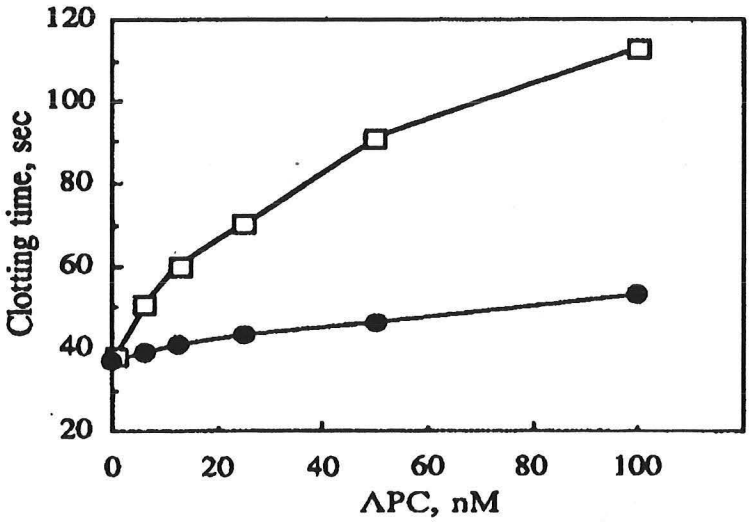
Resistance to Activated Protein C: The Most Common Inherited Defect Underlying the Prothrombotic State



Medical Grand Rounds
Parkland Memorial Hospital
University of Texas Southwestern Medical Center
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INTRODUCTION

Venous thromboembolism, with an annual incidence of 1 in 1000, is a major public health problem and represents a substantial source of morbidity and mortality among our hospitalized patients. It has been estimated that venous thrombosis and pulmonary embolism are associated with 300,000 to 600,000 hospitalizations and 50,000 deaths in the United States each year (1). Factors predisposing to thrombosis include surgery, fractures, immobilization, inflammatory states, cancer, congestive heart failure, pregnancy and oral contraceptive use. Genetic factors have been postulated to play a role, but until now were demonstrated in only a minority of patients.

The purpose of this Medical Grand Rounds is to introduce a new hereditary cause of venous thromboembolism, which is detected in clotting assays as a subnormal prolongation of the clotting time following the addition of activated protein C (APC) to test plasma. This abnormality, referred to as "resistance to activated protein C," is caused by a single mutation in the factor V gene. The importance of this finding is in the striking high incidence of the defect in patients with thrombosis (about 60% in referral populations and about 20% in unselected patients) and in the normal population (3-7%). This new genetic defect is five to ten times more common than the three previously recognized prothrombotic defects (protein C, protein S, and antithrombin III deficiencies) combined; furthermore, it appears that protein C and S deficiencies (in the heterozygous form) are asymptomatic unless combined with APC resistance. APC resistance may represent the most common known disease-producing genetic defect in man.

CASE REPORT: A NEW DEFECT ASSOCIATED WITH FAMILIAL THROMBOSIS (from ref. 2).

The index patient was a 53 year-old Caucasian male presenting with pain and swelling in the lower extremity. Clinical findings and non-invasive studies were consistent with deep venous thrombosis (DVT). The patient had experienced his first episode of DVT at age 19, but did well until age 40, after which he experienced one or more episodes of DVT for seven years. Episodes were treated with Vitamin K antagonists for periods of three months. He suffered from postphlebitic syndrome in both legs but was otherwise healthy. The patient was referred to Dr. Bjorn Dahlback of the Department of Clinical Chemistry and Coagulation Disorders, Malmo General Hospital, Malmo, Sweden. Values for antithrombin III, protein C, protein S, plasminogen, fibrinogen, thrombin and reptilase times and routine coagulation parameters were normal. Dr. Dahlback developed a new assay that measures the anticoagulant response in patient plasma to added activated protein C. The results are shown below:

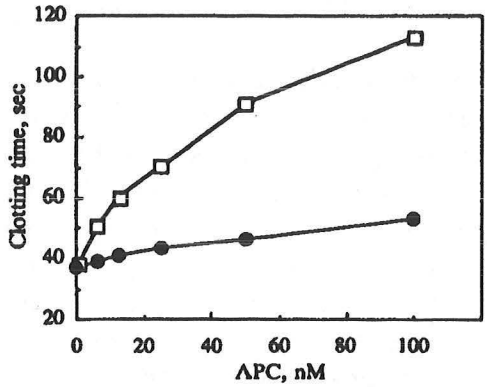

Fig. 1. Poor anticoagulant response to APC in patient (closed circles) as compared to control (open squares) plasma. From reference (2).

The patient's family history was remarkable (Fig. 2). His older brother (III-2) had leg DVTs on several occasions, as had an uncle (II-7) and aunt (II-5). A younger relative (IV-2) had a clinically evident DVT during her third pregnancy.

Fourteen out of nineteen of the patient's family members had an anticoagulant response that was subnormal (less than the fifth percentile) as compared to the normal population, suggesting an inherited basis for the biochemical abnormality.

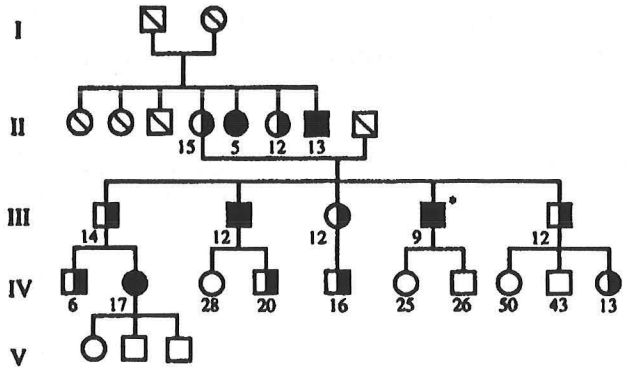

Fig. 2. Pedigree of a family with thromboembolic disease. The proband is indicated by an asterisk. Filled symbols indicate individuals with a history of thrombosis and APC-dependent prolongation of the clotting time below the fifth percentile of controls; half-filled symbols represent poor anticoagulant response to APC without a history of thrombosis. Values for the APC-dependent prolongation of the clotting time are indicated. From reference (2).

He postulated that the poor anticoagulant response to APC could be explained by a number of mechanisms: an autoantibody to protein C, a fast-acting protease inhibitor of APC; protein S deficiency, mutations in the genes for factors V and VIII (especially close to the regions encoding the cleavage sites); or some other mechanism. He performed a number of experiments that seemed to rule out all of these possibilities. This led him to postulate that the patients lacked an unknown

cofactor necessary for APC function, and he set out to demonstrate and purify such a factor. In pursuing these studies, he continued a long tradition of using factor-deficient plasma to purify novel clotting factors. In this approach, the novel factor is separated from other plasma components based on its ability to complement the activity of the factor-deficient plasma. The results of some of his experiments are shown in Fig. 3.

