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Thrombophilia as a multigenic disease

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ABSTRACT

Background and Objective. Venous thrombosis is a common disease annually affecting 1 in 1000 individuals. The multifactorial nature of the disease is illustrated by the frequent identification of one or more predisposing genetic and/or environmental risk factors in thrombosis patients. Most of the genetic defects known today affect the function of the natural anticoagulant pathways and in particular the protein C system. This presentation focuses on the importance of the genetic factors in the pathogenesis of inherited thrombophilia with particular emphasis on those defects which affect the protein C system.

Information sources. Published results in articles covered by the Medline® database have been integrated with our original studies in the field of thrombophilia.

State of the Art and Perspectives. The risk of venous thrombosis is increased when the hemostatic balance between pro- and anti-coagulant forces is shifted in favor of coagulation. When this is caused by an inherited defect, the resulting hypercoagulable state is a lifelong risk factor for thrombosis. Resistance to activated protein C (APC resistance) is the most common inherited hypercoagulable state found to be associated with venous thrombosis. It is caused by a single point mutation in the factor V (FV) gene, which predicts the substitution of Arg506 with a Gln. Arg506 is one of three APC-cleavage sites and the mutation results in the loss of this APC-cleavage site. The mutation is only found in Caucasians but the prevalence of the mutant FV allele (FV:Q506) varies between countries. It is found to be highly prevalent (up to 15%) in Scandinavian populations, in areas with high incidence of thrombosis. FV:Q506 is associated with a 5-10-fold increased risk of thrombosis and is found in 20-60% of Caucasian patients with thrombosis. The second most common inherited risk factor for thrombosis is a point mutation (G20210A) in the 3' untranslated region of the prothrombin gene. This mutation is present in approximately 2% of healthy individuals and in 6-7% of thrombosis patients, suggesting it to be a mild risk factor of thrombosis. Other less common genetic risk factors for thrombosis are the deficiencies of natural anticoagulant proteins such as antithrombin, protein C or protein S. Such defects are present in less than 1% of healthy individuals and together they account for 5-10% of genetic defects found in patients with venous thrombosis. Owing to the high prevalence of inherited APC resistance (FV:Q506) and of the G20210A mutation in the prothrombin gene, combinations of genetic defects are relatively common in the general population. As each genetic defect is an independent risk factor for thrombosis, individuals with multiple defects have a highly increased risk of thrombosis. As a consequence, multiple defects are often found in patients with thrombosis.

Key words: APC resistance, protein C, protein S, antithrombin, factor V, thrombosis

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Familial thrombosis is a well recognized medical entity, but the inherited risk factors underlying the disease have until recently remained elusive. During the last 30 years, several anticoagulant proteins have been discovered and biochemical studies in conjunction with identification of deficiency states in thrombosis patients, have been instrumental for the elucidation of their physiologic functions. The first genetic defect in thrombosis patients was deficiency of antithrombin (AT), which was described in 1965.[1] AT-deficiency is a rare genetic defect found in 1-2% of venous thrombosis patients. The unravelling of the protein C system in the 1970s and 1980s and the following identification of thrombosis patients with deficiency of protein C or protein S, provided a genetic explanation for 5-10% of cases with familial thrombosis. Two common genetic risk factors for venous thrombosis have been identified in recent years. In 1993, inherited resistance to activated protein C (APC resistance) was demonstrated in several families with thrombophilia.[2] Shortly thereafter, several extensive studies found APC resistance to be a very common inherited risk factor for thrombosis, present in 20-60% of the patients.[3-5] The APC resistance phenotype was found to be corrected by the addition of factor V (FV) to APC-resistant plasma, suggesting that the molecular defect is located in the factor V gene.[6] The exact genetic defect was reported, in the spring of 1994, to be a single point mutation, a G-to-A transition at nucleotide position 1691 in the factor V (FV) gene.[7-11] The mutation predicts the substitution of arginine (R) at position 506 by

glutamine (Q). In the mutant FV, which is referred to as FVR506Q, FV:Q506 or FV Leiden, one of three APC-cleavage sites is lost which results in the hypercoagulable state. In 1996, Poort *et al.* identified a variant of the prothrombin gene as a risk factor for venous thrombosis.[12] A G-to-A transition at position 20210 in the 3' untranslated region of the prothrombin gene was found to be present in 18% of probands of thrombophilic families, in 6% of unselected thrombosis patients and in 2% of healthy controls. The mutation is associated with slightly increased prothrombin levels in plasma, which may be the molecular mechanism behind the increased thrombosis risk. Several other candidate genes have been suggested to be linked to thrombophilia, such as the genes for thrombomodulin, fibrinogen, plasminogen, plasminogen activator inhibitor type I, and heparin cofactor II. However, abnormalities of these genes are infrequent and will not be discussed further.

