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Genetics and Molecular Pathophysiology of Thrombotic States

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1. Introduction

Venous thrombosis is a multifactorial disease frequently related to the interaction of genetic and environmental risk factors. Testing for specific mutations in these patients helps to determine the decision on the duration of anticoagulant therapy, risk stratification for primary or secondary prophylaxis. Some of the recently discovered genetic risk factors, such as factor V Leiden and prothrombin G20210A mutations, are quite common in the population. When compared to functional assays, molecular assays provide clear results without different cut-off values. Accordingly, laboratory investigation of thrombophilic disorders has expanded due to incorporation of modern molecular assays. Criteria used to select specific DNA methodologies reflect the issues of cost, automation, speed, reliability, and simplicity for specific diagnostics. A variety of currently used molecular methods fulfill many, but not all of these criteria. The new methods of real-time PCR and DNA microarrays offer the potential for widespread application and utility in the future. Problems arise with interpretation in many new polymorphisms without significant clinical relevance.

Let's look at the history of molecular diagnosis of thrombophilia. Since the very beginning of the diagnosis of thrombophilic disorders, which arose from the study of families with a high frequency of thrombophilic complications, it was apparent that in a number of cases, the disorder was due to dominantly inherited conditions. Already the discovery of the first families presenting a defect in antithrombin (AT) led to the description of the genetic causes of this defect. As such, over 150 causes of AT mutation were described.

Molecular genetic methods were implemented into the screening examinations for thrombophilic disorders in the 1990's along with the first discoveries of coagulation inhibitors (AT, protein C and protein S). The discovery of the molecular cause of activated protein C (APC) resistance by Bertina in 1994 greatly expanded their utilization.
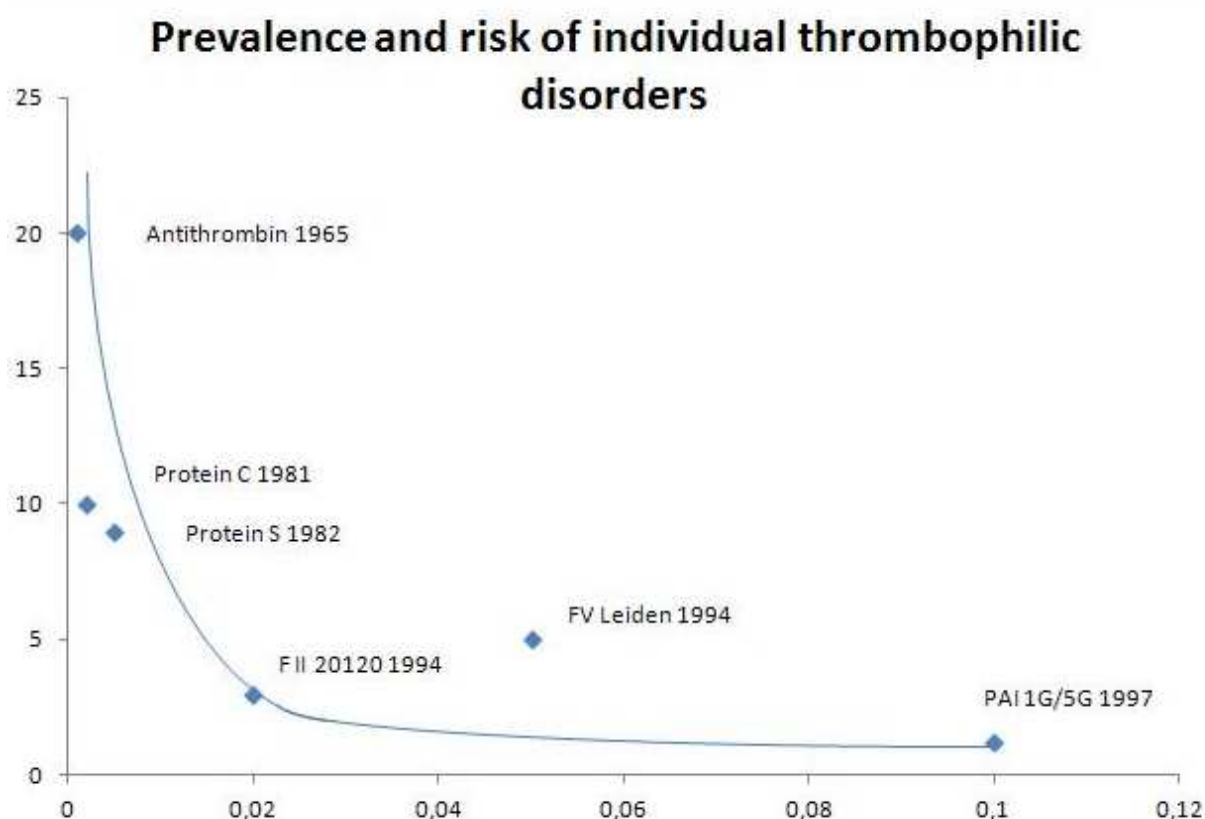
Figure 1. Prevalence and risk of individual thrombophilic markers with a time line representing their discovery[1] Despite the expansion of these methods, the following years brought forth discoveries of defects on a molecular basis, which posed decreasing risks of thrombosis, as can be seen in Fig 1. However, until the end of the last century, it has been assumed that further investigations of genetic causes of thrombophilia are needed to clarify the risk of this disorder in a more detailed manner. Currently, it is apparent that there is a deflection from this idea and attention is focused rather on the elucidation of the complex pathophysiology of coagulation at the molecular level.