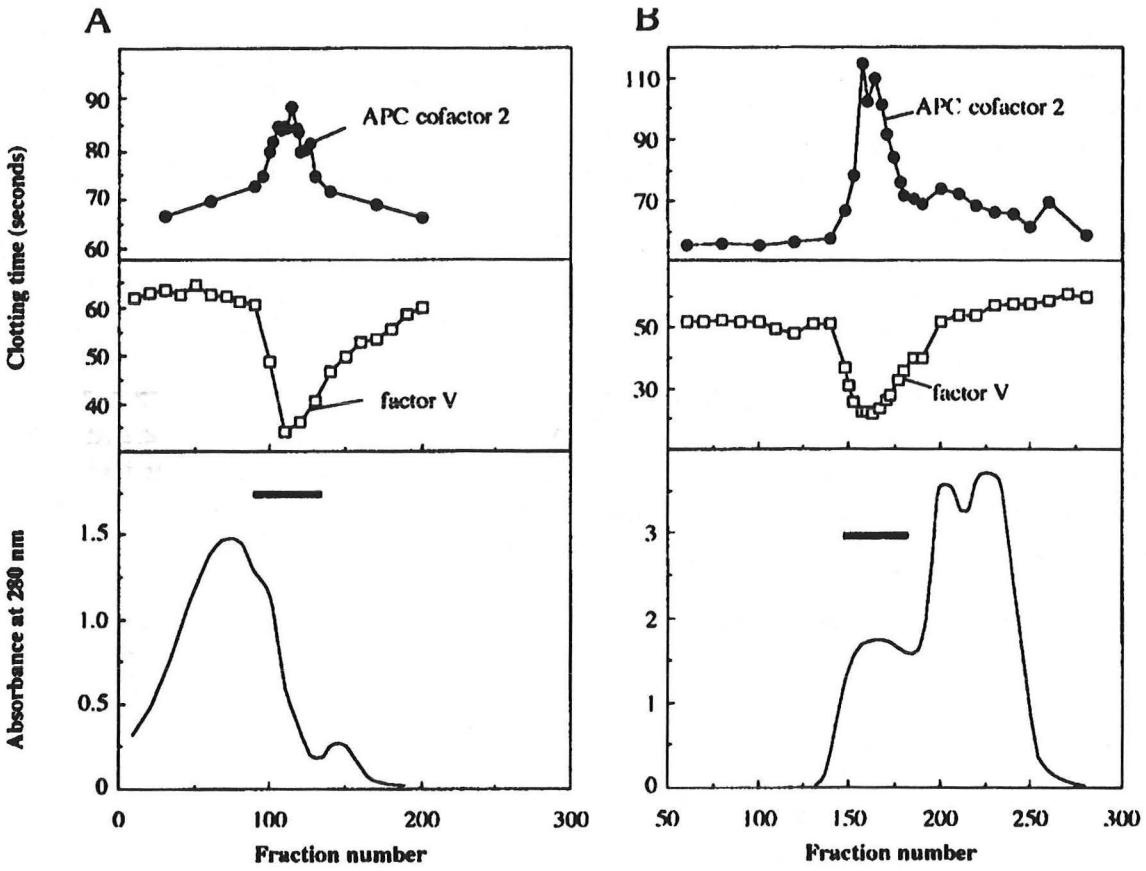

Fig. 3. Chromatography of APC cofactor on Q-Sepharose (A) and Sephacryl S-300 (B). APC cofactor activity was measured as a prolongation of the clotting time of APC-resistant (patient) plasma (upper panels) in the presence of APC; factor V activity was measured as a shortening of the clotting time in the presence of factor V-deficient plasma. From reference (3).

To his surprise, the APC cofactor was identical to coagulation factor V, a protein he had purified from human plasma some fifteen years earlier (4). His observations led him to conclude that Factor V serves a previously unsuspected function: as a cofactor for activated protein C. Later work demonstrated a mutation in the gene for factor V in these patients; this work will be described below. To put this work into perspective, however, we will first review the protein C anticoagulant system and the consequences of deficiencies of the major components of this pathway, protein C and protein S.

THE PROTEIN C ANTICOAGULANT PATHWAY

Procoagulant and anticoagulant mechanisms in the vascular system are delicately balanced, to achieve a lifetime of freedom from unwanted thrombosis in most people, yet nearly instantaneous hemostasis to prevent life-threatening hemorrhage when needed. Vascular injury produces a cascade of biochemical reactions involving serial proteolysis that ultimately results in the generation of thrombin (5) (Fig. 4). Thrombin has three major actions: 1) the cleavage of fibrinogen to fibrin to begin the formation of the fibrin clot; 2) the activation of platelets; and 3) the activation of factors V and VIII to Va and VIIIa, which serves as a positive feedback mechanism to amplify the coagulation cascade. To oppose these procoagulant mechanisms the intact vessel wall produces anticoagulant molecules, such as prostacyclin (an inhibitor of platelet activation), tissue plasminogen activator (an activator of the fibrinolytic system), and heparin-like molecules (cofactors with antithrombin III that inactivate procoagulant proteases). In addition, another major action of thrombin is to initiate a potent anticoagulation system, the protein C anticoagulant pathway, at the site of vascular injury. This system serves to limit the clot to the extravascular space and is a major reason why a breached vessel does not become completely occluded. In order to initiate this anticoagulant pathway, thrombin must first bind to a receptor (thrombomodulin) on the surface of the endothelial cell. The binding causes a change in the specificity of thrombin, so that now it cleaves and activates protein C, a potent anticoagulant.

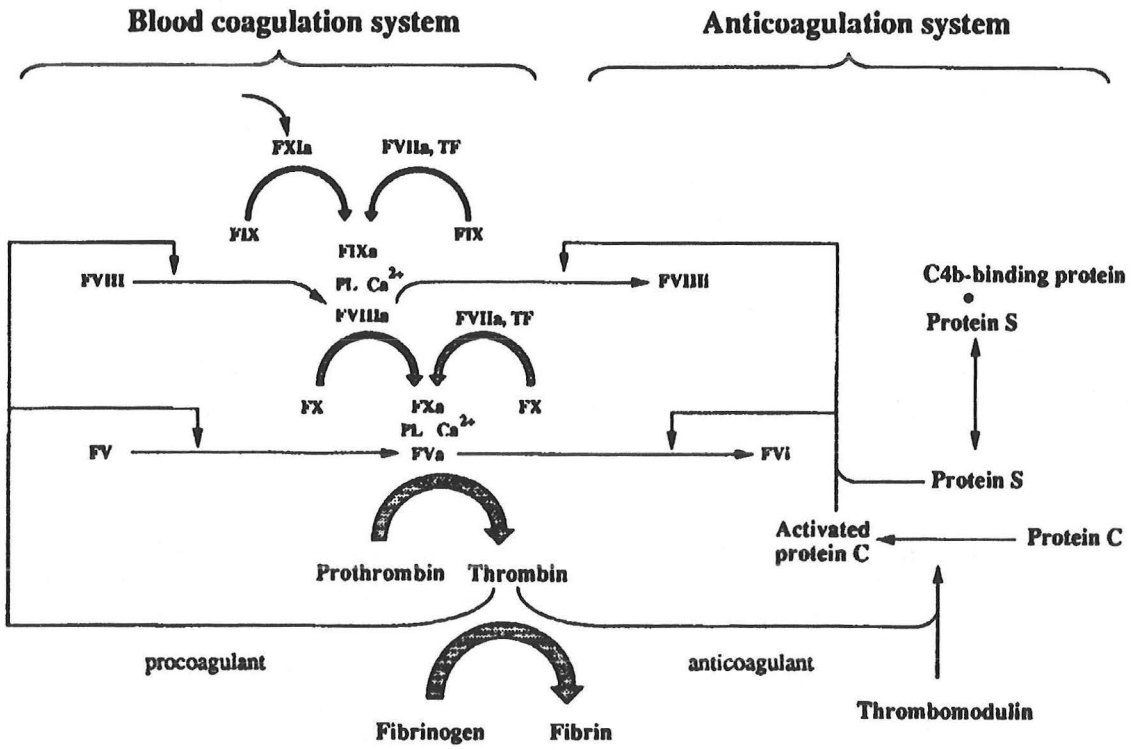

Fig. 4. Interplay between the reactions of blood coagulation and anticoagulation. From reference (5).

