Molecular Risk Factors for Thrombosis

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Introduction

A diagnosis of venous thrombosis is made each year in about 0.1% of Western populations. In the majority of cases, the diagnosis is based on objective evidence for an obstruction of the circulation by a blood clot that either formed locally or developed elsewhere in the circulation. The interest in the pathogenesis of this hemostatic process has increased enormously during the past 20 years and, as a consequence, substantial progress has been made in understanding the multifactorial nature of the disease, where interactions between genetic and environmental factors result in the formation of an obstructive thrombus at a specific time and at a specific location. Frequently, these thrombi are found in the elderly in the superficial and deep veins of the legs. Thrombi may also occur in younger individuals and in the veins of the brain, mesentery, liver, or retina.

The episodic nature of the disease points out the importance of acquired and/or environmental factors in thrombosis. The disease also has strong genetic components, given the many reports of familial clustering of thrombotic events and the fact that 20% to 30% of patients with a first thrombotic event report at least one first-degree relative with thrombosis.

The search for molecular risk factors for venous thrombosis remains intensive. Over the years, this research has been facilitated by new insights in the regulation of blood coagulation and by the development of new research methods, especially in the field of molecular genetics. Today, at least one genetic defect can be found in about 70% of the families with thrombophilia.

Initial studies focussed on patients and their relatives (family studies). Such studies are very useful but can be hampered by selection bias. In other words, we know these families because the proband and some of his/her relatives have experienced thrombosis. Therefore, later studies used groups of unrelated patients and healthy controls (patient-control studies). One of the limitations of these studies is that they need to be very large to identify the less common risk factors.

The concept of venous thrombosis as a multifactorial disease has received much attention in recent years. One of the reasons is that some of the newly discovered genetic risk factors concern single point mutations that are quite common in the general population (e.g., factor V Leiden, prothrombin 20210A allele). As a result, studies focussing on the interaction of these mutations with other relatively common risk factors (e.g., use of oral contraceptives, pregnancy, surgery, but also lupus anticoagulant or mild hyperhomocysteinemia) have become feasible. In addition, researchers will continue to learn more about the relationship between these common molecular risk factors and the clinical phenotypes, namely deep vein thrombosis, pulmonary embolism, superficial thrombosis, and first versus recurrent events.

The Thrombo-Hemorrhagic Balance

The key event in venous thrombosis is the formation of a blood clot in a vein. Locally, the coagulation system has been activated and, consequently, a thrombus is formed that may either develop into an obstructive clot or become unstable and form smaller emboli that may cause an obstruction at another location. In this context, the hypothesis that thrombus formation is the result of sufficient imbalance between local fibrin formation and fibrinolysis seems logical and has continued to feed the search for thrombosis risk factors since it was first formulated by Astrup in the late 1950s.

Fibrin formation and degradation are dynamic processes. The actual kinetics of these processes will determine whether a threshold will be crossed that leads to thrombus formation. Three types of disturbances of the vascular compartment can provoke such thrombus formation (Virchow’s triad): changes in the composition of the blood, changes in the properties of the vessel wall, or changes in blood flow. Changes in the composition of the blood include the number and quality of cells and the concentration of [activated] coagulation and fibrinolytic proteins. Such changes can be genetically determined or acquired, and their potential effect on the kinetics of thrombin formation/fibrinolysis is obvious.

Normally the endothelial lining of veins possesses strong antithrombotic properties, but these can change under the influence of inflammatory reactions or by direct damage to the vessel wall. Endothelial cells also produce many proteins that are important for the regulation of the hemostatic process (e.g., von Willebrand factor, thrombomodulin, tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor (PAI-1), tissue factor (TF)). The synthesis of most of these proteins is highly regulated.

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Finally, because blood flow is very important for the delivery of substrates and nutrients to a specific site and for the removal of active intermediates and products from that site, flow conditions will have a major impact on the local kinetics of thrombin formation and fibrinolysis. Flow may also indirectly contribute to changes in blood composition through the modulation of gene expression in endothelial cells.20

From the above, it is not difficult to see how changes in all three parameters defined by Virchow can contribute to local changes in the kinetics of thrombus formation and/or fibrinolysis. Furthermore, the composition of the blood, the properties of the vascular wall, and flow conditions are defined by gene-environment interactions. This results in a model that describes venous thrombosis as a multifactorial disease that is defined by multiple interactions between genetic and environmental components.

