LECTURE
ΔΙΑΛΕΞΗ

Genetic risk factors of thrombophilia

Venous thromboembolism is a common multifactorial disease. Varying combinations of risk factors play a role in the onset of the disease. These risk factors are often environmental, but also genetic risk factors are essential. These genetic risk factors include common gain-of function mutations in the procoagulant plasma protein factors V and prothrombin that occur with a relatively high prevalence in Caucasian populations. Mutations in anticoagulant factors are also important. These loss-of function mutations are characterized by an overwhelming diversity but low abundance in the population. This low prevalence in the population is probably caused by the loss of mutant alleles from the gene pool through critically ill homozygous subjects. The gain-of function mutations in procoagulant proteins differ from the loss-of function mutations in this respect, as homozygous individuals are relatively mildly affected.

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Γενετικοί παράγοντες κινδύνου θρομβοφιλίας

Περίληψη στο τέλος του άρθρου

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Antithrombin deficiency
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1. INTRODUCTION

Upon vascular injury the coagulation system becomes activated in order to stem the bleeding. This procoagulant mechanism is counteracted by intricate anticoagulant networks that protect from unnecessary and excessive clot formation. Under normal circumstances these systems are quiescent and in balance, and profuse bleeding or runaway coagulation are therefore rare events. Only in unusual circumstances (oral anticoagulant treatment, septic shock, hereditary coagulation defects, etc.) do major coagulation problems occur. In addition, apparently unprovoked thrombus formation may occur, particularly in older persons. Examples of this are deep venous thrombosis of the leg and pulmonary embolism.

Such venous thrombotic events do not occur randomly, and both acquired and genetic risk factors play a determining role. Classical acquired risk factors include advancing age, cancer, prolonged bed rest and surgery. In approximately 25% of the patients genetic risk factors are found. This review will focus on the spectrum displayed by the mutations that underlie these genetic risk factors.

Mutations in important segments of a gene are detrimental to function and lead to a so-called loss-of function abnormality. But there are rare cases where a mutation will improve the functionality of a gene or gene product, in other words leads to gain of function. In the genetic risk factors for venous thrombosis both types of mutation are significant. Predictably, the gain-of function mutations go with procoagulant factors, whereas the loss-of function abnormalities occur in anticoagulant genes.

2. COAGULATION INHIBITORS

2.1 Antithrombin

Antithrombin is the most important circulating inhibitor of the coagulation cascade. Not only thrombin, but also other serine proteases such as factors IXa, Xa, XIa and XIIa are, to a variable extent, inhibited by antithrombin.

Loss-of function mutations in the antithrombin gene cause antithrombin deficiency. Homozygosity for antithrombin deficiency is extremely rare, and probably zero antithrombin levels are not compatible with life.[1] Heterozygous antithrombin deficiency is associated with a predisposition to venous thrombosis.

On the basis of plasmatic tests, different classification schemes for antithrombin deficiency have been proposed.[2] In the most recent scheme, two principal types of deficiency are recognized. Type I is a quantitative deficiency, with plasma levels of antithrombin around 50% of normal. In type ll deficiency, levels are roughly normal, but functional activity is impaired. Type II can be further subdivided on the basis of the nature of the functional defect. Mutations in the reactive site are referred to as RS, and mutations in the heparin-binding site as HBS. The remaining functional mutations are classified as pleiotropic, or PE.

In 1983, a large deletion of the antithrombin gene was documented.[3] Since then, numerous mutations have been discovered in affected pedigrees (more than 250 to date).[2] Not surprisingly, type I deficiency is associated with a wide spectrum of mutations, including large deletions, frameshift mutations, premature stopcodons, and splice junction mutations. In type II RS deficiency, mutations are in (or influencing) the reactive site, whereas type II HBS mutations are in the heparin- binding region of antithrombin. The missense mutations that underlie type II PE deficiency tend to cluster in the C-terminal region of the protein.

2.2. Heparin cofactor II

This circulating anticoagulant protein is very homologous to antithrombin. Heterozygous deficiency appears to predispose patients to arterial and venous thrombotic disease, but solid evidence to support these claims is still lacking. Two frameshift mutations have been described in heterozygous deficiency. One, insertion of a T in exon 2, was discovered in a Japanese patient who suffered from coronary artery disease.[4] The second mutation, a deletion of two nucleotides in exon 5, was found in two apparently unrelated Italian patients with thrombophilia.[5]

2.3. Protein C

Protein C is the plasma zymogen of a serine protease that plays a central role in limiting the activity of the coagulation cascade.[6] Thrombin formed during coagulation is responsible for conversion of protein C to activated protein C (APC). This activation takes place on the surface of endothelial cells and monocytes by thrombin in complex with thrombomodulin. APC restrains blood coagulation by cleaving the activated cofactors Va and VIIIa into inactive fragments: this cleavage requires protein S as a cofactor in order to proceed efficiently.

Loss-of function mutations in the protein C gene lead to protein C deficiency. In 1981, i.e. well before the gene was cloned and sequenced, the first family with heterozygous deficiency was discovered.[7] Soon thereafter the first reports appeared on homozygous deficiency.[8] This condition is much more severe than heterozygous deficiency, and life-threatening thrombotic complications start immediately after birth. This differs sharply from the late-onset episodes of deep venous thrombosis or pulmonary embolism that arise in only a minority of heterozygous carriers.

Not all mutations lead to a complete disruption of gene function. Differences in severity between mutations can be evaluated only in homozygous or compound heterozygous patients. Severe mutations, like a premature termination codon, lead to purpura fulminans at birth. Mutations that leave some activated protein C to be produced, even if this is only several percent of normal, protect the patient from immediate symptoms and may delay the first episode of venous thrombosis until early adulthood.[9] It is not known whether these milder mutations also lead to less of a thrombotic risk in heterozygous form.