Anticoagulant Proteins: Protein C and Protein S. Protein C was initially discovered as a Vitamin K-dependent protein. Like many proteins involved in the coagulation cascade, it circulates as a precursor, and is cleaved to form an active proteolytic enzyme (5). A schematic diagram of the human protein C molecule is presented in Fig. 5. The molecule is composed of a domain containing γ-carboxyglutamic acid (Gla) residues, an EGF-like domain, and a serine protease domain containing the classic aspartic acid-histidine-serine catalytic triad. Protein C is activated by thrombin that has bound to thrombomodulin, an important anticoagulant molecule on the surface of endothelial cells (Fig. 6). Activated protein C (APC) slows the rate of activation of the coagulation cascade by cleaving factors Va and VIIIa. The reaction requires a cofactor protein, protein S, which is also a Vitamin K-dependent clotting factor (Fig. 7). Protein S is not a precursor to a protease, but may instead play a role in localizing APC to the endothelial surface through negatively-charged phospholipids. Protein S is active as a cofactor only in its free form (normally, 30-40% of the total). The majority of protein S is complexed in plasma to a regulator of the complement pathway, C4b-binding protein (Fig. 8), and it may also serve to localize the complement regulatory activity of C4b-binding protein to negatively-charged phospholipid membranes. Therefore, protein S is involved in both blood coagulation and the regulation of the complement system. C4b-binding protein is an acute phase reactant; higher levels of C4b-binding protein lead to lower levels of free protein S in plasma, thus providing an explanation for the prothrombotic predisposition observed in inflammatory states (6).

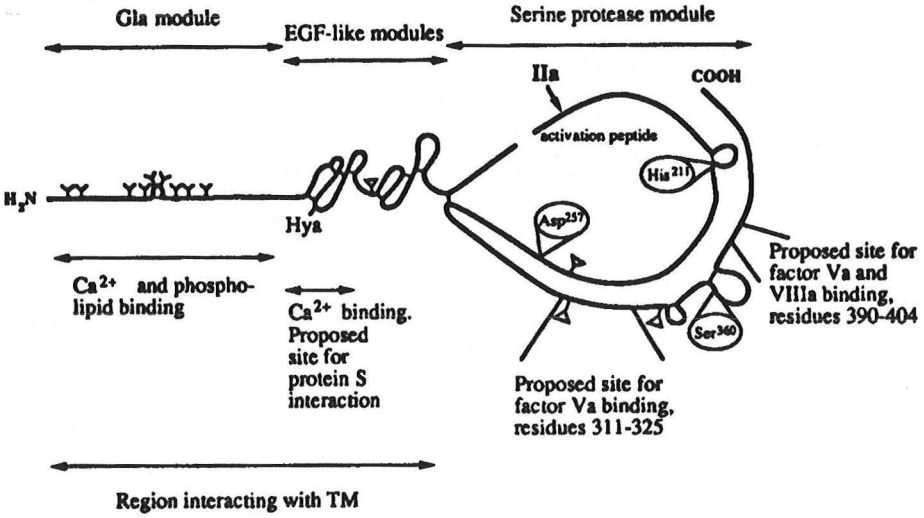

Fig. 5. Human protein C. The arrow indicates the thrombin (IIa) cleavage site, leading to activation of protein C. From reference (5).

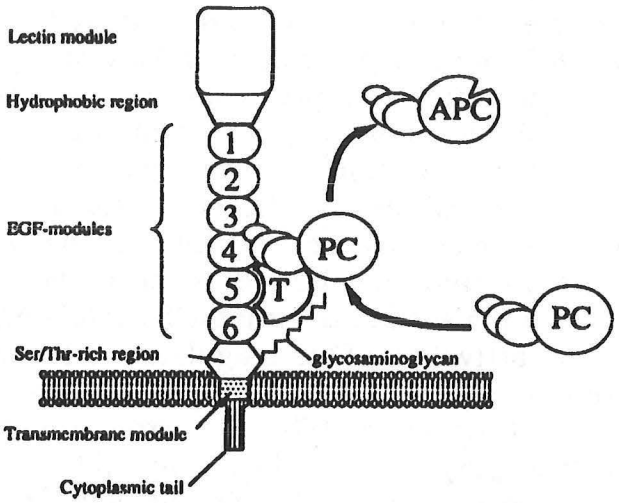

Fig. 6. Molecular interactions occurring during protein C activation. T, thrombin; PC, protein C; APC, activated protein C. From reference (5).

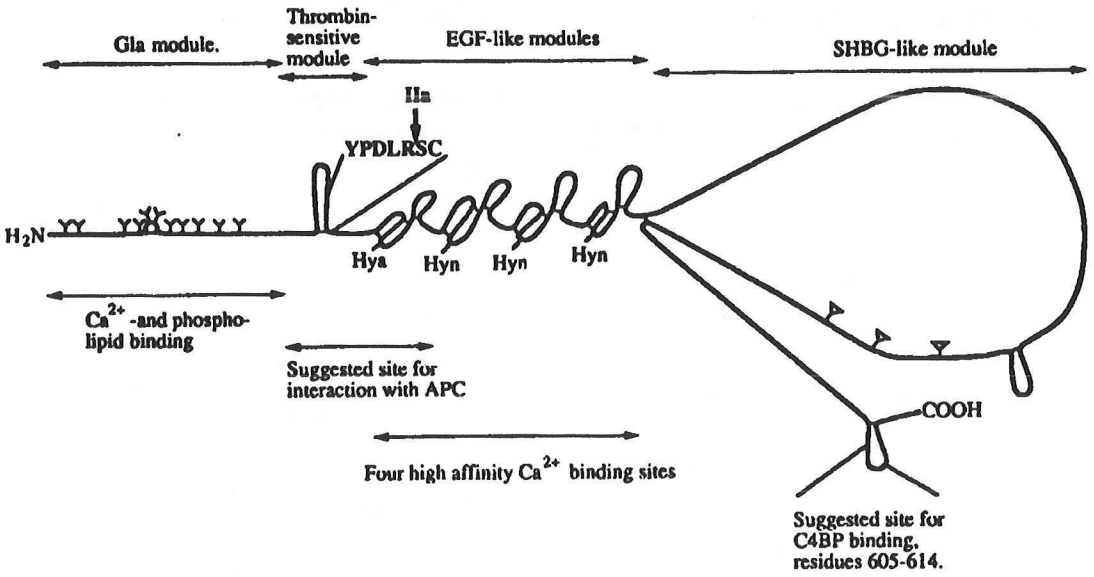

Fig. 7. Human protein S. The thrombin cleavage site (IIa) is indicated. From reference (5).

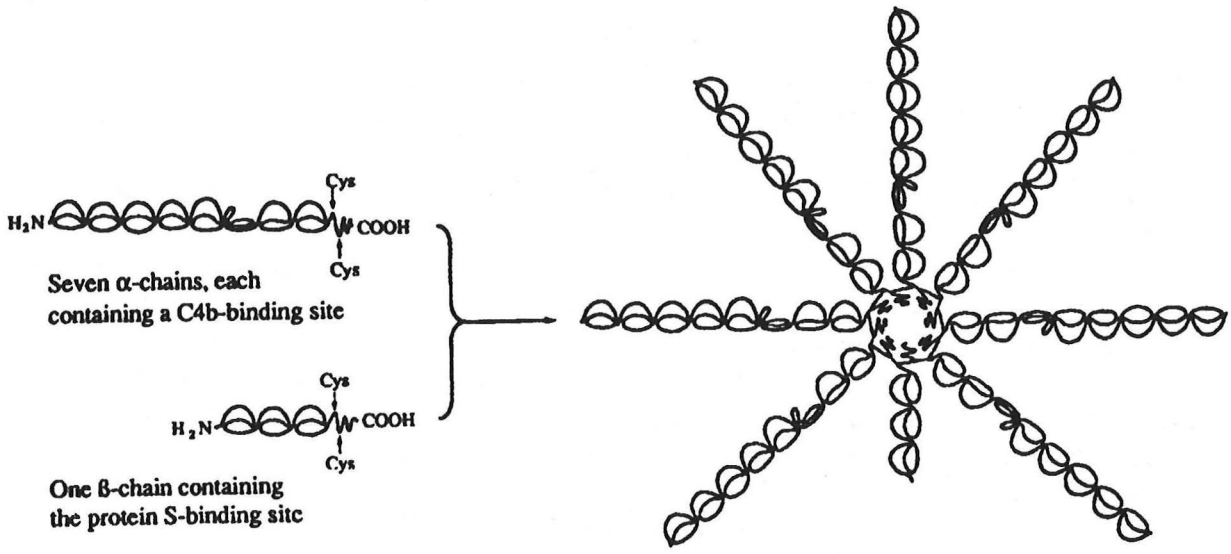

Fig. 8. C4b-binding protein. The protein contains seven α chains and one β chain that form an octopus-like structure visible by electron microscopy. From reference (5).