**Molecular Risk Factors for Venous Thrombosis**

**Identification of Molecular Risk Factors**

The search for molecular risk factors for venous thrombosis has focused on changes in the composition of plasma. Guided by our understanding of the hemostatic system, candidate abnormalities (mostly genetic defects) thought to be associated with an increased risk of thrombosis were defined.21 Initially, this approach was quite successful, for example, in the discovery of antithrombin deficiency and, after the discovery of the protein C anticoagulant system, also protein C and protein S deficiencies. Later, several failures illustrated the limitations of this approach. More recently, the first results obtained using a different approach have been reported.10,22 In this approach, genetic variations associated with an increased risk of venous thrombosis are identified through sequencing of candidate thrombosis genes in selected patients and healthy controls. A disadvantage of this approach is that it can be time consuming. New technical developments that allow for the rapid screening of several candidate genes in large groups of individuals may help to overcome this problem.23 At present, it is difficult to predict what the contribution of the use of (random) genomic markers in the identification of thrombosis susceptibility loci through linkage methods will be.24,25

**Classification of Molecular Risk Factors**

Two types of molecular risk factors for venous thrombosis can be recognized: genetic factors and abnormal laboratory phenotypes. Genetic factors comprise all mutations responsible for a loss or gain of function that are associated with an increased risk of venous thrombosis in families or in patient-control studies. Genetic factors also include polymorphisms, used as markers for risk alleles, in which the causative mutation has not yet been identified, as with the polymorphisms in the protein C gene promoter.26 Gene mutations that result in a loss of function of the encoded protein are responsible for hereditary deficiencies, like antithrombin, protein C, and protein S deficiency. Such mutations prevent the synthesis of a normal protein (type I deficiency) or result in the synthesis of an abnormal protein that either is degraded intracellularly (type I deficiency) or is released into the blood as a functionally defective protein (type II deficiency). In general, there is heterogeneity in the actual genetic defects,27-30 and the spectrum of mutations observed can be strongly influenced by local founders.31,32 Laboratory diagnosis primarily seeks to identify heterozygotes and relies on the measurement of protein concentrations and/or function in plasma.

Mutations that result in a gain of function also can be found among the thrombosis risk factors, for example, the mutation in exon 10 (1691 G→A) of the factor V gene delays inactivation of the activated factor V, and the mutation in the 3’ untranslated end [20210 G→A] of the prothrombin gene results in elevated plasma prothrombin levels. Of interest, these mutations are much more common than mutations that cause loss of function. In addition, both the mutation in the factor V gene and the mutation in the prothrombin gene probably concern unique mutational events.33,34 DNA analysis seems to be the first choice for the screening of thrombosis patients for these risk markers.

The identification of abnormal laboratory phenotypes as risk factors for venous thrombosis relies almost exclusively on the analysis of large patient-control studies. Examples of these phenotypes include activated protein C (APC) resistance (reduced sensitivity to APC), mild hyperhomocysteinemia,37 and elevated factor VIII levels.38 Often these abnormal phenotypes serve as a starting point for further studies into the molecular basis of the observed abnormality. In general, this is a tedious process, partly because different unrelated mechanisms (both genetic and acquired) can produce the same abnormal phenotype.