Successive determination of relative risk of individual factors and their prevalence in the population led to the gradual definition of the panel of genetic thrombophilia. Currently, they can be divided into two groups i.e.1/ a well-defined genetic thrombophilic risk factors and 2/ potential risks factors of thrombophilia.Well-defined VTE risk factors include resistance to activated protein C (APCR) often due to factor V Leiden (FVL), prothrombin G20210A gene mutation (FII G20210A), high factor VIII (FVIII) activity or homocysteine level, and natural anticoagulant deficiencies: antithrombin (AT), protein C (PC), and protein S (PS). Patients with laboratory-confirmed thrombophilia are at greater risk for VTE, but most will never have such an event[2]. VTE risk increases synergistically as other risk conditions are acquired (eg, pregnancy, trauma, immobilization). More than 60% of patients with idiopathic (spontaneous or unprovoked) VTE have inherited thrombophilia[3].

An individual's risk for VTE would be determined by the combination of baseline propensity for thrombosis and the magnitude of the acute insult. In the face of genetically increased baseline hypercoagulability (major genetic thrombophilic state) even a relatively weak insult (e.g., blood stasis during a flight) can be sufficient to precipitate DVT. Likewise, in an indi-

vidual with a relatively low level of baseline genetic hypercoagulability (potential genetic thrombophilic state) a relatively strong thrombogenic event (e.g., pregnancy) may be required to provoke an episode of VTE[4]. Thus, the precipitating event in such individuals is often clinically overt. In most cases, such thrombophilic individuals never suffer VTE throughout their lifetimes, and when they do have an episode, it is unlikely to recur. In contrast, an individual with a high level of baseline genetic hypercoagulability is at such high risk that relatively minor acquired triggers can initiate a thrombotic episode. These triggers are therefore subclinical, giving the appearance that the patient has "idiopathic", "spontaneous", or "unprovoked" VTE.

Thrombophilia	Prevalence* (%)	Relative risk
Antitrombin deficiency	0.02	10
Protein C deficiency	0.2 - 0.4	10
Protein S deficiency	< 1	10
FV Leiden (G1691A) homozygosity	0.02	50
FV Leiden (G1691A).heterozygosity	5-7	5 - 7
F II (G20210A)	2 - 7	2 - 3
Fibrinogen gamma 10034T	6	2

* prevalence in Caucasian population

Table 1. Prevalence and relative risk of venous thromboembolism associated with well defined major genetic risk factors[5]

2. Major genetic thrombophilic states

Major genetic thrombophilic states include defects with clinically confirmed risk for VTE and an understanding of the pathophysiological action of these defects.

2.1. Antithrombin

Egeberg first described familial antithrombin III deficiency, now termed antithrombin deficiency, in 1965[6]-[8]. This first work already pointed out that antithrombin deficiency is a significantly more serious risk factor for developing thrombosis than protein C and S deficiency, and that the majority of patients show clinical manifestation before the age of 25[7], [9]. This does not pertain to changes of the heparin binding site, which occurs frequently, and does not present a risk in the heterozygous form[9].

Based on extensive studies, the thrombotic risk for patients with AT deficiency was determined to be increased five-fold, based on the 1.1 % prevalence of this deficiency in patients with venous thrombosis compared to 0.2 % prevalence in the control group[10]. Other studies

determined the prevalence of AT deficiency to be between 1 – 0.5 % [6]. There are two primary types of antithrombin deficiency: type I and type II. Type I antithrombin deficiency is characterized by an inadequate amount of normal antithrombin present. In this case, there is simply not enough antithrombin present to inactivate the coagulation factors. In type II antithrombin deficiency, the amount of antithrombin present is normal, but it does not function properly and is thus unable to carry out its normal functions. In many cases, the antithrombin in type I deficiencies has a problem binding to heparin, although there have been multiple other changes to the antithrombin molecule described.

Antithrombin deficiency may be assessed by chromogenic or clotting laboratory methods. The chromogenic assay is the most simple and usually preferred. Overall, there are fewer confounders with antithrombin activity assays than with protein C or protein S activity assays; partly because chromogenic antithrombin activity assays are performed rather than clot-based[11]. For antithrombin activity, chromogenic (IIa- or Xa-) based assays which are not affected by heparin are available. Thrombin (IIa)-based assays, in theory, can be falsely elevated by elevated heparin cofactor II because heparin cofactor II is a natural inhibitor of thrombin. Factor Xa-based assays might be less sensitive to type II deficiencies than the IIa-based assays[12]. Direct thrombin inhibitors falsely increase results in IIa-based assays but not with Xa-based assays, because they inhibit factor IIa but not factor Xa[13].

A false-positive result for a type II deficiency may occur in the presence of a heparin-binding site (HBS) mutation. Extending the incubation time of the activity assay to 300 sec may help normalize results, since the extra time allows the assay to overcome this limitation and normalize results, whereas other (clinically important) mutations remain abnormal[14]. In general, HBS mutations are thought to be not significant except in homozygotes[14].

Another caveat with antithrombin testing involves different type II mutation called antithrombin Cambridge II A384S[15],[16]. Heterozygotes and homozygotes with this mutation were found to have normal activity in an anti-Xa assay (and appropriately low activity with an anti-IIa-based assay). Heterozygosity was found in 0.1–0.2% of the general population and 0.4–1.7% of patients with venous thrombosis. Heterozygosity was associated with a ninefold increased risk for venous thrombosis. The main disadvantage of screening for deficiency using an antithrombin antigen assays is that type II (qualitative) deficiencies will not be detected.

2.2. Protein C

The first studies demonstrating increased risk of thrombosis in patients with heterozygous protein C deficiency were presented in 1981[17],[18]. No difference between patients with various types of deficiency (I or II) and basic mutation were noted. These studies showed that a large majority of patients already has clinical manifestations of the disease at a young age[19]. In addition, it is interesting to note that in some patients, APC resistance was also present as an additional factor increasing the risk of thrombosis[20].