Natural anticoagulation

Owing to the potency of the blood coagulation system strict regulation is vitally important. During activation of the coagulation system, several serine proteases with high procoagulant capacity are produced. The activity of these proteases is regulated by the composition of the phospholipid surface upon which the coagulation reactions occur, by protein cofactors and by protease inhibitors. Antithrombin (AT) is a regulator of several of the coagulation enzymes. It circulates as a single-chain glycoprotein (M_r = 58,200) with a plasma concentration of 2 µM, which is far higher than the concentrations of the target enzymes.[13] AT is a member of the serpin family, which is a large family of proteins with similar structure and mechanism of action. Serpins inhibit the target enzymes by acting as pseudo-substrates and the serpin-enzyme interaction results in the formation of stable, enzymatically inactive bimolecular complexes between the enzyme and the serpin.[14] AT in itself is a relatively inefficient inhibitor and the rate of thrombin inactivation by AT alone is too slow to prevent coagulation. The activity of AT is stimulated by heparin, which accelerates the rate of inhibition of the enzyme.[15] During inhibition of thrombin, heparin functions as a bridge between thrombin and AT.[16] In addition, heparin induces conformational changes in AT, transforming it into a more efficient inhibitor. In the inhibition of FXa, the conformational change of AT appears to be more important than the bridging mechanism.[17,18] Under normal physiological conditions, there is no heparin exposed to circulating blood. However, heparan sulfate proteoglycans present on the endothelial cell surface play a part similar to that of heparin and stimulate the activity of AT.

The protein C system is a natural anticoagulant pathway which regulates the activity of the procoagulant factor Va (FVa) and factor VIIIa (FVIIIa), key enzyme cofactors of the coagulation process.[19] Thrombin activates the protein C system on the surface of intact endothelial cells, where it binds with high affinity to a cell-bound receptor, thrombomodulin. Upon binding to thrombomodulin, thrombin loses its procoagulant properties and its substrate specificity is shifted towards protein C. Activated protein C (APC) inhibits coagulation by cleaving and inactivating membrane-bound FVa and FVIIIa. These reactions are potentiated by the non-enzymatic cofactor protein S. In plasma, protein S circulates both as free protein (40%) and bound to C4b-binding protein, a regulator of the complement system.[19] Only the free form of protein S is active as an APC cofactor.[20] Recently, FV has been found to function as a cofactor to APC in the degradation of FVIIIa.[6,21] In the presence of protein S, inactivation of FVIIIa by APC was found to be enhanced 2-7-fold by intact FV, but not by FVa.[22-25] The conclusion from these studies was that intact FV and protein S act synergistically to potentiate the APC-mediated degradation of FVIIIa.[26] The anticoagulant properties of FV may explain the relatively mild bleeding symptoms observed in patients with FV deficiency (parahemophilia) and the paradoxical thromboembolism found in FV deficiency.[27]

Molecular basis of inherited APC resistance

One of three APC-cleavage sites in the heavy chain of normal FVa (Figure 1) is lost as a result of the FVR506Q mutation.[7,28-31] During the degradation of membrane-bound normal FVa, the APC-mediated cleavage at Arg 506 occurs at a 10-fold higher rate than that at Arg 306 or Arg 679.[28-34] As a consequence, mutated FVa is inhibited at a 10-fold lower rate than normal FVa (Figure 2).[28-31,33,34] The decreased rate of APC-mediated degradation of FVa results in increased thrombin formation, as reflected by elevated levels of prothrombin activation fragments (F1+2) and thrombin-antithrombin complex (TAT) in plasma of APC resistant patients.[32-37] An additional explanation for the prothrombotic effect of the FV:R506Q mutation may be that FV:Q506 works as a poor APC cofactor in the degradation of FVIIIa.[22] The decreased APC-cofactor activity of FV:Q506 presumably contributes to the increased thrombin generation and may explain why the addition of normal FV to APC-resistant plasma results in a correction of the poor response to APC (Figure 2).[6] A third possible procoagulant effect conferred by FV:Q506 is mediated through enhanced activation of TAFI (thrombin actifiable fibrinolysis inhibitor), resulting from the increased thrombin generation.[38] As TAFI inhibits fibrinolysis through the release of carboxy-terminal lysines from fibrin, clots formed in APC-resistant individuals may have an increased resistance to fibrinolytic attack (Figure 2).[38,39] It is not yet known whether increased TAFI activation explains why thrombosis patients with APC resistance appear to be less prone than other thrombosis patients to developing pulmonary embolism (see below).[40-43]