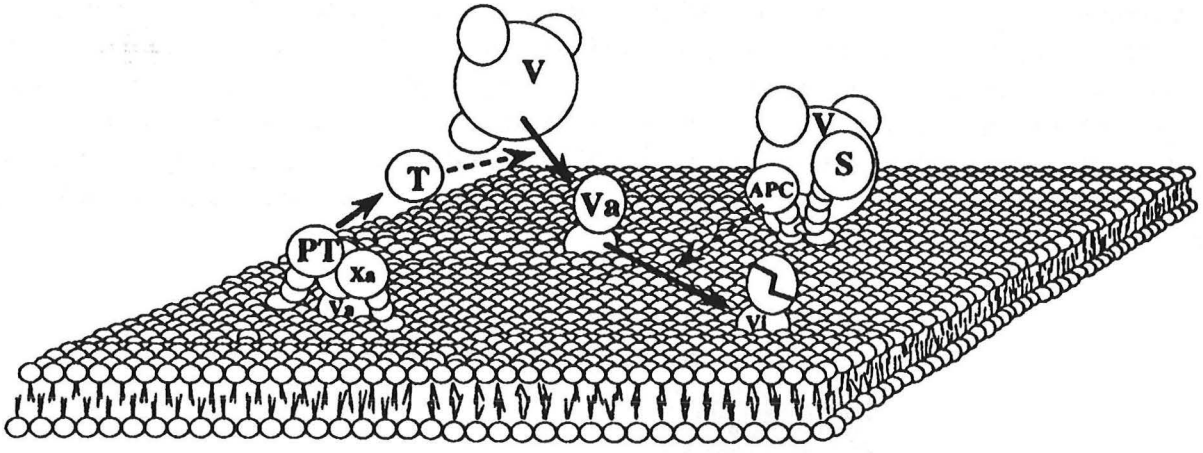

Fig. 9. Theoretical molecular model of reactions involved in the degradation of factor Va. II, prothrombin; IIa, thrombin; Va, activated factor V; Vi, APC-inactivated factor Va. Factor V forms a complex with protein S and APC on the surface of platelets and endothelial cells. APC mediates degradation of factor Va and VIIIa. Thrombin activation of factor V results in loss of APC cofactor activity and a gain of factor Xa cofactor activity. From reference (7).

Factor Va and VIIIa are both substrates for APC. The scheme for activation of factor V by thrombin or factor Xa and its inactivation are presented in Fig. 9. Factor V is a single-chain glycoprotein of 330,000 kDa present at a concentration of 0.7 mg/dl in human plasma. A second pool of factor V, about 30% of the total, is found in storage granules in human platelets; functional differences between these two pools do exist, potentially as a result of differences in post-translational modifications. After activation by thrombin or Xa, factor Va (in the presence of negatively-charged phospholipid and Ca^{2+}) functions as a high-affinity receptor for factor Xa. This complex, referred to as the "prothrombinase complex", plays a central role in the generation of thrombin from prothrombin and offers a million-fold rate enhancement over the cleavage of prothrombin by Xa alone. Note that this scheme includes factor V as a cofactor for the APC reaction, per Dahlback's observations; this aspect of the scheme awaits experimental confirmation.

THE MECHANISM OF INACTIVATION OF HUMAN FACTOR Va FROM NORMAL AND APC-RESISTANT INDIVIDUALS

How do we explain the prothrombotic tendency in APC resistance? And how does factor V "factor" into the equation?

In addition to the finding that factor V corrects the APC resistance measured *in vitro*, Dahlback and others also discovered that APC resistance was "linked" to the factor V gene by genetic linkage studies involving two large families (8, 9). Independently, Bertina and coworkers and other groups found a single nucleotide substitution, a G to A transition at position 1691 in the factor V cDNA, in APC-resistant individuals (8, 9) (Fig. 10). This nucleotide substitution leads to the replacement of glutamine for arginine at amino acid position 506 in the protein. The arginine at this position occurs just N-terminal to an APC cleavage site, making this mutated Factor Va resistant to cleavage by APC. The procoagulant activity of the mutant, however, is normal (Fig. 11).

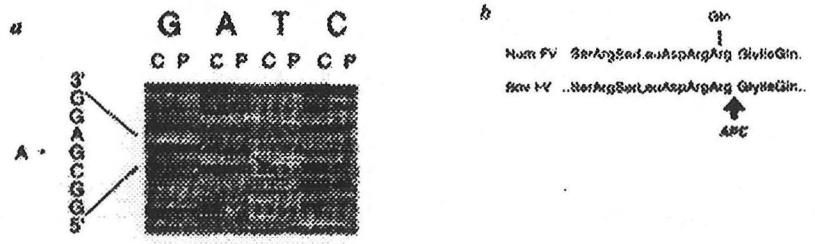

Fig. 10. Mutation in the factor V gene leading to APC resistance and schematic representation of the factor V molecule. Cleavage of factor V by activated protein C occurs at the site of the mutation. From reference (8).

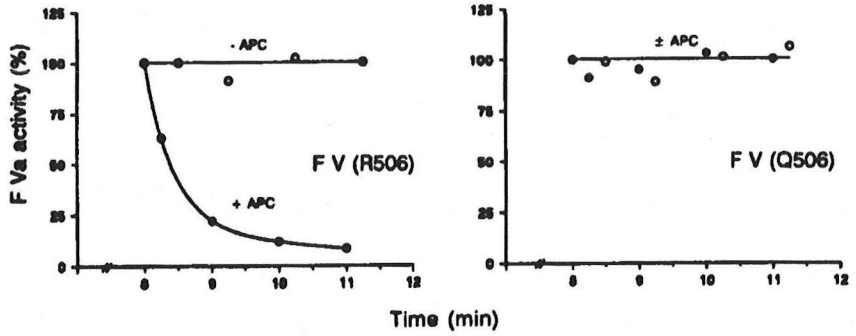

Fig. 11. Resistance of factor Xa-activated factor V (Q506) to inactivation by APC. From reference (8).

Fig. 12 illustrates the pattern of proteolytic inactivation of factor Va by APC in normal and APC-deficient patients. The salient features of the scheme are:

- 1. the substrate for APC is factor Va, not factor V.
- 2. the physiologically important inactivation takes place at cell membranes.
- 3. the inactivation is an ordered and sequential event. The first cleavage occurs at Arg 506 of the heavy chain, followed by cleavage at Arg 306 and Arg 679, which results in loss of prothrombinase cofactor activity.

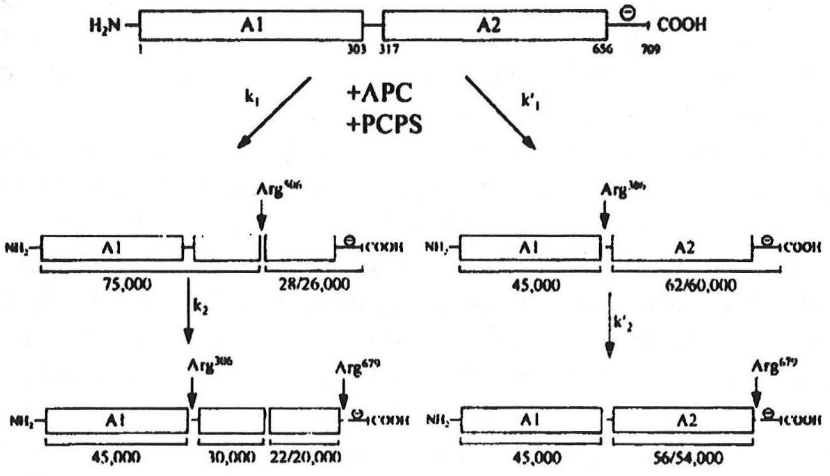

Fig. 12. The cleavage of human factor Va heavy chain during inactivation by APC. A, wild-type factor Va; B, mutant factor Va. From reference (10).