The prevalence of protein C deficiency in patients with venous thromboembolism has been determined to exist approximately 3%[21]-[23] compared to 0.2 % prevalence in the healthy population[18]. When compared with the control group, the relative thrombotic risk for

protein C deficiency is apprixomately 6.5 fold[24]. Despite the relatively low population prevalence, the high risk of thrombogenicity results in a 1–2% contribution of protein C deficiency on all thrombophilic conditions.

Protein C deficiency can be detected by chromogenic or clotting methods. Both assays may have pitfalls in testing. Clot-based assays are aPTT- or Russell viper venom (RVV) based, either of which can give falsely high results in the presence of direct thrombin inhibitors. With aPTT-based assays, lupus anticoagulants can cause falsely high results and elevated factor VIII or factor V Leiden can cause falsely low results. Heterozygous factor V Leiden did not appear to affect an RVV-based assay, but artificially low protein C results in homozygous factor V Leiden patients could not be excluded due in part to the small number of such patients[25]. Lupus anticoagulants did not falsely increase protein C levels using an RVV-based assay[25], but according to the manufacturer, the possibility of lupus anticoagulant interference cannot be excluded.

There are two primary types of antithrombin deficiency: type I and type II. Type I antithrombin deficiency is characterized by an inadequate amount of normal antithrombin present. In this case, there is simply not enough antithrombin present to inactivate the coagulation factors. In type II antithrombin deficiency, the amount of antithrombin present is normal, but it does not function properly and is thus unable to carry out its normal functions. In many cases, the antithrombin in type I deficiencies has a problem binding to heparin, although there have been multiple other changes to the antithrombin molecule described. None of these conditions affect chromogenic assays, but there is a rare type II variant that might not be detected by chromogenic assays but is detected by clot-based assays. More recently, another rare variant, Asn2Ile, has been identified that is missed by chromogenic assays, and it is also missed by some clot-based assays[26]. Testing protein C while on warfarin therapy is not recommended because warfarin decreases protein C (and protein S) levels. However, if testing is inadvertently sent while on warfarin, the result with a chromogenic assay is typically higher than with clot-based assays.

2.3. Protein S

Protein S is an important anticoagulant protein, which acts as a non-enzymatic co-factor of activated protein C (APC) during inactivation of factor Va and VIIIa. Laboratory screening of protein S deficiency is complicated by the fact that protein S circulates in bloodstream in two forms, i.e. bound and free[27]. Forty percent of total protein S is represented by free protein S, which acts as the APC co-factor.

Protein S deficiency may be divided into three basic forms. Type I is characterized by a decrease in total protein S most often due to decreased synthesis, type II is characterized by decreased protein S activity and type III by a decreased level of free protein S and normal activity of total protein S.

Most deficits are type I or a combination of types I and III. At present very few cases of type II protein S deficiency have been described. The prevalence of protein S deficiency represents 1-2% of patients with deep venous thrombosis and 6% of families with thrombophilia. From

a genetic viewpoint, 70 different mutations in the gene for protein S have been described to date.

The detection of protein S deficiency is complicated, because protein S circulated in the blood stream in two forms, i.e. bound protein S associated with C4b binding protein (60%) and free protein S (40%). The thrombotic risk potential constitutes only free protein S. Protein S activity may be determined using clot-based (aPTT, PT, Xa or RVV) assays. A number of factors may interfere with clot based assays. Lupus anticoagulants or direct thrombin inhibitors[28] can cause falsely elevated results. Factor V Leiden can cause falsely low results with PT-, Xa-, RVV-, and some aPTT-based assays[29]-[31]. With aPTT-based assays, elevated factor VIII can cause falsely low results because factor VIII shortens the aPTT in the assay.

Protein S free or total antigen assays do not suffer from similar limitations. Acute phase reactions (e.g., illness, inflammation) can falsely decrease protein S in vivo, and it is a common cause of low protein S activity or decreased free antigen results (but not total protein S antigen). This effect is attributed to C4b-binding protein, which becomes elevated during acute phase reactions. C4b-binding protein binds to protein S, thus reducing the amount of free protein S. When protein S is bound to C4b-binding protein, it is essentially inactive. The main disadvantage of free or total protein S antigen assays is that type II deficiencies will not be detected[32], but the majority of hereditary protein S deficiencies are type I (quantitative).

3. Factor V Leiden and APC resistance

The detection of Factor V Leiden (FVL) is usually performed as genetic confirmation of activated protein C (APC) resistance positivity. The classical assay for activated protein C resistance detects a ratio between a baseline activated partial thromboplastin time (aPTT) and the aPTT with APC. A variety of conditions can cause "falsely elevated" or "falsely decreased" ratios and interfere with this test, rendering it somewhat insensitive and nonspecific for diagnosing FVL, However, a positive result of APC resistance, due to acquired factors, may pose the same risk as the presence of the FVL mutation [33].

Elevated factor VIII (FVIII), the presence of a lupus anticoagulant, and certain drugs including oral contraceptives, estrogen, vitamin K antagonists, heparin, or direct thrombin inhibitors may interfere with the traditional aPTT-based APC assay. Improved assays are available, including the second-generation assay for activated protein C resistance, with sensitivity and specificity approaching 100% for the diagnosis of factor V Leiden[34]. The modification involves diluting the patient plasma into factor V-deficient plasma, thus minimizing the effect of factor V deficiencies and factor FVIII elevations that alter the baseline aPTT. Despite these changes, lupus anticoagulants may cause falsely low ratio results and direct thrombin inhibitors can cause falsely normal ratio results[35],[66].

Newer test options include Russell viper venom (RVV)-based assays, which uses the factor X activator from RVV. The reagent contains phospholipid designed to reduce lupus anticoagulant interference.

A factor Xa-based clotting assay is a third type of new assay for APC resistance. The method includes dilution into a proprietary reagent containing purified factors II, fibrinogen, protein S, and activated protein C. The inclusion of these factors presumably eliminates interference due to deficiencies or increased levels of these proteins.