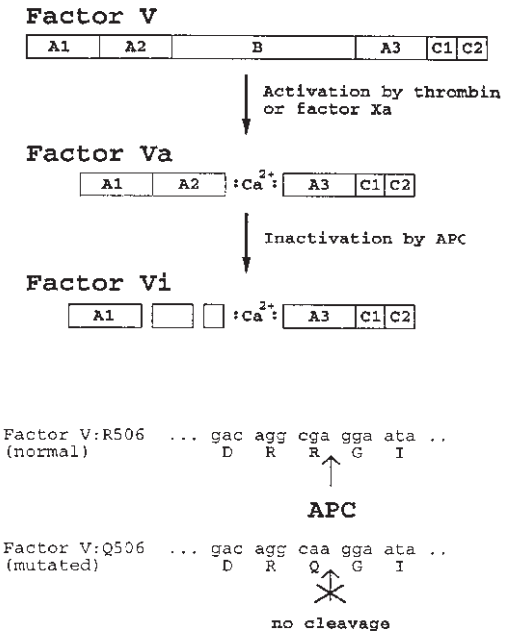

Figure 1. Schematic representation of proteolytic processing of factor V. Factor V is a large single chain glycoprotein with the domain organization A1-A2-B-A3-C1-C2. It is activated to factor Va via limited proteolysis mediated by thrombin or factor Xa. The factor Va molecule is composed of heavy (A1 and A2 domains) and light chains (A3, C1 and C2 domains). During the inactivation process, activated protein C (APC) cleaves factor Va at Arg506, Arg306 and Arg679. The nucleotide and the translated amino acid sequences around the APC-cleavage site at Arg506 in the A2 domain are shown. A single guanine (g) to adenine (a) mutation at nucleotide position 1691 in the factor V gene predicts the replacement of arginine 506 (R) by a glutamine (Q). This substitution prevents the APC-mediated inactivation of factor Va at this cleavage site and is the molecular basis of APC resistance.

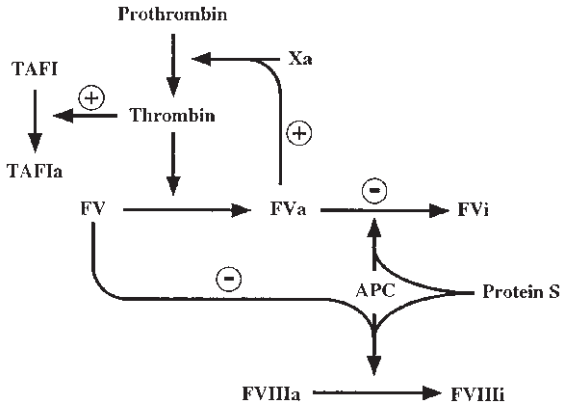

Figure 2. Schematic representation of the prothrombotic effect on the blood coagulation, the fibrinolytic system and the protein C anticoagulant system of the FV:Q506 mutation. The affected steps in the various proteolytic reactions are shown. Plus (+) and minus (–) signs denote increased and decreased activities, respectively. The mutation leads to a decreased inactivation of the factor Va and thus an increased factor Va activity. The increased factor Va activity results in increased thrombin generation which in turns leads to an increase in the overall blood coagulation activity. The increase in thrombin generation will also result in increased formation of TAFIa, an inhibitor of the fibrinolytic system. The FV:Q506 also has impaired anticoagulant activity in the protein C system. The mutated FV:Q506 has been reported to be a poor APC cofactor in the APC-mediated degradation of FVIIIa.

Other genetic defects in factor V associated with APC resistance

Individuals with a quantitative deficiency in FV display an APC resistant phenotype only if they are homozygous.[44] Moreover, the combination of heterozygosity for both FV deficiency and FV:Q506 results in pseudohomozygous APC resistance.[45] A FV haplotype denoted HR2, a constellation of 6 polymorphisms in FV, is associated with slightly decreased APC response.[46,47] The mechanism involved is not clear and may be related either to the amino acid substitutions predicted by the HR2 haplotype or to linkage disequilibrium with another mutation in the FV gene. However, as the frequency of the HR2 haplotype was found to be similar in thrombosis patients and healthy controls it does not appear to be a risk factor for thrombosis.[46] Mutations affecting other APC cleavage sites of FVa or FVIIIa could potentially give rise to APC resistance, hypercoagulability and an increased risk of venous thrombosis. Recently, Williamson *et al.* reported a new FV mutation (FV Cambridge, FV:R306T) associated with APC resistance.[48] In a group of 17 patients with thrombosis and unexplained APC resistance they found a G-to-C mutation in the codon of Arg306. As the mutation was neither found in 585 thrombotic patients nor in 226 healthy blood donors it can be concluded that the mutation is rare. Chan et al. found another mutation affecting Arg306 (FV Hong Kong, FV:R306G).[49] This mutation was present in two of 43 Hong Kong Chinese patients with venous thromboembolism and in one of 40 healthy controls. Thus, it remains to be demonstrated whether FV Hong Kong is a risk factor for thrombosis.

