common genetic variation in the 3' untranslated region of the prothrombin gene has recently been described which is associated with an increased tendency to thrombosis. The mutation responsible can readily be detected by a PCR based test and segregates both with the APCR and the thrombophilic phenotypes. Prothrombin G20210A is a single nucleotide polymorphism in the 3' untranslated region of the prothrombin gene. It is a common genetic variation and has been associated with an increased risk of venous thrombosis.
with raised plasma prothrombin levels and venous thrombosis.\(^2\) 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.\(^3\)

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 hyperhomocysteinemia are also associated with an increased risk of thrombosis and are present in approximately 20% of cases.\(^2\) 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 \(\beta\)-cystathionine synthase and methylene tetrahydrololate reductase (MTHFR).\(^2\) 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.\(^3\) A number of other mutations have been described subsequently and some of these do appear to have a relationship with arterial disease.\(^31\) 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, \(\alpha\)-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.\(^31\) 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-
Pulmonary embolism

Much lower than in those with DVT or DVT+. The incidence of FVL in patients with isolated PE is much lower than in those with DVT or DVT+ PE.45

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.