While APC resistence positivity caused by presence of lupus anticoagulants is often considered to be „falsely elevated", several studies report that this parameter has some clinical signifi‐cance.

While APC resistance positivity caused by lupus anticoagulants is often thought of as an "falsely elevated", some studies report that it have clinical significance.

Factor V Leiden can be detected by several DNA assays. In recent years, different molecular approaches, including restriction fragment length polymorphism (RFLP) and real-time PCR, have been developed to genotype single nucleotide polymorphisms (SNPs). Compared with more traditional methods such as RFLP, real-time PCR is a fast, simple, and accurate procedure for SNP genotyping of medium to large collections of samples. Real-time PCR analysis can be performed using various strategies[5]. In the hydrolysis assay, the probe and fluorescent chromogen is released by hydrolysis during PCR amplification and the free probe quantity is proportional to the fluorescence[37]. Fluorescence resonance energy transfer (FRET) is a method that distinguishes alleles by melting the products and monitoring the loss of fluores‐cence using an allele-specific oligonucleotide probe which hybridizes to specific sequen‐ces[38]-[40].

High-resolution melting analysis with LCGreen™ I is a newly designed analysis that de‐tects heteroduplexes during homogeneous melting curve analysis with a new fluorescent DNA dye[41]. Genotyping of single-nucleotide polymorphisms (SNPs)1 by high-resolu‐tion melting analysis in products as large as 544 bp has been reported. This allows closed-tube, homogeneous allele-specific PCR genotyping [45],[46], on real-time PCR instruments without fluorescently labeled probes[42]-[44]. Heterozygotes are identified by a change in melting curve shape, and different homozygotes are distinguished by a change in melting temperature (Tm). However, it is still not clear whether all SNPs can be genotyped by this method[45]-[47].

Among the Caucasian population, factor V Leiden (factor V 1691G-A) is the most com‐mon genetic defect causing thrombosis[48]-[54]with a frequency between 2 and 15 % [49].. Factor V mutation was first described by Bertina et al in 1994 at the University of Lei‐den[55], based on the discovery of resistance to activated protein C (APC), which was first described in 1993[56]. The heterozygous form of factor V Leiden increases the risk of thrombosis 3 – 8x [49],[57],[58], while the homozygous form presents a risk which may be up to 80 times greater[59].

Factor V Leiden is present in up to 20% of patients with venous thrombosis[50],[57] and in over half of probands in selected families with thrombophilia making it the most common genetic abnormality in patients with thrombosis.

4. Pathophysiology of action FV Leiden mutation

FV plays a key role in both the procoagulation and anticoagulation cascade processes. In the activated form, it acts as a co-factor for FXa in the prothrombinase complex and as such catalyses the conversion of prothrombin to thrombin. In the inactive form FV acts as an APC co-factor in the regulatory activity of FVIIIa. In inherited and acquired defects, this double role allows FV to influence the manifestation of these disorders into hemorrhagic or thrombotic forms [60],[61]. To determine this manifestation, it is necessary to recognize precisely the procoagulation and anticoagulation forms of FV[63].
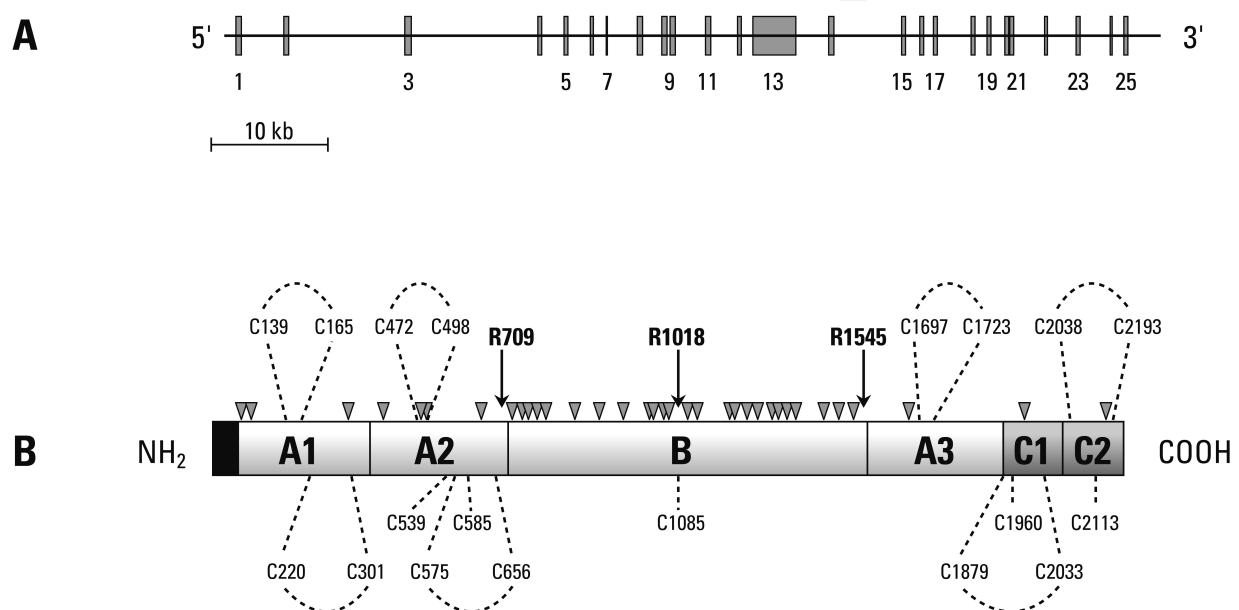
Figure 2. Schematic representation of the structure of FV

Unlike FV, FVa increases the FXa activated conversion of prothrombin in the prothrombinase complex. It may be expected that the cleaved B-domain allosterically inhibits the binding of FV in the active site of FXa [63].