Clinical epidemiology of APC resistance

The main clinical manifestation of inherited APC resistance is deep venous thrombosis, although superficial thrombophlebitis and pulmonary embolism also occur.[11,50] In families with inherited APC resistance, the thrombotic risk was found to be determined by the FV genotype.[11] The annual incidence of venous thromboembolism among relatives was 0.18% for those with normal FV genotype, 0.37% for heterozygous and 1.0% for homozygous carriers of FV:Q506.[11] Similar annual incidences of venous thromboembolism were reported in a recent family study; 0.45%

in those with heterozygosity for FV:Q506 and 0.10% in those without the mutation, giving a relative risk of 4.2.[51] From case-control studies, the increase in risk of venous thrombosis has been calculated to be 5-10-fold for heterozygous individuals and 50-100-fold for homozygous individuals.[7,52] A large prospective study suggested the calculated relative risk for venous thrombosis to be 2.7 for heterozygotes.[52a] Similar results were obtained in a prospective study of out patients with thrombosis (relative risk of 3.1, CI 1.7-5.5).[53,54] Though the penetrance of symptoms is high among homozygotes, some homozygous individuals remain asymptomatic throughout their lives.[11,52,55,56]

The FV:Q506 allele is confined to Caucasians, while it is absent in indigenous populations of Asia, Africa, America and Australia.[57-59] In the Western world the average prevalence of the mutation is approximately 5%. The high incidence of thromboembolism in Western communities, as compared to those of Asian and African populations, is at least in part due to the high prevalence of the FV:Q506 allele. Haplotyping of individuals homozygous for the FV:Q506 allele suggested a founder effect and that the single mutational event occurred 21,000 to 34,000 years ago.[60-62] Thus, all carriers of the FV:Q506 allele have descended from a common ancestor. The high prevalence of the FV:Q506 allele makes it reasonable to suppose that the mutation has conferred selective advantage(s). The hypercoagulability associated with the mutation may have provided protection against fatal blood loss after injuries, childbirth or against iron deficiency, especially during periods of famine. In accordance with this hypothesis, Lindqvist *et al.* recently demonstrated that carriers of the FV:Q506 allele have significantly lower risk of intrapartum bleeding complications.[63]

The G20210A mutation in the prothrombin gene

A G-to-A transition at nucleotide 20210 of the prothrombin gene has been identified as the second most common independent risk factor for venous thrombosis.[12] The mutation is located in the 3' untranslated region of the gene (Figure 3) and thus it does not alter the amino acid sequence of the prothrombin molecule. The mechanism by which this mutation leads to an increased risk of thrombosis is not fully understood even though it has been shown that the mutation is associated with increased plasma levels of prothrombin. The prevalence of the mutation in the general population is between 1-4% and it is more common in southern than in northern Europe.[64] From clinical studies, it has been concluded that the 20210 A allele of the prothrombin gene is associated with an approximately 3-fold increased risk of venous thrombosis.[64-68]

Protein C deficiency

Heterozygous deficiency of protein C is present in 2 to 5% of thrombosis patients and in 0.3-0.5% of healthy controls, suggesting an associated 10-fold increased risk of venous thrombosis.[69] Type I protein C deficiency is characterized by a parallel reduction in protein C antigen and functional activity, whereas the less common type II is associated with functionally abnormal protein C molecules. Homozygous, and compound heterozygous, protein C deficiency is

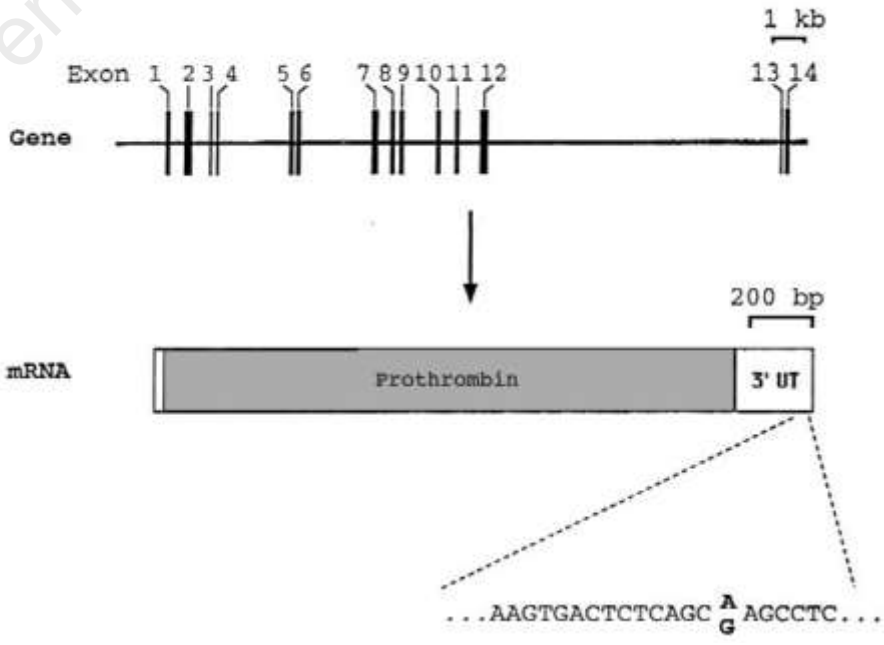

Figure 3. Structure of the human prothrombin gene and localization of the prothrombin 20210 G→A mutation. The human gene for prothrombin comprises 14 exons and spans approximately 20 kb of DNA on chromosome 11. Exons are denoted by black bars and introns by lines. The prothrombin gene mRNA is approximately 2.1 kb in size and it is composed of a translated region (shaded box) and a 5' and 3' untranslated regions (open boxes). The nucleotide sequence flanking the G→A transition at nucleotide 20210 (indicated in bold) in the 3' untranslated region of the gene is shown below.