**Defective Pathways**

In the thrombo-hemorrhagic balance, four pathways can be recognized that may harbor defects resulting in thrombotic tendency: the pro- and anticoagulant pathways and the pro- and antifibrinolytic pathways. In the past 20 years, all four pathways have been studied extensively in an attempt to identify molecular risk factors. However, all of the presently known molecular risk factors are located in the anticoagulant and procoagulant pathways. In the fibrinolytic pathways, both genetic defects (dysfibrinogenemias, hypo and dysplasminogenemias) and abnormal phenotypes (increased levels of PAI-1, decreased levels of t-PA) have been reported. Except for some rare genetic variants of fibrinogen,39 none of these are considered risk markers for venous thrombosis. Especially noteworthy is the absence of intravascular fibrin deposits in severely homozgyous plasminogen-deficient patients.39 These patients do not suffer from thrombosis but show signs of local excessive extravascular fibrin formation (e.g., ligneous conjunctivitis). Of interest is the recent observation that the clinical symptoms of these patients can be reversed by infusion of plasminogen.40 Although almost half of deep vein thrombosis (DVT) patients have increased plasminogen activator inhibitor (PAI)-1 levels, elevated PAI-1 is not considered a risk marker for venous thrombosis. Attempts to find a genetic explanation for the elevated PAI-1 have been unsuccessful.41 Such attempts are hampered by the strong influence of different environmental factors on PAI-1 gene expression42 and the very short half-life of PAI-1 in the circulation.
Mechanism Unknown

The mechanisms of two well-established molecular risk markers for thrombosis are still unknown: lupus anticoagulant (LAC) and mildly elevated plasma homocysteine levels.43 LAC is rather rare among DVT patients and refers to a group of autoantibodies that recognize epitopes on protein-lipid complexes (e.g., \( \beta_2 \) glycoprotein, prothrombin). Mild hyperhomocysteinemia (>18.5 \( \mu \text{mol/l} \)) is found in 10% of all DVT patients and is associated with a 2.5-fold increased risk of thrombosis.44 The intake of vitamins \( \text{B}_2 \) (e.g., folic acid) and a polymorphism in the methylene tetrahydrofolate reductase gene (MTHFR C677T)45 seem to be important determinants of homocysteine levels. The molecular basis of this abnormal phenotype is still unknown.

Defects in the Anticoagulant Pathways

Thrombin formation can be controlled on four different levels: tissue factor pathway inhibitor (TFPI) controls the availability of active tissue factor (TF)-factor VIIa complex; serine protease inhibitors control the activity of the activated clotting zymogens (factors Xa, IXa, Xa, Ila); the protein C anticoagulant system controls the activity of the activated cofactors factor Va and factor VIIIa; and proteins like \( \beta_2 \)-glycoprotein may be involved in the control of catalytic surface availability (phospholipid membranes). Almost all of the known genetic risk factors for venous thrombosis concern components of these anticoagulant pathways.

Antithrombin Deficiencies

Antithrombin deficiency was first reported by Egeberg in 196526 in a family with thrombophilia where partial antithrombin deficiency was found to segregate with the thrombotic tendency. From this report, we learned that heterozygotes were at-risk for developing the disease and that the penetrance of the disease in carriers of the defect is incomplete. In addition, Egeberg’s findings were consistent with the general notion that hereditary disorders of blood coagulation were single gene disorders. Since 1965, many families with antithrombin defects have been reported, confirming Egeberg observations.47,48 Laboratory findings in the plasma of the patient and, later, the type of mutation have been used for the classification of antithrombin deficiencies.49,52 No homozygous antithrombin deficiencies have been reported, which underlines the important role of antithrombin in the regulation of thrombin generation. The observation that homozygotes for a heparin-binding defect in antithrombin have increased risk of arterial and venous thrombosis at young age50 confirms the important role of the interaction of antithrombin with glycosaminoglycans in the control of thrombin formation in vivo. Numerous reports on mutations in antithrombin deficiency have been published. At present, more than 79 different mutations in the antithrombin gene have been reported.52 Heterozygosity for antithrombin deficiency, which is associated with a five-fold increased risk of thrombosis,51 is found in 0.05% to 1.0% of healthy individuals,52 1% of consecutive patients with a first DVT,51 and 4% of families with inherited thrombophilia.23,53