The mechanism by which FVa acts on FXa is not completely understood. Based on the latest works, it may be assumed that FVa increases the binding affinity of FXa to phospholipids by about 100x. In addition, it was determined that FVa in the prothrombinase complex does not change the binding site of FXa, but instead increases the affinity of the prothrombinase complex to prothrombin and ensures an increase in binding sites.

The co-factor activity of FVa is balanced by APC, which proteolytically cleaves FVa at the Arg306, Arg506 and Arg679 sites of the heavy chain. The weakest inactivation is seen during cleavage at the Arg679 site. Latest discoveries suggest two pathophysiological models of FVa cleavage. The first model assumes preferential cleavage at the Arg506 site followed by cleavage at the Arg306 site. Alternatively, FVa may be inactivated directly by cleavage at the Arg306

site. Cleavage at the Arg506 site also decreases the affinity of FVa to FXa, while cleavage at the Arg306 site causes complete inactivation of FVa [64].

5. Anticoagulation function of FVa

Aside from its procoagulation function, FVa also possesses an anticoagulation function, which is expressed as APC activation. In this case it is a cofactor in the proteolytic cleavage of FVIIIa[65]. Recent experimental works supports this model where the addition of purified FV renewed the function of APC in both healthy patients and in families with the Leiden mutation. The experiments utilized measured generation of thrombin.
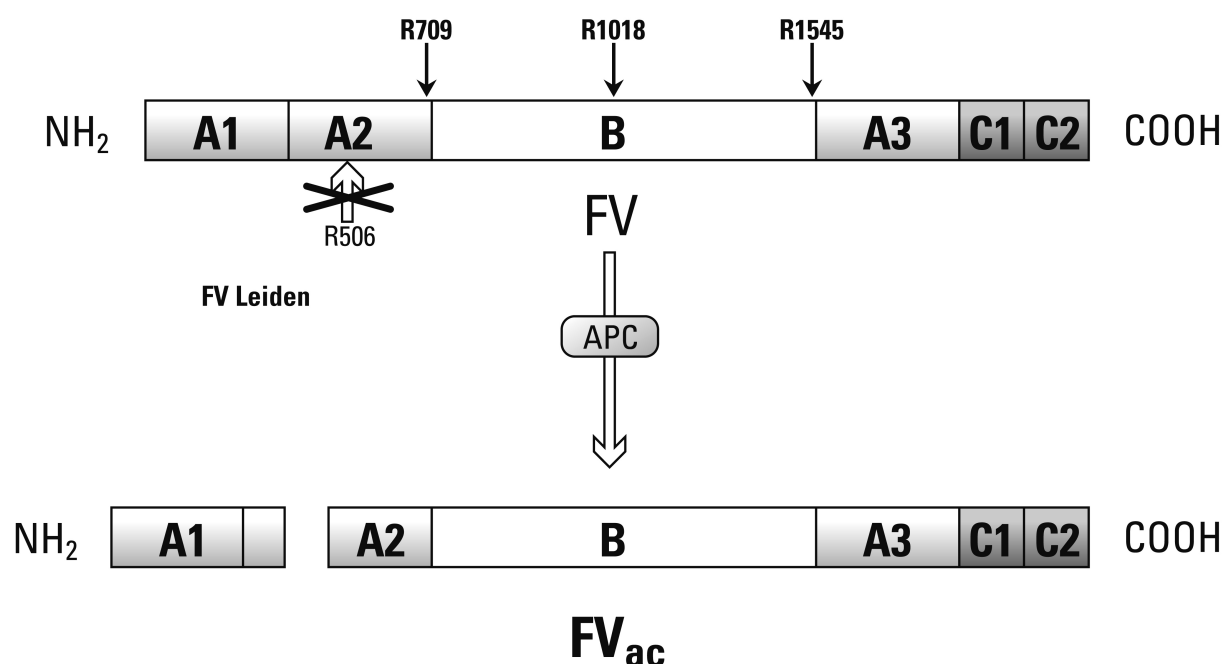

Figure 3. Anticoagulation structure of FV[59]

Thus if the anticoagulation function of FV is required, cleavage must occur in position Arg506. Unlike APC mediated cleavage in sites Arg306 and Arg679, which does not exhibit anticoagulation activity.

6. FV and thrombophilia

Such described pathways of FV activation point to the balance of pro- and anticoagulation activity of factor V and its significance in maintaining haemostatic equilibrium. The pathways of FV activation describe the mutual balance of both pro- and anticoagulation activity of factor V and its significance in maintaining haemostatic equilibrium.

APC resistance is an in vitro described phenomenon, which is characterized by a slight anticoagulation response to APC in plasma. Such decreased sensitivity to APC leads to inadequate regulation of thrombin production. As such, APC resistance is associated with an increased risk of developing thrombosis. APC resistance is associated with FV Leiden mutation in up to 90 % of cases.