a rare condition associated with neonatal coagulopathy resulting in purpura fulminans with skin lesions, disseminated intravascular coagulation and potentially irreversible brain damage. Genetic analysis of a large number of cases with protein C deficiency (160 different mutations known) has demonstrated missense mutations, resulting in single amino acid substitutions, to be the most common genetic problem (Figure 4).[70,71] Mutations in the promotor region affecting the concentration of protein C in plasma and mutations affecting RNA splicing have also been found. Type II deficiency accounts for approximately 10% of protein C deficient patients. Most of the mutations leading to type II deficiency are located in the phospholipid binding Gla domain or in the serine protease domain.

Protein S deficiency

Heterozygous protein S deficiency is present in 2 to 5% of thrombosis patients, but its prevalence in the general population is not known.[72] However, family studies have suggested the associated risk of venous thrombosis to be similar to that in patients with protein C deficiency or APC resistance.[72-74] It has been found that the level of free protein S in plasma discriminates better between those with and without protein S deficiency than the level of total protein S.[75-77] Type I deficiency is characterized by low levels of both free and total protein S. A variant of protein S deficiency with low free protein S and normal total protein S has been believed to constitute a separate genetic type (type III). However, the demonstration of coexistence of the two types in several protein S defi-

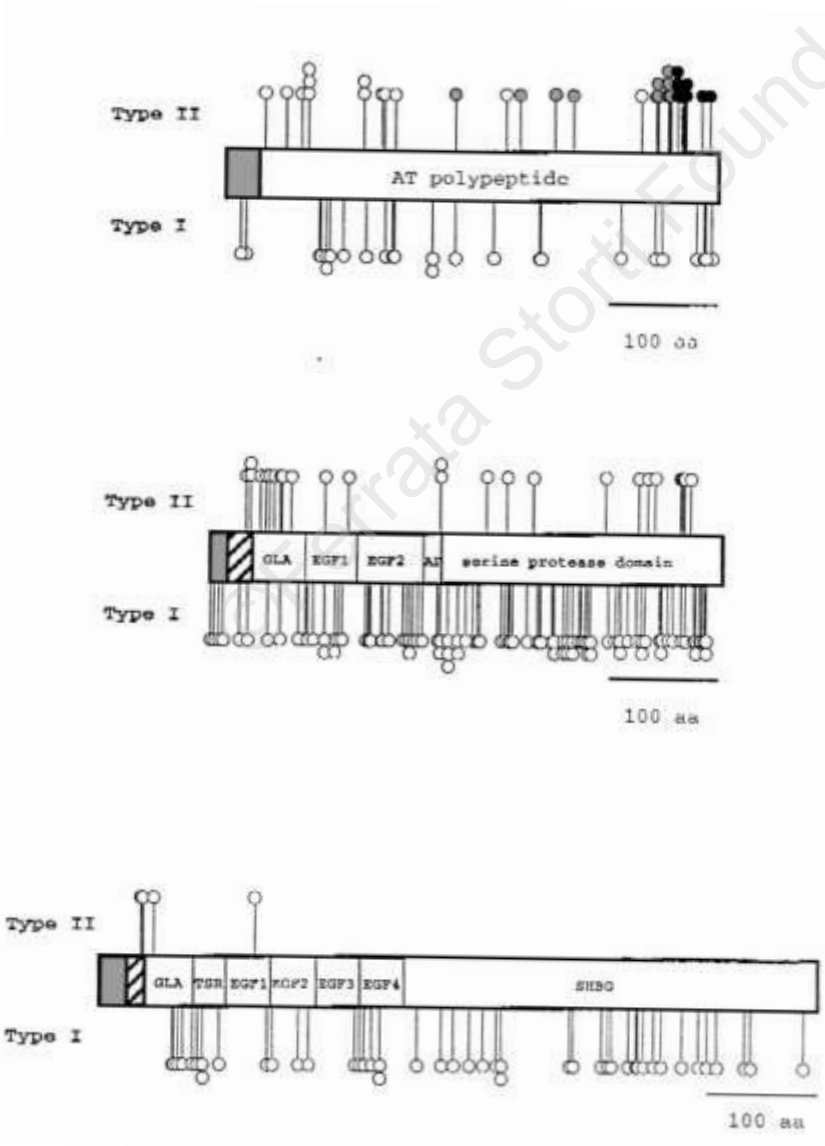

Figure 4. Detrimental missense mutations in protein C, protein S and AT. Protein C is synthesized as a 461 amino acid residue long prepro molecule (upper part). The presequence (shaded) serves as a signal peptide and the pro-sequence (hatched) functions as a signal for proper γ-carboxylation of the protein. The mature protein consists of 419 residues and can be divided into a γ-carboxy glutamic acid (Gla) domain, two epidermal growth factor (EGF)-like domains and a serine protease domain. The activation peptide (AP) is released upon protein C activation. The circles indicate the localization of known missense mutations, leading to amino acid substitutions and type I or type II deficiency. Protein S is synthesized as a 676 amino acid residue long pre-pro molecule (middle part). The polypeptide chain can be divided into a signal peptide (shaded), a pro-peptide (hatched), a γ-carboxy glutamic acid (Gla) domain, a thrombin sensitive region (TSR), four EGF-like domains and a large carboxy-terminal domain homologous to sex hormone-binding globulin (SHBG). The circles indicates the localization of known missense mutations, leading to amino acid substitutions and type I or type II deficiency. AT (lower part) is synthesized as a single polypeptide chain composed of a 432 amino acid residue long mature protein and a signal peptide (shaded) of 32 amino acid residues. Missense mutations leading to amino acid substitutions associated with type I deficiency (open circles indicated below the polypeptide chain) or type II deficiency (open, shaded and filled circles denote HBS, RS and PE variants, respectively).

cient families suggests the two types to be phenotypic variants of the same genetic disease.[77] Very few cases of qualitative protein S deficiency (type II deficiency) have been found (Figure 4), which presumably reflects the poor diagnostic performance of available functional protein S assays. Homozygous protein S deficiency is extremely rare, but appears to give a similar clinical picture to that of homozygous protein C deficiency with purpura fulminans in the neonatal period.