Protein C Deficiencies

The first reports on families with protein C deficiency (type I or type II) and thrombotic disease date from the early 1980s.54-55 Essentially, the findings were identical to those in antithrombin deficiency: an autosomal dominant disorder, where heterozygous deficient individuals are at-risk; incomplete penetrance of the disease in carriers of the defect; and support for the concept that familial thrombophilia is a single gene disorder. These observations were confirmed in many subsequent reports on protein C deficiency in thrombotic families and have been reviewed elsewhere.56 Later, strong arguments were presented proposing that protein C deficiency is, in fact, an autosomal recessive disorder and that only the homozygous or double heterozygous individuals will develop thrombosis.57-59 For a long time, the molecular basis of these apparently conflicting observations was not understood, although it was hypothesized that, in thrombophilic families, epistatic interactions of the protein C deficiency with other unknown genetic defects might explain the thrombotic risk of heterozygous carriers of the protein C gene defect.5 Only in 1995 was the first firm support for this hypothesis published. In about 20% of the thrombophilic families with protein C deficiency, factor V Leiden was also segregating, and the penetrance of thrombosis was much higher in individuals carrying two defects than in those carrying only one defect.50

The first report on protein C gene mutations was published in 1987. Using aberrant Pvu II restriction patterns as a guide, Romeo and colleagues61 identified the protein C gene mutations in two Dutch, protein C-deficient patients. After the introduction of polymerase chain reaction (PCR) techniques, screening for genetic defects in the protein C gene became much easier,31 and many different mutations were identified. The last published database of protein C gene mutations includes 160 different independent entries.28 Mutation analysis also demonstrated that identical protein C gene mutations could be found in symptomatic individuals from thrombophilia families and in the asymptomatic protein C-deficient relatives of homozygous protein C-deficient patients.28 Heterozygosity for protein C deficiency is associated with a seven-fold increased risk of venous thrombosis51,62 and is found in 0.3% of healthy individuals,60,63 3% of consecutive patients with a first DVT,51 and in about 6% of selected thrombophilia patients.53

Protein S Deficiencies

Protein S is an important anticoagulant protein. It is the nonenzymatic cofactor of APC in the inactivation of factors Va and VIIIa and also has an APC-independent anticoagulant activity. Protein S deficiency, therefore, is a good candidate risk factor for thrombosis. Unfortunately, the laboratory screening for such deficiencies is complicated by the fact that circulating protein S (PS) exists in two forms.54 Free PS, which is active as APC cofactor, forms about 40% of the total PS, whereas 60% consists of a complex of PS with C4 binding protein, which has no APC-dependent anticoagulant activity. Unfortunately, the laboratory screening for such deficiencies is complicated by the fact that circulating protein S (PS) exists in two forms.54 Free PS, which is active as APC cofactor, forms about 40% of the total PS, whereas 60% consists of a complex of PS with C4 binding protein, which has no APC cofactor activity. Discussions have focussed on the specificity of the various assays (free or total PS) and discovering which protein S form should be measured to identify PS deficiencies (PS activity, free PS, or total PS). Only recently, it has been convincingly
demonstrated that free PS measurements are better able to discriminate between heterozygous PS-deficient and normal individuals than total PS measurements.\textsuperscript{55,66}

The first reports on the possible association of (partial) PS deficiency with venous thrombosis appeared in December 1984.\textsuperscript{67-69} In the following years, more systematic analysis of collectives of families with PS deficiency and thrombophilia confirmed that, in these families, a partial deficiency of PS (type I deficiency) was associated with the occurrence of venous thrombosis.\textsuperscript{70-72} In fact, the results of these analyses very much resembled those in thrombophilia families with antithrombin or protein C deficiency: autosomal dominant inheritance, heterozygous carriers of the deficiency are at-risk (incomplete penetrance, relatively late-onset of symptoms, and a high percentage of spontaneous events). So far, only two patients with severe homozygous PS deficiency have been reported.\textsuperscript{73,74} These patients have symptoms very similar to those in homozygous PC deficiency.\textsuperscript{75} Only one of these is a compound heterozygote,\textsuperscript{74} suggesting that the prevalence of PS gene mutations in the population is much lower than that of PC gene mutations. This might explain why PS deficiency was not identified as a risk factor for venous thrombosis in the Leiden thrombophilia study.\textsuperscript{74} A recent observation has indicated that, in about 40% of the thrombophilia families with a documented PS deficiency, the factor V Leiden mutation also segregates.\textsuperscript{75,76} In addition, in these families, carriers of two gene defects have thrombosis earlier in life and more frequently than carriers of a single defect.