FV Leiden mutation significantly influences the pro- and anticoagulation balance of FVa. The Leiden mutation, which leads to the disappearance of the Arg506 cleavage site, causes an insufficient decrease of the procoagulation activity of FVa, which explains the presence of procoagulation states in carriers of the Leiden mutation.
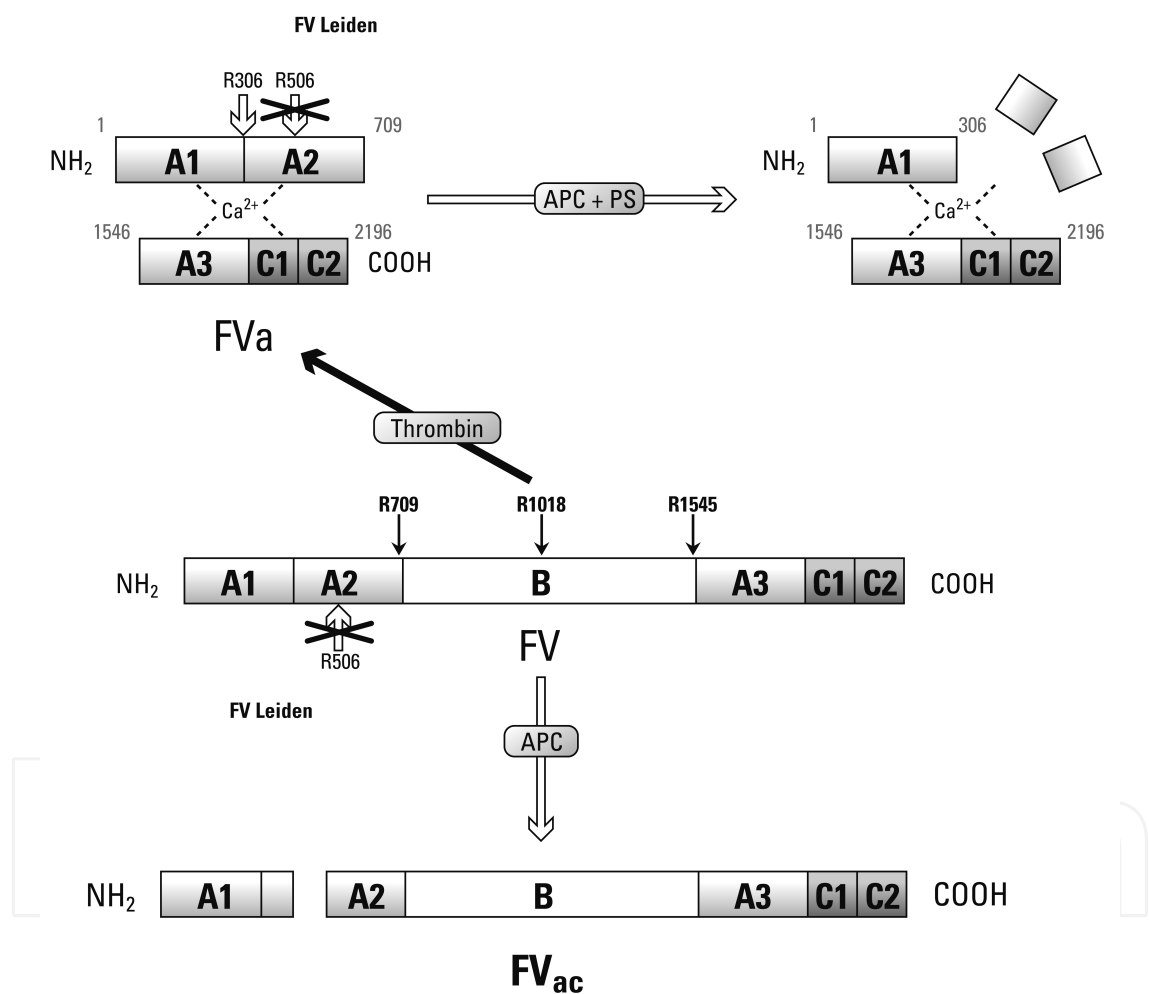
Figure 4. A schematic illustration of the activation of FV to procoagulation and anticoagulation forms

The discovery of a second pathway of action of FVa, where via APC it acts as a co-factor during proteolytic cleavage of FVIIIa aids us in establishing the effect of the Leiden mutation. Since FV with the Leiden mutation does not contain a cleavage site in the position Arg506, the anticoagulation form FVac cannot be produced and therefore there is only weak co-factor activity of APC during FVIII proteolysis.

7. Prothrombin 20210A

Mutations in the 3′- untranslated region of the prothrombin gene in the position 20210 G-A are associated with an increased level of prothrombin and as such present an increased risk of developing thrombosis[66]. This mutation was described with a high prevalence (up to 18%) in families with thrombosis and in 6.2% of patients with first thrombosis[66].

F II 20210G-A mutation in the gene for prothrombin was found through linkage analysis in families with a history of venous thrombosis and no other congenital or acquired thrombophilic risk factors[67]. The mutation is located in the 3 'gene region, which already does not overwrite to the protein. The mutation is located in the 3 'gene region, which already does not overwrite to the protein. Therefore, it appears difficult to develop a specific functional coagulation tests based FII activity determination. However, in large clinical studies found significantly elevated levels of prothrombin mutations in patients with FII 20210 GThere is no evidence that the homozygous mutation carriers have a higher level of prothrombin than heterozygous. The pathophysiologic influence of the mutation is not yet fully explained. The original view was that the mutation alters the conformation of the pre-mRNA 3'UTR, which has an increased affinity for the production of polyA end. This causes an increased concentration of mRNA with an increase in protein synthesis, so the greater the level of prothrombin in plasma[68]. Later work demonstrated that the mutation does not affect the mRNA level, but instead 3' cleavage/polyadenylation, which affects abnormal properties of mRNAs[69]. Finally, recent work showing abnormal properties mRNA 20210A mutation in the 3'UTR, which cause the emergence of polyA preferentially in the presence of this mutation in the mRNA[70].

The prevalence of this mutation in the normal population is 1.7% in northern Europe and 3% in southern Europe[71],[72]. This mutation is very rare among people of African and Asian origin. It was found that originated as a founder effect until after the separation of Caucasian populations [73]-[76].

Apart from this, the mutation may represent an additional risk factor for spontaneous abortions especially during the first trimester[77]. The risk of venous thrombosis is significantly increased in women with prothrombin mutation during pregnancy and after childbirth,and the frequency of thrombosis further increases in the presence of an additional risk factor, which - among others - can be factor V Leiden[78]. Prothrombin mutation may also affect the risk of arterial thrombosis, but this has not been clearly demonstrated yet. In a large group of 3028 patients, a statistically significant relationship between the mentioned mutations and ischemic stroke (odds ratio 1.44) [79] was demonstrated. Ingeneral the risk of spontaneous recurrent thromboembolic events is the same in patients with mutations without such mutations[80].