Deficiency of antithrombin

Heterozygous AT deficiency is found in 1-2% of thrombosis patients, whereas the incidence in the normal population is between 1/2,000 and 1/5,000. This suggests AT deficiency is associated with a 10-20-fold increased risk of thrombosis, i.e. somewhat higher than that estimated for APC resistance. Type I AT deficiency is characterized by reduced levels (~50%) of both immunologic and functional AT, whereas type II denotes functional defects.[73,74,78,79] Type II cases are divided into three subtypes, HBS (heparin binding site mutants), RS (reactive site mutants), and PE (mutants giving pleiotropic effects). The last group is characterized by multiple abnormalities affecting the heparin binding site, the reactive site and the plasma concentration. A large number of AT deficiencies have been genetically analyzed (Figure 4).[78] Type I deficiencies are caused either by point mutations, deletions or insertions. Whole gene or partial gene deletions are relatively uncommon causes of type I deficiency. Type II HBS deficiencies are caused by mutations in the heparin binding site whereas type II RS mutants are defective in protease inactivation with amino acid substitutions in the vicinity of the reactive site. Type II PE mutants are caused by a limited number of mutations located between amino acids 402 and 429 near the carboxy-terminal end of the molecule. HBS mutations are associated with a less severe thrombotic tendency than the other groups.

Gene-gene interactions

For several years a puzzling observation was that protein C deficiency in some families appeared as a strong risk factor for thrombosis, whereas in other families the associated risk appeared to be mild.[80] This difference was also found between families having the same mutation, suggesting additional genetic risk factors segregating in the thrombosis-prone protein C deficient families. After the discovery of APC resistance, this multigenetic theory was confirmed in families with deficiency of protein C, protein S or AT.

Protein C deficiency. Several reports have demonstrated a high frequency of the FV:Q506 allele among symptomatic protein C deficient patients.[81-83] Koeleman *et al.* found 19% of symptomatic protein C-deficient probands carried the FV:Q506 allele and thrombosis was more common among carriers of both defects (73%) than in carriers of only protein C (36%) or the FV:Q506 allele (10%) (Figure 4).[81] That protein C deficiency in itself is a mild risk factor is demonstrated by a low incidence of thrombosis among blood donors with isolated protein C deficiency.[84]

Protein S deficiency. The penetrance of thrombotic symptoms is highly variable among protein S deficient patients.[77] The youngest protein S deficient patient with venous thrombosis found in our laboratory was a 11-year-old boy with combined deficiency of protein S and homozygosity for FV:Q506.[85] We found the FV:Q506 allele to be present in 39% (7/18) of Swedish families with protein S deficiency.[86] The thrombotic risk was much higher among individuals with combined defects (72%) than in patients with single gene defects (19%) (Figure 5). The annual incidence rate in individuals with combined genetic defects was 2.1% (calculated from ref. #86).

Antithrombin deficiency. van Boven *et al.* identified the FV:Q506 allele in 18 of 127 (14%) thrombophilic families with AT deficiency.[87] Eleven of 12 individuals with both AT deficiency and the FV:Q506 allele developed thrombosis. The median age of first thrombotic event among individuals with combined genetic