In 1987, it was reported that the human genome contained one active PS gene and one inactive PS pseudogene.\textsuperscript{77} After the resolution of the structures of these two genes, PS-deficient patients were screened for mutations in the PS gene.\textsuperscript{78,79} As a result, the database on PS gene mutations now contains 70 different mutations.\textsuperscript{29} Some investigators reported PS gene mutations in only some patients with PS deficiency.\textsuperscript{79} Others seem to be more successful.\textsuperscript{80}

An interesting variant of PS is PS (Pro460→Ser) or PS Heerlen.\textsuperscript{81} Heterozygous carriers of this variant, which lacks one of three glycosylation sites, have slightly reduced free PS concentrations,\textsuperscript{82,83} probably due to the more rapid clearance of free PS Heerlen. Analysis of the Leiden thrombophilia study showed that the PS Heerlen genotype is not associated with an increased risk of thrombosis. Most of the PS deficiencies are type I or mixed type I/type III (type III: reduced free PS, normal total PS). Very few type II PS deficiencies have been reported.\textsuperscript{29} In family studies,\textsuperscript{70,72} heterozygous carriers of a PS gene defect have a six- to ten-fold increased risk for venous thrombosis. However, this could not be confirmed in patient-control studies.\textsuperscript{51,83} The prevalence of heterozygotes in the population is not known. It may be 1% to 2% in consecutive patients with DVT\textsuperscript{4,51} and 6% in families with thrombophilia.\textsuperscript{84}

**Thrombomodulin Defects**
Thrombomodulin is a key protein in the PC anticoagulant pathway.\textsuperscript{85,86} Unfortunately, its location in the endothelial cell membranes lining the vessel wall and its absence from circulating blood have severely hampered the study of thrombomodulin defects in relation to thrombotic disease. Sequencing of thrombomodulin genes in 28 selected patients with a family history of unexplained DVT did not reveal any sequence variation (data not shown). Later studies in a larger group of thrombosis patients revealed several sequence variations that may affect thrombomodulin expression or function.\textsuperscript{87,88} At present, family studies are incomplete, and no conclusions can be drawn on the association of these mutations with venous thrombotic risk. At a maximum, 5% of thrombosis patients may have defects in one of their thrombomodulin genes. Interestingly, there are some recent reports on thrombomodulin mutations and polymorphisms that might increase the risk of arterial thrombosis, especially in combination with other risk factors. Confirmation of these data will be needed.

**Tissue Factor Pathway Inhibitor**
A defect in the tissue factor pathway inhibitor (TFPI) gene is an important candidate risk factor for venous thrombosis. Unfortunately, TFPI is distributed over different compartments.\textsuperscript{91} A large fraction binds to glucosaminoglycans of the endothelium, while in the blood, more than 80% of the circulating TFPI is bound to lipoproteins. This makes it very difficult to relate plasma TFPI levels (functional, free, or total) to gene activity.\textsuperscript{92,93} Nevertheless, there is one report on a family with thrombophilia with two siblings with partial TFPI deficiency.\textsuperscript{94} In our laboratory we have tried an alternative approach. After resolution of the intron-exon organization of the human TFPI-gene, we have sequenced the TFPI genes of the 28 patients of our thrombophilia panel. No single sequence variation was observed. Recently Kleebsink et al.\textsuperscript{95} reported a mutation (Pro151→Leu) in exon 7 of the TFPI gene that might be considered a candidate risk factor. More extensive studies are needed, however.

**Defects of Other Anticoagulant Proteins**
Deficiencies in two other anticoagulant proteins have been considered as candidate risk factors of thrombosis: heparin cofactor II\textsuperscript{96} and \( \beta_2 \) glycoprotein-I.\textsuperscript{97} Heterozygosity for deficiencies of these proteins is rather common (heparin cofactor II deficiency: 1% to 2%; \( \beta_2 \) glycoprotein-I deficiency: 8% to 10%). These genetic defects are not considered risk factors for thrombosis, although they may be found in thrombophilia families.