Therefore, while the cleavage at Arg 506 is not the inactivating cleavage, mutation at 506 greatly slows the rate of factor V inactivation. Therefore, it seems intuitive that the inability to inactivate the procoagulant factor Va should lead to a prothrombotic state. However, it is harder to understand, in light of Dahlback's model, how the mutation leads to defective APC cofactor activity, and how the

addition of normal factor V complements the defective activity. This is still a matter of intense debate, and more experimental work is needed before this mechanism is fully understood.

RESISTANCE TO APC AS A MAJOR RISK FACTOR FOR THROMBOSIS

A landmark study (11) (published by Svensson and Dahlback in NEJM before the availability of a genetic test for APC resistance) looked at the prolongation of clotting time in the presence or absence of activated protein C (expressed as the APC ratio) in a cohort of 104 consecutive patients referred to the study center with thromboembolic disease, and in 211 members of 34 families with thrombosis and APC resistance. Patient characteristics included a predominance of females (72 females, 32 males), with a mean age for the cohort of 37 years (range, 14 to 74) and a mean age of the time of the first thrombotic episode of 34 years (range, 14 to 71). This was a highly selected group; fully 45% of the patients has a family history of thrombosis; 29% in a first-degree relative and 16% in a second-degree relative. Patients on oral anticoagulant therapy were excluded from the study; unfortunately, the number of patients excluded and the potential impact of the exclusions was not discussed. Values for the patients were bimodal, with about one-third below the 5th percentile of the control group, and about half with APC ratios less than 2. About half of the members of families with thrombosis and APC resistance had low APC ratios, suggesting autosomal dominant inheritance. Of particular interest was a cluster of low APC ratios in the "normal" controls, suggesting a high prevalence of APC resistance in the normal population. The conclusion of the study was that 60% of the patients in their referral population and 3-7% of their control population had plasma that showed a poor response to APC.

This initial finding, performed before genetic testing, was confirmed in another smaller study (16 of 25 thrombophilic patients has APC ratios less than 2.15) (12). In France, the rate was 19% (9 out of 48) in one referral population (13) and 16% (14 out of 87) in another referral center (14). These studies established resistance to APC as the most common prothrombotic genetic defect found to date (Fig. 13).

What is the frequency of APC resistance in an unselected population (i.e., consecutive patients with thrombosis)? Koster, et al. (15) provided information on this question in a study (again, before the availability of genetic testing) in a series of 301 consecutive thrombosis patients and age-matched controls. The only exclusion criteria were age > 70 years and the presence of malignancy. These investigators found an incidence of 21% in the thrombosis population and 5% in normal controls. All patients resided near Leiden in the Netherlands.

In the U.S. population the incidence has been found to be similar (16), with an overall incidence of 24% (9/37) in referred thrombosis patients and a 5% incidence in normal controls (2/39). This study was rather small, however, and the results should be confirmed in larger studies.

ESTIMATED PREVALENCE OF INHERITED THROMBOTIC DISORDERS

Disorder	*Prevalence*
Antithrombin III deficiency	1–4%
Protein C deficiency	5–6%
Protein S deficiency	5–6%
APC resistance	20–60%

Fig. 13. Estimated prevalence of inherited thrombotic disorders among patients with thrombosis. From reference (17).

Following the recognition of mutation in factor V as a cause for the APC resistance, it now became possible to analyze for the frequency of the causative mutation (factor V Leiden) in the general population. Dahlback's group (18) has identified the factor V Leiden mutation in 47 out of 50 thrombosis-prone APC resistant families; an unknown genetic defect accounts for the other 3 families. Beauchamp, et al. (19) found an allelic frequency of the G1691A mutation of 1.7% (which translates to a heterozygote frequency of 3.5%) in the U.K. population. The most comprehensive genetic study to date is a re-analysis and update of the Leiden thrombophilia study, analyzed for the presence of factor V Leiden mutation (20). A total of 471 patients and 474 controls were enrolled. Eighty-five (18%) were found to be heterozygotes, and seven (1.5%) homozygotes for the factor V Leiden mutation among thrombosis patients. Fourteen heterozygotes were identified among control individuals, for an allele frequency of 0.015, and a heterozygote frequency rate of 3%. Homozygotes were found at a frequency expected by Hardy-Weinberg equilibrium, suggesting no strong survival disadvantage (in sharp contradistinction to homozygous protein C or S deficiency, which are fatal disorders). The homozygous patients tended to be younger at first presentation, and more tended to be women. It is remarkable that the incidence of a predisposing factor to thrombosis was somewhat less in this group, although the clinical course of the deep vein thrombosis in the homozygous patients was unremarkable.

The values for the APC ratio in normal family members, genetically determined heterozygotes and homozygotes for the factor V Leiden mutations, and unrelated controls are given in Fig. 14. Note that there is considerable overlap between heterozygotes and controls, and that more thrombi occurred in the subset of heterozygotes with APC ratios less than two. Also, note the higher prevalence of thrombosis in family members, as opposed to unrelated controls, even in the absence of the APC mutation, suggesting the presence of a second genetic defect.

What are the biological consequences of being heterozygous for the factor V Leiden mutation? This question is addressed in Figs. 15-17. The risk of thrombosis for homozygous individuals was about 80-fold increased as compared with normal individuals. The absolute risk is 82 per 10,000 person-years for people under age 30 and 227 per 10,000 patient-years for those over 50. These results imply that most homozygous patients will experience at least one thrombotic episode in a lifetime, as opposed to a 12% lifetime risk of the heterozygote and a 2% risk for the normal individual.

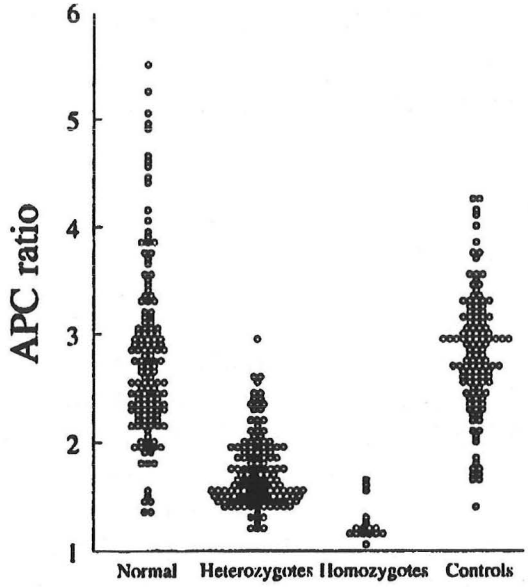

Fig. 14. APC ratios in family members and in unrelated control subjects. Filled circles denote individuals with a history of thrombosis. From reference (18).

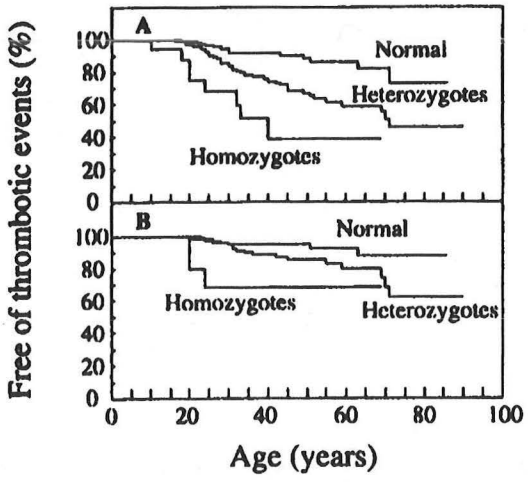

Fig. 15. Thrombosis-free survival curves for 146 normals, 144 heterozygotes, and 18 homozygotes carrying the factor V Leiden mutation. Panel B represents the same analysis after exclusion of 33 APC-resistant probands and 18 protein S-deficient family members. From reference (18).