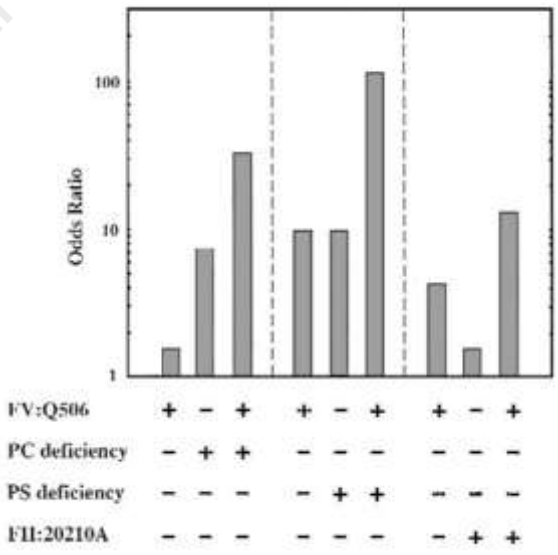

Figure 5. Gene-gene interactions and the risk of thrombosis. Families with two genetic defects, described by Koeleman et al.,[81] and Zöller et al.,[86, 90] were used to calculate the risk of thrombosis associated with isolated or combined genetic defects. Combinations of FV:Q506 (FV) and protein C deficiency (PC), protein S deficiency (PS) and the 20210A allele of the prothrombin gene (FII) were analyzed. The calculated odds ratios were: 1, (left) FV = 1.6 [CI 0.2-12], PC = 7.6 [CI 1.5-38], FV+PC = 32 [CI 5.9-173]; 2, (middle) FV = PS = 10 [CI 1.1-97], FV+PS, 112 [CI 12-1044]; 3, (right) FV = 4.2 [CI 1.2-15], FII = 1.6 [CI 0.2-16], FV+FII = 13 [CI 2.3-71]. The limited number of thrombosis cases among relatives without a genetic defect explain the wide 95% CI.

defects was 16 years (range 0-19 years). Because the AT gene, like the FV gene, is located on chromosome 1, it is expected that the FV:Q506 allele is less frequently associated with AT deficiency than with deficiency of protein C or protein S. In rare cases mutations causing AT deficiency may affect the chromosome carrying the FV:Q506 allele resulting in linkage of the two genetic defects.[87]

Prothrombin A20210 allele. The A20210 allele is present in 5-7% of venous thrombosis patients and in 1-4% of healthy controls. Alhenc-Gelas and co-workers found no patients with the A202210 allele among 26 thrombophilic families with APC resistance and suggested a lack of gene-gene interaction between the prothrombin and FV gene defects.[88] We and others have not been able to confirm this lack of gene-gene interaction.[67,89-92] In contrast, the prothrombin A20210 allele appears to be quite common in thrombophilic families with APC resistance and the risk of thrombosis is high in individuals with combined defects (Figure 5). In our study, 50% (4/8) of individuals with combined defects had suffered venous thrombosis; the mean age at first thrombotic event was 28 years.[90]

Interaction between FV:Q506 and acquired prothrombotic states

The high prevalence of the FV:Q506 allele facilitates studies of gene-environment interactions, which will be important for establishing evidenced based guidelines for the management of APC resistant patients.

Oral contraceptive usage has been extensively debated as a risk factor for thrombosis.[93] A recent World Health Organisation case-control study showed that the relative risk among persons using oral contraceptives was 4.15 in Europe whereas it was 3.25 in developing countries.[94,95] The lower risk in developing countries may be due to the lower prevalence of the FV:Q506 allele. Rosing *et al*. demonstrated that oral contraceptives, especially third-generation pills, were associated with increased thrombin generation and reduced sensitivity to APC.[96] Moreover, the reduction in APC sensitivity caused by oral contraceptives was found to be more pronounced in individuals carrying the FV:Q506 allele.[96] In Western societies, women suffering from thrombosis associated with oral contraceptive usage are often APC-resistant, e.g. in a cohort of such patients, Hellgren *et al*. found the frequency of APC resistance to be 30%.[97] Vandenbroucke *et al.* calculated that heterozygous women using oral contraceptives have a 35-fold increased risk of thrombosis, compared to women who did not use oral contraceptives and who had normal FV genotype.[98] The third generation progestagen may be worse in this respect (50-fold increased risk of thrombosis) than the second generation.[99] In homozygotes, oral contraceptive usage is associated with a several hundred-fold increased risk of venous thromboembolism, which is consistent with the observation that oral contraceptive usage is common among homozygous women with thrombosis (80%).[100] A study by Schambeck *et al.* confirmed that the FV:Q506 allele is an important risk factor for development of venous thromboembolism in association with oral contraceptives (odds ratio 4.9).[101] In addition, they showed that acquired risk factors such as surgery, leg fractures, and prolonged immobilization are significant risk factors for developing thrombosis during oral contraceptive use (odds ratio 10). The authors suggest that knowledge about the additional risk associated with carrriership of the FV:Q506 allele could possibly contribute to the prevention of thrombosis in risk situations. Schambeck *et al.* also demonstrated that a positive family history of thrombosis is an insufficient predictor not only of the FV:Q506 allele but also of who will develop thrombosis during oral contraceptive usage.[101]

Pregnancy is a well established risk factor for thrombosis among patients with inherited deficiency of AT, protein C or protein S.[102] Pregnancy is a also a common risk factor in APC resistant patients with thrombosis.[11,103] In a case-controlled study, 60% of women with pregnancy-associated thrombosis were found to be APC resistant.[97] Similar high frequencies of APC resistance among patients with pregnancy-associated thromboembolism have been reported by several groups.[104,105] However, it should be kept in mind that the majority of heterozygous women will not develop thrombosis in conjunction with pregnancy.[106]