8. Potential genetic thrombophilic states

8.1. Plasminogen activator inhibitor-1 mutation

The plasminogen activator inhibitor-1 (PAI-1) mutation is a single base insertion or deletion at position -675 (4G/5G) in the promoter region of the PAI-1 gene, which is associated with
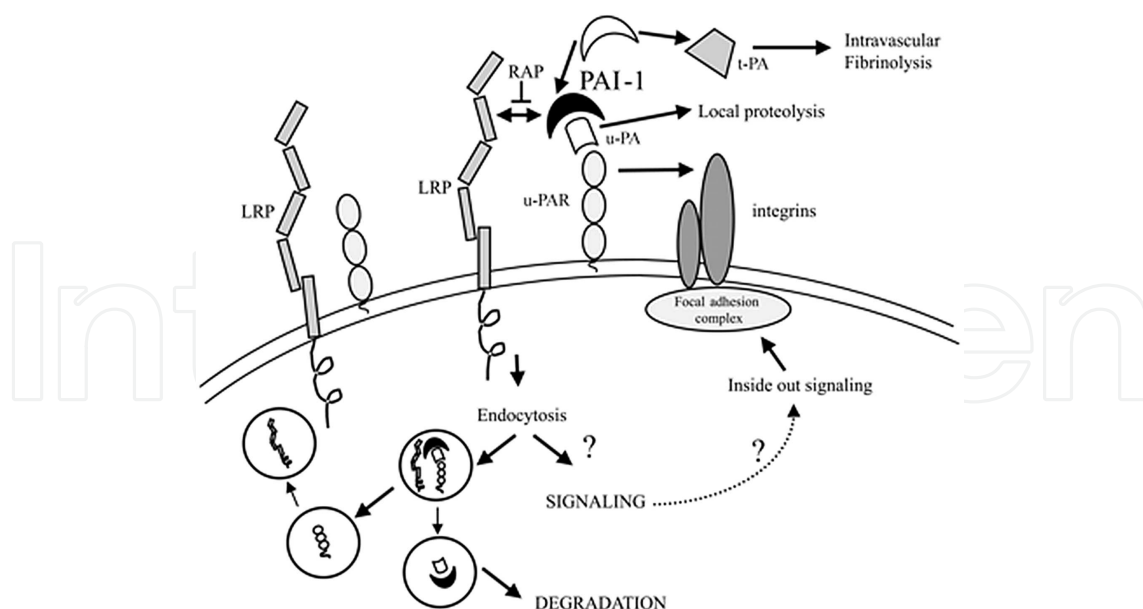
Figure 5. Schematic action PAI-1 in regulation mechanism of fibrinolytic system

higher levels of PAI-1 transcripts and also higher plasma activity of PAI-1[81]. In the PAI-1 - 675 4G/5G mutation, the 4G site binds an enhancer element only, whereas the 5G allele binds both enhancer and suppressor elements. As a result individuals with 4G/5G or 4G/4G geno-types have an increased level of transcription and consequently a higher PAI-1 protein level than individuals with 5G/5G polymorphism. Because PAI-1 is a rapid inhibitor of tissue plasminogen activator (t-PA), the 4G/5G variant of mutation can increase the activity of PAI-1 and decrease the conversion of plasminogen to plasmin, which causes decreased fibrin degeneration and increased clot stabilization.

Under physiological conditions, PAI-1 is released into the circulation and the extracellular space by only a few cells: liver cells, smooth muscle cells (SMC), adipocytes, and platelets are the major sources of PAI-1. This results in plasma levels of only 5–20 ng/ml of active PAI-1, sufficient to control fibrinolysis and extracellular proteolysis.

Under pathological conditions, however, several other tissues secrete quite large amounts of PAI-1: tumor cells, endothelial cells in response to inflammatory cytokines, and other inflam-mation-activated cells. High PAI-1 plasma levels are consistently found in patients with severe sepsis but also with other acute or chronic inflammatory diseases such as atherosclerosis. PAI-1 is upregulated by inflammatory cytokines and may therefore be regarded as a marker for an ongoing inflammatory process. It is of major importance, however, that no classic inflamma-tory response element was found in the PAI-1 promoter region, and it is still unclear how PAI-1 expression is upregulated during inflammation[82].

8.2. Fibrinogen

Fibrinogen is a 340-kDa glycoprotein produced in the liver with a plasma concentration of 180 - 450 mg/dl. It consists of two monomers linked by a disulfide bond. Each monomer consists

of three polypeptide chains encoded by three distinct genes: Aa, Bb and γ. It binds to the platelet through the glycoprotein IIb/IIIa receptor and, therefore, is highly involved in the thrombotic processes. It also has a direct effect on the vascular wall and blood viscosity. An association between increased circulating levels of fibrinogen and the development of arterial thrombosis has been demonstrated[83],[84]. The mechanisms that explain the association between increased fibrinogen and AMI include increased fibrin formation, increased blood viscosity, platelet hyperaggregability and an increased proliferation of vascular endothelial and smooth muscle cells[85]. In addition, fibrinogen is an acute phase reactant and possibly an indicator of chronic inflammation associated with atherosclerosis.

There are several polymorphisms identified in the genes that code the three polypeptide chains and are associated with increased concentration of circulating fibrinogen (>500 mg/dl). From the functional point of view, the most relevant are those in the β chain: Arg448Lis, -148 C/T, -455 G/A and 854 G/A, with the last two having the most pathophysiological interest due to their association with the development of vascular disease. The genotype -455A is present in 10-20% of the general population and is correlated with an increase of 10% high concentration of fibrinogen, prevalence of other mutations not specifically described in current literature. Some studies[86] showed an association between the -455 polymorphism and an increased risk for arterial thrombosis.