Age (yrs)	Genotype: Patients (GG/AG/AA)	Genotype: Controls (GG/AG)	E_{AA}	OR_{AA} (CI95%)	OR_{AG} (CI95%)	Person-years	Incidence Rates/10^4 yrs: GG	AG	AA
0-29	61/17/2	70/3	.0164	140 (9.5-2,049)	6.5 (1.8-23)	1,134,681	0.6	5.1	78.4
30-49	176/35/4	217/6	.0502	98 (15-652)	7.2 (3.0-17)	1,006,733	1.8	11.8	176.2
50+	142/33/1	173/5	.0400	30 (2.2-428)	8.0 (3.1-21)	682,939	2.1	16.4	110.0

Fig. 16. Odds ratios and absolute risk of first thrombosis by age in individuals with and without the factor V Leiden mutation. From reference (20).

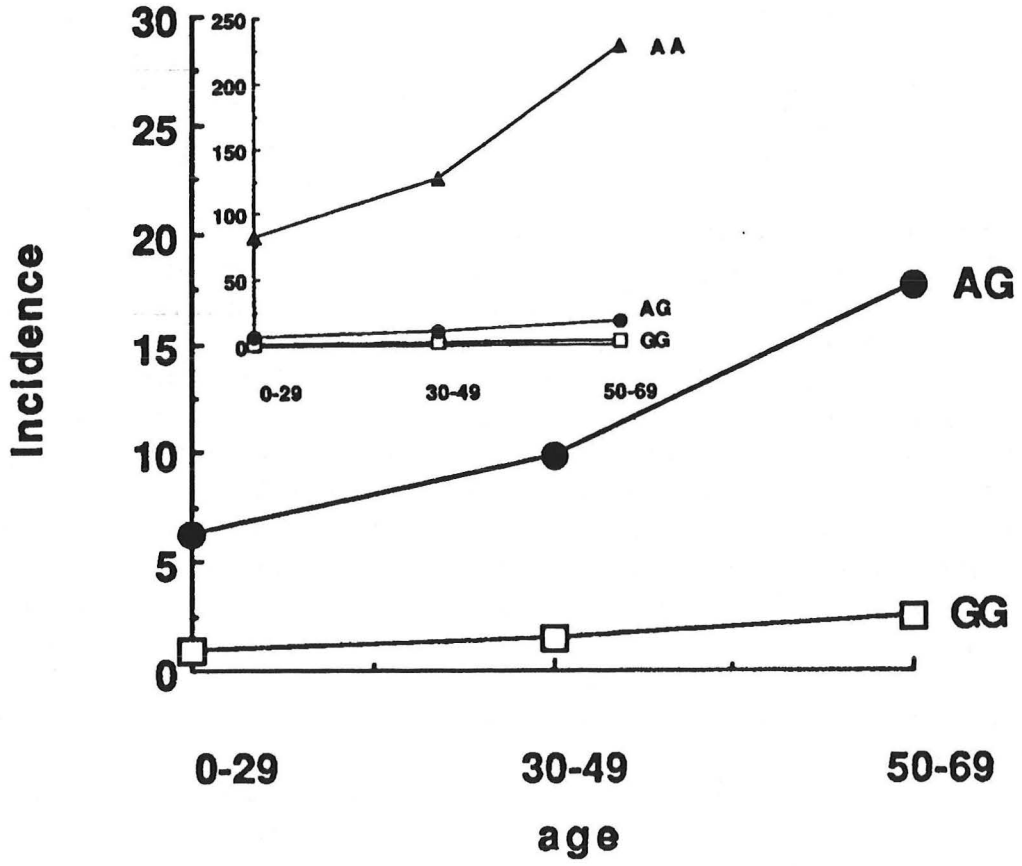

Fig. 17. Incidence rate estimates per 10,000 person-years for factor V Leiden genotypes by age. Adapted from reference (20).

TENDENCY TO THROMBOSIS: A POLYGENIC TRAIT

Hereditary Deficiencies of protein C and protein S. The importance of the protein C-protein S anticoagulant system is illustrated by the clinical syndromes of hereditary protein C and protein S deficiencies. Homozygous protein C deficiency leads to the severe disorder neonatal purpura fulminans, which is fatal unless treated with protein C concentrates and coumadin. The disorder is characterized by widespread thrombosis in the vascular system, with tissue ischemia and necrosis of the skin and other tissues. A rare complication of coumadin initiation, termed warfarin skin necrosis, is also associated with heterozygous protein C deficiency. Protein C has a shorter biological half-life than factors IX, X, and prothrombin, and the syndrome probably results from a transient and severe lowering of protein C levels in the heterozygous protein-C deficient individual (21). The incidence of heterozygous protein S deficiency in the general population is unknown, but the prevalence in patients with thromboembolic disease is similar to the frequency of protein C deficiency (22, 23). Homozygous protein S deficiency also causes neonatal purpura fulminans, and several cases of warfarin skin necrosis in protein S-deficient patients have been described (see (5) and references therein).

The association between heterozygous protein C deficiency and prothrombotic tendency has been well documented, as shown in Fig. 18.

Co-inheritance of APC-resistance and protein C or S deficiency. The common occurrence of APC resistance in the population resolves an important paradox in protein C and protein S epidemiology. This is the paradox: in most families with homozygous protein C deficiency that have been studied, the defect behaves purely as an autosomal recessive trait. That is, the parents and relatives of an infant (heterozygotes) with neonatal purpura fulminans do not have an increased frequency of thrombosis (25-28). Furthermore, the prevalence of heterozygous protein C deficiency in random healthy blood donors (0.1 to 0.5%) is consistent with a recessive disorder (29). However, heterozygous protein C deficiency is much more prevalent in patients with thrombosis (2 to 9%) as compared to the general population (22, 30-33). And heterozygous first degree relatives of symptomatic probands have a much higher risk than the nonheterozygous first-degree relatives (34). How does APC resistance resolve this paradox?

The answer is to be found in the co-inheritance of protein C or S deficiency in certain thrombosis-prone families. Recently, several protein C-deficient families have been reevaluated for mutation at the factor V locus, and thrombosis was strongly associated with the inheritance of two defective genes. In this study, 9 of 48 symptomatic protein C-deficient probands (19%) were found to carry the factor V Leiden mutation. An example of six of these families is shown in Fig. 19. A high percentage of family members with both mutations had developed a thrombus (73%), compared to those with either the protein C mutation (36%) or the Factor V Leiden mutation (10%) alone (Fig. 20). Interestingly, 7% of patients without either mutation had a history of thrombosis. Sibships were also analyzed; this removes bias that is introduced by the inclusion of sibships in which the factor V Leiden mutation is not segregating. These results are also presented in Fig. 20.

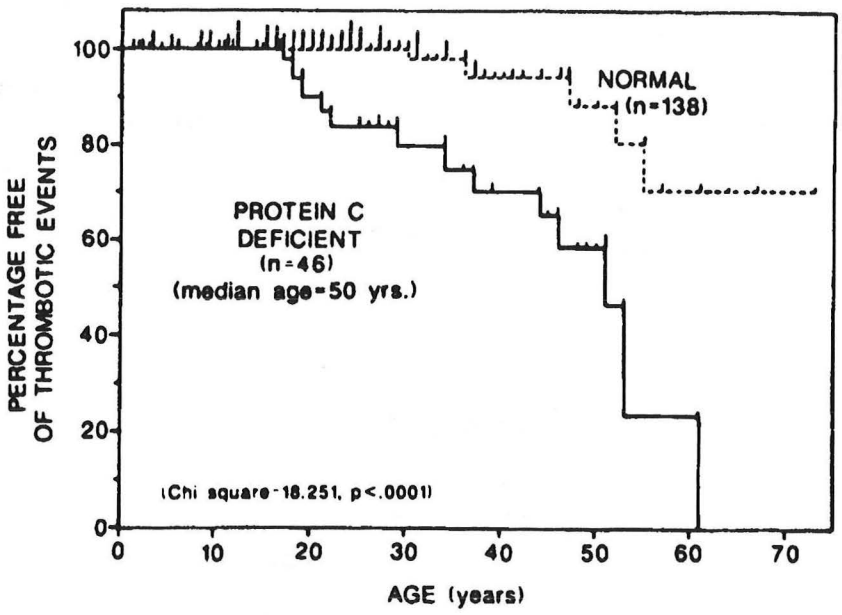

Fig. 18. Percentage of patients free of thrombosis in relation to age, in members of a large kindred with heterozygous protein C deficiency. The frequent occurrence of thrombosis in genotypically "normal" family members suggests the existence of a second prothrombotic gene. From reference (24).