Systemic lupus erythematosus and antiphospholipid antibodies. Episodes of venous or arterial thromboembolism are frequent in patients with systemic lupus erythematosus (SLE) and are often associated with the presence of antiphospholipid antibodies, notably anticardiolipin antibodies or lupus anticoagulants.[107] The combination of a thrombotic tendency, an increased risk of recurrent fetal loss and the presence of phospholipid antibodies has been named the phospholipid syndrome, which may also occur in a primary form not associated with SLE. In a Dutch study, the FV:Q506 allele was found to be an independent risk factor for venous (odds ratio 4.9; CI 1.2-19.6), but not for arterial thrombosis, among SLE patients.[108] However, other studies have failed to show a significant link between the FV:Q506 allele and venous thrombosis among patients with antiphospholipid antibodies secondary to SLE[109-111] or with patients with primary antiphospholipid syndrome.[105,109,112]

Phenotypic variability in thrombophilia

The clinical manifestations of the different inherited thrombophilias demonstrate some variability, which may be related to the specific pathogenetic mechanisms involved in each disease (reviewed in ref. #50). A noteworthy observation is the relatively low risk of pulmonary embolism in individuals with the

FV:Q506 allele.[40-43] Thus, in patients with the FV:Q506 allele, Manten *et al.* found a relative risk of isolated pulmonary embolism of 3.3, as compared to a relative risk of 6.9 for venous thrombosis.[41] Moreover, chronic thromboembolic pulmonary hypertension (CTEPH) is not found to be associated with the FV:Q506 allele.[113] The molecular explanation for the lower tendency to embolize in individuals with FV:Q506 is unknown. Possibly, increased generation of thrombin in individuals with FV:Q506 may lead to a rigid clot structure with reduced tendency to embolize. In addition, increased activation of the thrombin-actifiable fibrinolysis inhibitor (TAFI) in patients with APC resistance could contribute to decreased fibrinolytic activity.[38]

The tendency to develop emboli may be influenced by several genetic factors. Recently, we found an association between pulmonary embolism and a 4G/5G polymorphism in the PAI-1 promotor in protein S deficient individuals.[114] The conclusion was that individuals having protein S deficiency combined with homozygosity for the 4G allele were at increased risk of pulmonary emobolism whereas individuals with either of the two genetic traits had no increased risk of thrombosis.

Venous thromboembolism rarely occurs during childhood (annual incidence of 0.7/100 000) even in individuals carrying one or more genetic defects. Thus, thrombosis is rare before the age of 15 years in carriers of the FV:Q506 allele.[11] However, this does not mean that the FV mutation is not a risk factor for thrombosis during childhood. In fact, there are several case-reports of the FV:Q506 allele in children and even neonates with thrombosis.[85,115,116] According to several case-controlled studies, the FV:Q506 allele is a risk factor for childhood thrombosis, but other genetic or acquired risk factors are often present. In a study by Gurgey *et al.*, 6/12 (50%) children with thrombosis carried the FV:Q506 allele.[117] Similar results were reported by Nowak-Göttl *et al.*[118] who found the FV:Q506 allele in 10/19 (52%) of children with venous thromboembolism and in 7/18 (38%) children with arterial thromboembolism.

Management of patients with inherited thrombophilia

The realization that thrombophilia is a multifactorial disease, with both circumstantial and genetic risk factors being involved in its pathogenesis, is presumably going to influence the future management of the thrombophilic patient. However, available data are not sufficient for calculation of the thrombosis risk associated with combinations of genetic defects. As most studies are made on selected populations, while accurate prevalence numbers of the different defects in the general population are still lacking, it can only be concluded that individuals with combined defects have higher thrombosis risk than those with individual defects.[119]

No evidenced-based guidelines are available for the handling of symptomatic or asymptomatic individuals with APC resistance.[120] The following practical guidelines for the diagnosis and treatment of APC resistance are based on experience rather than on controlled studies. To diagnose inherited APC resistance, a modified APC resistance test specific for the FV:Q506 allele is used as screening assay. DNA-based assays for the FV genotype are performed to confirm a positive result of the APC-resistance test. Asymptomatic individuals with heterozygosity for the FV:Q506 allele are given prophylactic treatment only in situations known to predispose for thrombosis, e.g. major surgery. Patients with inherited APC resistance and a history of thrombosis are handled like other patients with genetic defects, i.e. preventive anticoagulation therapy is given in risk situations and long-term therapy is considered if thrombosis is spontaneous, life-threatening or recurrent. Homozygous cases, and heterozygous patients with another prothrombotic genetic defect, are given therapy in all risk situations and prolonged or lifelong therapy is considered even after a single thrombotic event.

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The authors contributed equally during the preparation of this paper and the order of appearance of the names does not reflect differences in amount of work put into the writing of this review.

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