9. Coagulation factor VII (FVII)

Factor VII is a single polypeptide chain, 406-amino acid glycoprotein with a weight of ~50,000 Da. It is vitamin K dependent protein, synthesized in the liver and has a plasma concentration of 500 ng/mL. FVII is transformed into its activated form (FVIIa) by binding with tissue factor (TF). The TF:FVIIa complex converts Factor IX and Factor X to their active forms, FIXa and FXa. Recent studies have described an association between an increased plasma concentration of FVII and development of arterial thrombosis[87]

In contrast, polymorphism Arg (R) 353Gln (Q) in the FVII gene is associated with a 20-25% decreased plasma concentration of FVII.[88]

In addition, several studies[89],[90] have demonstrated that plasma concentration of FVII is lower in subjects homozygous for the 353Gln allele compared with those homozygous for the 353Arg allele.

In contrast, other studies have been unable to corroborate the association of the polymorphism R/Q353 with AMI or increase in plasma concentration of FVII in subjects with Arg/Arg genotype[91].

10. Polymorphisms in platelets

Platelets play an important role in the development of acute coronary syndromes. Platelets possess glycoprotein receptors on the surface membrane which participate in platelet activa-

tion, degranulation and aggregation. These receptors belong to the family of integrins (aIIb β3) and are highly polymorphic, which often produces an antigenic alteration of the glycoprotein. The most frequent polymorphisms present in these glycoproteins are receptors for von Willebrand factor (vWF) (GPIb-IX, GIIb-III), a in the protein receptor for collagen (GPIa-IIa), and in the receptor for fibrinogen (GPIIb-IIIa).

The most abundant glycoprotein on the platelet membrane is the GPIIb-IIIa complex. Inactivated platelets bind through GIIb-IIIa complex to vWF and to fibrinogen. This polymorphism consists of a thymine substitution for cytosine in the 1565 position in exon 2 of the GPIII gene[92]. Approximately 25% of the population of northern Europe is a carrier of at least one allele PLA2 (PLA1/A2). Several studies described an association between this polymorphism and AMI in patients <60 years[93], ACS[94] and atherothrombosis[95]. Many studies have attempted to establish an association between the presence of allele PLA2 and coronary artery disease, but results are still contradictory.

11. Hyperhomocysteinemia

Increased levels of homocysteine are associated with an increased risk of thrombosis[96]-[98]. Two studies performed on an undivided group of patients showed that a level above 18.5 μmol/l in 5 % (10 % respectively) of tested subjects led to a two-fold increase in the risk of thrombotic episodes[99]-[101]. This means that hyperhomocysteinemia represents 5 – 10 % of all thrombotic episodes.

Hyperhomocysteinemia may develop due to genetic or acquired dispositions[99]. Acquired dispositions primarily include low vitamin intake or resorption (B6, B12, folic acid), which leads to an increase in homocysteine level[101],[102]. Genetic factors include the very rare cystathione β-synthase deficit, whose homozygous form represents classical hyperhomocysteinemia with very high levels[103], and on the other hand the very common variant of the methyltetrahydrofolate reductase (MTHFR) gene, which leads to the thermolabile enzyme variant with a slightly increased homocysteine level[104]-[106]. Currently, direct association between the MTHFR 677TT variant, increased homocysteine level and risk of developing thrombosis is not distinctly established[107].

12. Conclusion

The elucidation of the precise pathogenesis of action leads to the conclusion that to explain the clinical expression of thrombophilic conditions, it is most important to understand the interaction of at least one genetically dependent thrombophilia with one or more acquired conditions.

Determination of thrombophilic markers is necessary to evaluate the risk of thrombophilia in the examined patients. The degree of risk depends largely on the choice of methods for testing

individual markers and their specificity. Another important reality in the interpretation of tests are interfering factors for each test.

Uncritical interpretation of laboratory results can lead to misdiagnosis and thrombophilia testing exemplifies this. If possible errors and overdiagnosis are to be avoided, the following points should be respected: Normal ranges for antithrombin and proteins C or S levels are wide and patients with deficiency may have levels that are only slightly below normal. Repeated testing is often required for diagnostic accuracy. For example, laboratory quality assurance data have shown that for protein S in particular the rate of laboratory error in diagnosis can be as high as 20%. Pregnancy induces a state of resistance to the anticoagulant effect of activated protein C, which mimics the presence of factor V Leiden. Pregnancy and OCP use lead to a fall in plasma protein S concentration. Antithrombin concentration is reduced in acute thrombosis, by heparin treatment and in pre-eclampsia state. Proteins C and S are vitamin K dependent and their concentrations are reduced by warfarin treatment. Even if the above potential pitfalls are avoided, there is no indication to routine testing for heritable thrombophilia at presentation with acute VTE, as clinical management is not influenced by the results.

Concerning the determination of mutation FII 20210 G / A, the situation is quite the opposite. Screening test shows high interference with the current status, therefore it is completely unusable and direct molecular genetic analysis of point mutations FII 20210 G-A.

The identification of potential genetic causes of thrombophilia, such as mutation of fibrinogen, platelet receptor, PAI-1, F VII and homocysteine are designed for testing the rare causes of thrombophilia in thrombotic centers. Howeever, they can be beneficial to clarify complex cases of thrombophilia especially if associated with repeated manifestations in the family.

Genetic testing is a separate part of the diagnosis of thrombophilia. Generally, it is not intended for screening the universal population, but only to determine the causes of thrombophilia in defined groups of patients - young patients with a positive family history of thrombophilia, in patients with previous thrombosis under 45 years of age, patients with the manifestation of thrombosis in an unusual site (e.g. portal vein), and at women in pregnancy or with using oral contraceptives.

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