**Activated Protein C (APC) Resistance and Mutations in Factor V**
In 1993, Dahlbäck et al.\textsuperscript{98} described three unrelated patients with thrombosis who carried a newly identified defect in the protein C anticoagulant pathway, resulting in the poor anticoagulant response of plasma to activated protein C (APC resistance). In the family of one of these patients, 13 relatives with APC resistance were observed—four of which had experienced thrombotic episodes. The investigators proposed that the APC resistance was caused by an inherited deficiency of a
previously unrecognized cofactor to APC. Later that year Griffin et al\(^9\) reported that about 60% of selected unrelated thrombosis patients have APC resistance, and Koster et al\(^10\) demonstrated that the laboratory phenotype of APC resistance is a common, and often hereditary, risk factor for a first deep vein thrombosis. The nature of the new APC cofactor, which was deficient or defective in APC-resistant individuals, was identified early in 1994. Dahlbäck and Hildebrand purified the protein from plasma and arrived at the conclusion that the new APC cofactor was identical to the procofactor factor V.\(^9\) The same conclusion was reached by Bertina et al\(^11\) using plasma mixing experiments and linkage analysis in a large family with APC resistance. They also found that 80% of all individuals with APC resistance carried a mutation in exon 10 of the factor V gene (1691 G → A) that results in the replacement of Arg506→Gln in one of the three cleavage sites for APC (factor V Leiden). Within 2 months, the same mutation was reported independently by two other groups.\(^100,101\) Obviously, the Arg506→Gln mutation in factor V delays its inactivation by APC.\(^102,103\) and, thus, prolongs the life span of the procoagulant factor Va. On the other hand, the mutation clearly abolishes the APC cofactor activity of factor V,\(^104\) which was found to act synergistically with protein S in the inactivation of factor VIIIa.\(^105\) So, the same mutation in factor V may affect both procoagulant and anticoagulant pathways. The anticoagulant properties of factor V have been related to a region in the B domain of factor V.\(^106\)

The factor V Leiden mutation is rather common in Caucasian populations,\(^107\) but there are important regional differences in prevalence (2% to 16%). Heterozygous carriers have a seven-fold increased risk of venous thrombosis, whereas homozygous carriers have an 80-fold increased risk.\(^108\) About 18% of patients with a first DVT\(^108\) and about 40% of thrombophilia families carry the mutation.\(^9\) The high prevalence of this mutation among thrombosis patients, helped to establish familial thrombophilia as a multifactorial disease.\(^60,7,8\) There are strong interactions with PC deficiencies,\(^60,109\) PS deficiencies,\(^75,76\) and the prothrombin 20210A allele.\(^110\) Factor V Leiden is not only a risk factor for DVT but also for cerebral vein thrombosis\(^111\) and superficial vein thrombosis,\(^112\) but not for retinal vein thrombosis.\(^113\) The mutation is not a strong risk factor for (primary) pulmonary embolism.\(^114,115\) Its role in the pathogenesis of arterial thrombosis remains controversial.

When using diluted plasma to measure sensitivity to APC, 20% or more (depending on the actual assay and cut-off point) of all APC-resistant cases identified will not carry the factor V Leiden mutation.\(^9\) A very few will carry the factor V Cambridge (Arg306→Thr) mutation,\(^116\) and homozygosity for the HR2 haplotype of the factor V gene\(^117\) also might cause APC resistance (and thrombotic risk).\(^118\) Other findings indicate that another mutation at Arg306 in factor V (Arg306→Gly), which has been found among Hong Kong Chinese both in thrombosis patients and in healthy controls, is not associated with APC resistance.\(^119\) In addition, there are several conditions associated with APC resistance not caused by factor V defects (as reflected by a normal APC sensitivity after dilution in factor V-deficient plasma): pregnancy, oral contraceptive use, elevated factor VIII levels, lupus anticoagulant, and stroke. The molecular basis of these types of APC resistance is mostly unknown. Recently, De Visser et al\(^120\) demonstrated, in the Leiden Thrombophilia Study, that this type of APC resistance (non-Factor V Leiden) is also a risk factor for DVT, although less strong than the APC resistance associated with factor V Leiden. The thrombosis risk was not influenced by variables, such as gender, current use of oral contraceptives, or PS or factor X levels, but was, importantly, reduced after adjustment for factor VIII levels, which itself is a risk factor for venous thrombosis.\(^38\) Whether it is important to screen patients for this type of APC resistance needs to be discussed, especially after the recent report that this phenotype might be a prominent predictor of advanced atherosclerosis and arterial disease.\(^121\)