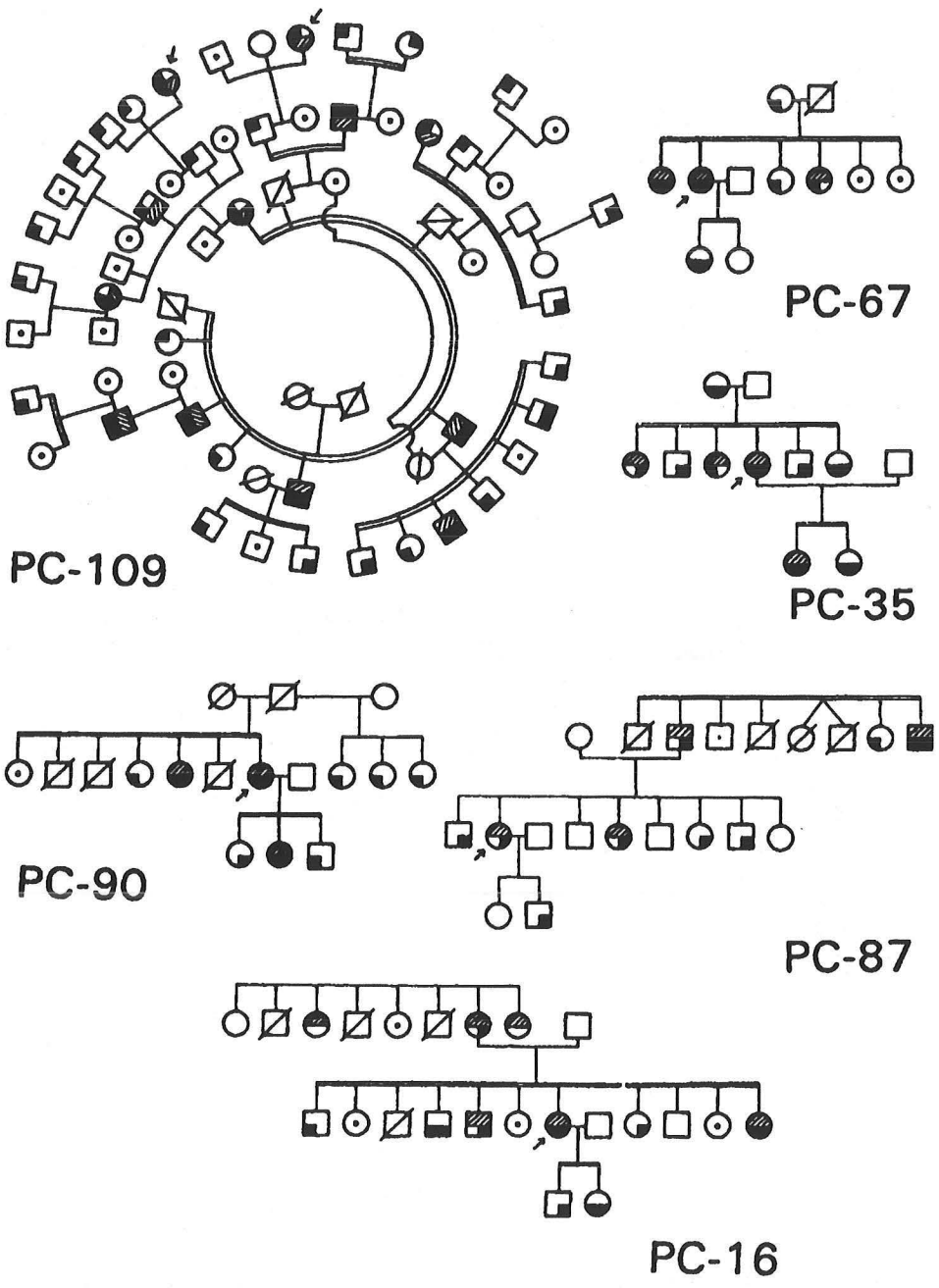

Fig. 19. Pedigrees of six families in which both a protein C gene mutation and the Factor V Leiden mutation are segregating. Hatched upper bars, symptomatic patients; solid lower right quadrant, protein C mutation; solid lower left quadrant, Factor V Leiden mutation; solid dot, tested healthy subjects. From reference (35).

The Number of Symptomatic (Thrombotic) and Asymptomatic Individuals of the 6 Pedigrees in Which the Protein C Gene Mutation and the FV Leiden Mutation Are Segregating

Gene Mutation	Symptoms of Thrombosis	
	Present	Absent
Protein C and FV	16 (73)	7 (27)
Protein C	12 (36)	22 (64)
FV	2 (10)	18 (90)
None	2 (7)	28 (93)

Percentages are in parentheses. The presence of a protein C gene mutation is indicated as protein C. The presence of the FV Leiden mutation is indicated as FV.

The Number of Symptomatic (Thrombotic) and Asymptomatic Individuals of the Sibships in Which the Protein C Gene Mutation and the FV Leiden Mutation Are Segregating

Gene Mutation	Symptoms of Thrombosis	
	Present	Absent
Protein C and FV	16 (73)	6 (27)
Protein C	5 (31)	11 (69)
FV	2 (13)	13 (87)
None	0 (0)	11 (100)

Percentages are in parentheses. The presence of a protein C gene mutation is indicated as protein C. The presence of the FV Leiden mutation is indicated as FV.

Fig. 20. The number of symptomatic and asymptomatic individuals of the 6 pedigrees (left) or sibships (right) in which the protein C gene mutation and the factor V Leiden mutation are segregating. From reference (35).

To further complicate matters, APC resistance may be misdiagnosed as functional protein S deficiency (36-38). Some investigators have found that APC-resistant plasma gives a non-linear response in a functional protein S assay, leading to falsely low protein S levels, especially at low dilutions of serum. This artifact was not a problem in the study cited above.

In conclusion, it appears that each single gene defect (protein C, protein S, or factor V Leiden) is a relatively weak risk factor for thrombosis in isolation, but the effect of having two defects is multiplicative, leading to high risks in individuals carrying two mutations. These risks are similar to the risks associated with homozygosity for the factor V Leiden mutation.

DOES APC RESISTANCE PREDISPOSE TO ARTERIAL THROMBOSIS?

It would be interesting to know whether APC resistance is associated with clinical events other than DVT and PE, such as presentations of thrombosis at unusual sites, or arterial thrombosis. Several studies have begun to address these questions. The factor V Leiden mutation does not appear to be a risk factor for myocardial infarction (39). A population of 643 patients with MI vs. 726 age-matched controls participating in the ECTIM study in Europe were investigated; there was no difference in the incidence of the mutation in MI patients (5.1%) vs. age-matched controls (4.6%). However, there have been two case reports of myocardial infarctions occurring in young women later documented to lack coronary artery disease who were found to be homozygous for the factor V Leiden mutation. These patients had no other hemostatic abnormalities (40).

Heterozygotes for factor V Leiden do not appear to be represented more frequently among patients with arterial thrombosis as compared to the normal population (16). No increased incidence of APC resistance was found in 44 patients with premature arterial disease (2/44). The patients had peripheral vascular disease (17), stroke or TIA (15), MI (5), amaurosis fugax (3), peripheral vascular disease and MI (3), retinal artery occlusion (1) and aortic thrombus (1). However, another study in Austria found a 20% incidence of APC resistance (6 out of 30) in juvenile or

recurrent stroke patients. In addition, no strikingly high incidence of factor V Leiden mutation in a group of 13 patients with SLE and the antiphospholipid syndrome was found (41). These rather small studies will need to be confirmed. Two patients with Budd-Chiari syndrome were found to have APC resistance (42, 43). It will be interesting to know whether there is an unusally high incidence of APC resistance in patients with thrombosis of the inferior vena cava or other unusual sites.

APC RESISTANCE AND ORAL CONTRACEPTIVE USE

An important question is whether APC resistance interacts unfavorably with oral contraceptives to increase the risk of thrombosis, and whether knowing the APC status of women should guide decisions regarding OCP use. In the Leiden thrombophilia study, the risks of thrombosis associated with oral contraceptive use were assessed and reported in an independent paper (44). In this case-control study, 155 women with thrombosis aged 15 to 49 were compared with 169 age-matched controls for comparison of oral contraceptive use. Seventy percent of the 155 women were found to be oral contraceptive users, as compared to 38% of the controls, a significant difference. Analysis of the data led to a conclusion that oral contraceptive use led to a relative increase in risk of 3.8-fold (in agreement with other previous studies) and that the relative risk of the factor V Leiden mutation was 7.9-fold, and that the relative risk of having both risk factors was 34.7-fold. An additional 2 cases of thrombosis per 10,000 person-years is attributable to oral contraceptive use alone; the additional number of cases among heterozygotes for the factor V Leiden mutation was 23 per 10,000 person-years, a significantly higher number, but still small in absolute terms (Fig. 21). A woman's APC status could be taken into account in the event of a personal or strong family history of venous thrombosis. Whether the test has any predictive value (over and above the clinical history) remains to be seen.

	Patients	Person-years*	Incidence per 10 000 person-years
Factor V Leiden negative			
No OC use	36	437 870	0·8
Current OC use	84	275 858	3·0
Factor V Leiden positive			
No OC use	10	17 515	5·7
Current OC use	25	8757	28·5

*A total of 740 000 person-years (yielding 155 patients) was partitioned according to the distribution of the control group: 100/63/4/2.

Fig. 21. Population incidence of first venous thrombosis in women aged 15 to 49, according to presence of factor V Leiden mutation and use of oral contraceptives. From reference (44).

PRACTICAL CONSIDERATIONS AND RECOMMENDATIONS

Assays for activated protein C resistance and the Factor V Leiden mutation at Parkland. A standardized test for activated protein C resistance has been added to the "Thrombotic Panel" available through the coagulation laboratory. It is extremely simple and the cost is $75. It should not performed in the presence of heparin or coumadin, so (as with functional protein C and S assays) patients must be brought back to the clinic off anticoagulants. The test measures the ratio of the clotting time (the APTT) in the presence and absence of a fixed amount of activated protein C. A normal ratio is greater than 2.1.

Alternatively, a genetic test for factor V Leiden is now available (or will be in the next few weeks) through the Department of Pathology (Dr. Brian Dawson). The test is PCR-based (and therefore, anticoagulants do not interfere). The test should be close to 100% specific. Based on preliminary data, it should detect 85% of mutations responsible for APC resistance, but will miss patients who are APC resistant and do not have the factor V Leiden mutation. Cost will be around $150. The assay is illustrated in Fig. 22.

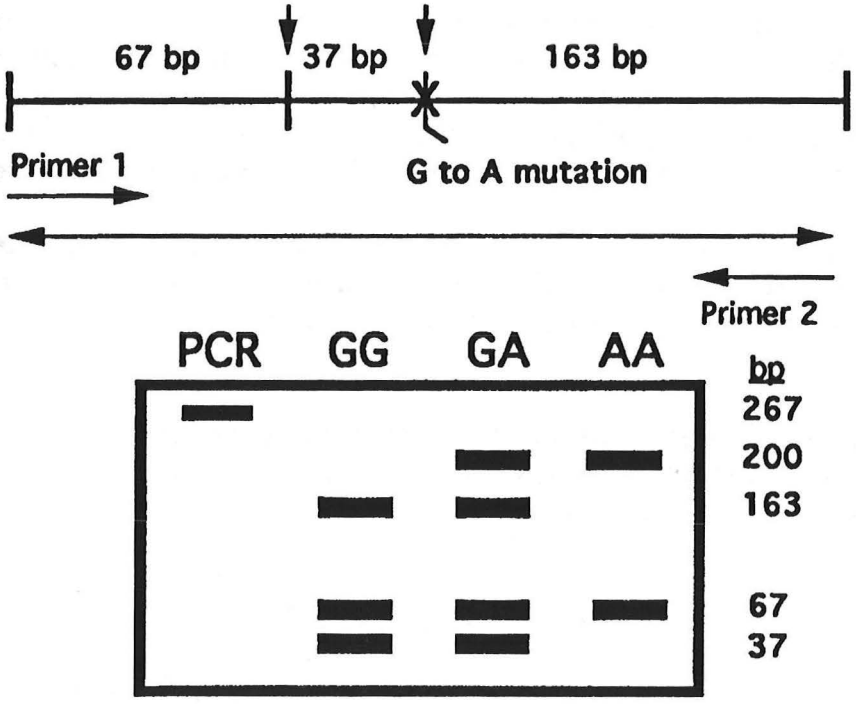

Fig. 22. A PCR-based assay for the factor V Leiden mutation. A G to A mutation causes the loss of a restriction enzyme site, leading to differences in banding patterns of amplified DNA among normal (GG), heterozygous (GA), and homozygous (AA) individuals. Courtesy of Dr. Brian Dawson, Department of Pathology.

Recommendations. The laboratory diagnosis of APC resistance will only be useful if effective intervention to prevent thrombosis is available, practical, and carries an acceptably low risk of side effects. There are three settings in which diagnosis could conceivably be useful: in avoidance of high-risk situations (such as the use of oral contraceptives), in the prevention of thrombosis during unavoidable high risk situations (such as major surgery or prolonged immobilization), and for the long-term prophylaxis of individuals at extremely high risk due to multiple genetic or other risk factors. The latter group would include individuals that currently would be evaluated for a genetic defect leading to a prothrombotic state: young patients (under 45) with thrombosis, patients with recurrent thrombosis, thrombosis at unusual sites, or strong family histories, or who seem to be unresponsive to therapy. There is insuffient data to determine whether individuals should be screened for APC resistance prior to surgery, oral contraception, or pregnancy. However, Dahlback has made several reasonable recommendations based on the available data (45):

1. APC resistance should be confirmed by a genetic assay for the Factor V Leiden mutation. Both tests are available at our institution.

2. Heterozygous individuals having no other anticoagulant defect and no personal or family history of thrombosis (i.e., asymptomatic family members) should be given prophylactic anticoagulant therapy in situations known to provoke thrombosis, such as major surgery. (Whether these patients should be actively sought is an open question and has not been addressed outside the research setting).

3. Heterozygous individuals with a history of thrombosis should be treated like protein C, S or anti-thrombin III-deficient patients. Preventive anticoagulation should be given in risk situations and long-term therapy is given if thrombosis is recurrent.

4. Homozygotes and individuals with combined defects are given preventive therapy in risk situations; long-term therapy should be considered early and tailored to the clinical circumstances.

UNANSWERED QUESTIONS

Prospective trials are needed to determine the natural history of thrombosis in APC-deficient individuals, with and without other genetic defects. If a patient has one thrombus, what is the risk of second thrombus? Is it higher in APC-deficient individuals as compared to those who are not (and may not be normal, but have some other defect). Would a test for APC-deficiency have a predictive value greater than that of the clinical presentation alone? And if long-term prophylactic anticoagulation is indicated, what is the most efficient way to manage chronic anticoagulation in these patients? These and other questions must be resolved in future well-designed clinical trials.

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