### Defects in Procoagulant Pathways

Abnormalities in procoagulant factors, which result in enhanced fibrin formation, mostly will concern elevated levels and/or function of procoagulant factors. These can be genetically determined (e.g., variations in gene-promoter sequences) or be the result of an interaction with the environment. The first report on the possibility of a genetic defect in a procoagulant factor was published in 1966 and concerned a family with thrombophilia and elevated levels of factor V activity.\(^112\) However, in a recent study, we found no support for the hypothesis that elevated factor V antigen is a risk factor for venous thrombosis (unpublished data). To determine whether an increased level of a procoagulant factor is a risk factor for thrombosis requires the analysis of large patient-control studies. Measurements in families are difficult because of the often unknown contributions of non-genetic factors (gender, age, body mass index, oral contraceptive use) to the protein levels. Nevertheless, there is now sufficient evidence that elevated levels of factor II, factor VIII, and fibrinogen can be considered independent risk factors for venous thrombosis.\(^10,123\) Presently, however, there is no support for the hypothesis that elevated levels of factor VII, factor XII, or factor X could be risk markers of venous thrombosis\(^123,124\) (unpublished data).

### Elevated Prothrombin Levels

The mutation in the prothrombin gene that is associated with an increased risk of venous thrombosis was identified by sequencing the prothrombin genes of selected patients with, at that time, unexplained familial thrombophilia.\(^10\) It concerns a G→A transition in nucleotide 20210 in the 3’ untranslated region of the prothrombin gene. The mutation is associated with elevated prothrombin levels and elevated prothrombin levels (>115%) are associated with a 2.1-fold increase in thrombosis risk.\(^30\) These data have been confirmed independently in a number of studies.\(^110\) It is not yet known whether this mutation itself causes the elevated plasma prothrombin or whether it is in linkage disequilibrium with the causative mutation. No other functional sequence variations have been identified in the coding and flanking regions and in 1 kb of the promoter region of the prothrombin 20210A allele. Like in the case of the factor V Leiden mutation, there is strong evidence for a single founder haplotype of the prothrombin 20210A allele.\(^34\)
Heterozygous carriers of the 20210A allele have a 2.8-fold increased risk of thrombosis, both deep vein thrombosis and cerebral vein thrombosis. This mutation interacts strongly with some other genetic risk factors (factor V Leiden and PS deficiency), but surprisingly, not with all risk factors (i.e., PC deficiency). In addition, the interaction with current oral contraceptive use is prominent. The role of the prothrombin 20210A allele in the pathogenesis of arterial thrombosis (especially stroke) is still a matter of debate and has been reviewed elsewhere. The prevalence of the mutation in Caucasian populations is about 2%. The prevalence is 6% among unselected patients with thrombosis and about 18% in families with unexplained thrombophilia.

**Elevated Factor VIII Levels**

It has been known for a long time that there is a relatively high prevalence of non-O blood groups among patients with venous thrombosis. This is of interest because these blood groups are associated with elevated levels of von Willebrand factor and factor VIII, the procofactor for factor IXa. Von Willebrand factor is the carrier protein of factor VIII and protects factor VIII against inactivation by APC.

Recently, Koster et al. reported that in the Leiden thrombophilia study blood group (non-O), elevated vWF (≥150%) and elevated factor VIII activity (≥150%), all were associated with increased thrombotic risk, and that after adjustment for blood group and von Willebrand factor level, the risk associated with elevated factor VIII was not affected. This identified elevated factor VIII as a common molecular risk factor for venous thrombosis. Approximately 25% of all DVT patients and 10% of healthy controls have a factor VIII:C above 150%. Later, similar findings have been reported by others. The molecular defects and/or mechanisms that underlie these elevated factor VIII levels are still largely unknown. Study of the aggregation of factor VIII levels in families points to a familial, possibly genetic influence on factor VIII levels, independent of von Willebrand factor (vWF) and blood group.

**References**