Around 200 different mutations in protein C deficiency are known.[10] This fact serves to illustrate once more that loss-of function mutations have a propensity to be very heterogeneous. It is as if every family possesses its private mutation, and the mutations that have been discovered cover the whole spectrum of imaginable abnormalities. These include missense mutations, promoter mutations, RNA-processing mutations and frameshift mutations.

Although families often have private mutations, some mutations occur more frequently. For example, the Arg230Cys replacement has been documented in at least 28 independent families. Two factors play a role in the recurrence of mutations.[11]

Firstly, recurrent mutations typically occur at so-called CpG dinucleotides, which are known hot spots where mutations preferably take place. Secondly, apparently distinct families may have a common ancestor (founder) in the past. In one example, this ancestor was traced back to the eighteenth century.[12]

2.4. Protein S

Protein S is the cofactor of protein C in the inactivation of factors Va and VIIIa. The human genome contains

two copies of the protein S gene on chromosome 3.[13] Only one of these copies is normally intact and active. The second copy is heavily mutated and does not produce any mRNA and is therefore considered a pseudogene. Although clinically irrelevant, the pseudogene complicates the genetic analysis of protein S deficiency.

The first cases of heterozygous deficiency were published in 1984.[14] Clinical symptoms in heterozygous deficiency are similar to those in heterozygous protein C deficiency, thus being of late onset and relatively mild. Only few cases of homozygous deficiency have been thoroughly documented, and these were associated with extremely severe thrombotic disease in newborns.[15]

Around 100 different mutations have been documented in protein S deficiency, and the spectrum is wide–as expected for loss-of function mutations–and comparable to that observed for protein C deficiency.[16] The mutations are spread across the gene and include splice-junction abnormalities, premature stopcodons, frameshift mutations, missense mutations, and large deletions.

Several polymorphisms, i.e. sequence variations not clearly associated with altered gene function, are known for the protein S gene. The Heerlen polymorphism is a notable example.[17] This polymorphism was originally considered to be neutral and of little clinical importance because plasmatic tests failed to show abnormal activity or levels. However, the neutrality of the Heerlen polymorphism has been recently challenged.[18,19] Using a variety of assays, two independent studies showed that the Heerlen polymorphism may be associated with low levels of free protein S (type III deficiency). It was also claimed that protein S Heerlen displays a higher affinity for its plasma companion C4b-binding protein. Therefore, it cannot be excluded that this polymorphism may turn out to be a risk factor for thrombotic disease after all. It is likely that the contribution to risk will be modest at best, however, because compound heterozygotes carrying a truly deficient allele and a Heerlen allele are not severely ill.[12]

2.5. Thrombomodulin

Thrombomodulin is an integral membrane protein of endothelial cells and monocytes. When thrombin binds to thrombomodulin, it loses its procoagulant activity but becomes capable of activating protein C. Given this crucial function in the protein C pathway, a hereditary deficiency of thrombomodulin might very well play a role as a risk factor for thrombotic disease.

At present, a handful of missense mutations are known in the thrombomodulin gene of patients with venous thrombosis.[20,21] It remains difficult, however, to judge the relationship between these mutations and disease. One reason for this is that simple functional assays are not in place to prove that these mutations are detrimental to thrombomodulin gene function. Indeed, *in vitro* data obtained with the first mutation ever reported in the thrombomodulin gene, Asp468Tyr, did not reveal any abnormality in production levels or functional activity.[22]

A particular amino acid dimorphism, Ala455Val, occurs with frequencies of 0.81/0.18 in Caucasians.[23] It is well established that this dimorphism is not a risk factor for venous thrombosis. There is one claim that the A allele is more frequent in survivors of myocardial infarction (MI), but this was not confirmed in a second study.[24,25] The latter study also reported on the prevalence of three novel promoter variations in MI survivors. Although the results are not robust, there was an indication that the promoter variations were more common in the patients. The potential role of thrombomodulin mutations in MI recently received further support from the documentation of a frameshift mutation in a family with arterial disease.[26] Again, the data are not final, but add further to the notion that thrombomodulin mutations may be more important in arterial thrombotic disease than in venous disease.

2.6. Endothelial-cell protein C receptor (EPCR)

The recently discovered EPCR on endothelial cells is an important regulator of the protein C anticoagulant pathway.[27] Recently, the EPCR gene has been characterized, which opens up the possibility of searching for mutations in this gene.[28] Indeed, a 23-base-pair insertion in exon 3 has been identified which may predispose patients to venous thrombosis.[29] Further studies are clearly needed in order to evaluate the importance of these findings.

2.7. Tissue factor pathway inhibitor (TFPI)

TFPI is a so-called Kunitz-type inhibitor that plays a major role in the inhibition of the intrinsic coagulation pathway.[30] Inhibition takes place by the formation of a quaternary complex between tissue factor, factor VIIa, factor Xa and TFPI. Because of its inhibitory function, TFPI is a candidate risk factor for thrombotic disease; however, with plasmatic assays, no clear qualitative or quantitative deficiency states have been found.

Recently, systematic sequencing of the TFPI gene has yielded four different polymorphisms (Pro151Leu, Val264Met, T384C exon 4, and C-33T intron 7).[31,32] It is not known whether these sequence variations affect plasma levels of antigen and/or activity, with the possi-

ble exception of the Val264Met mutation, for which lower TFPI levels have been claimed.[32] It is also uncertain whether these polymorphisms influence thrombotic risk. There has been one claim that the Pro151Leu replacement is a risk factor for venous thrombosis, but this could not be confirmed in a second study.[33,34]

3. PROCOAGULANT PROTEINS

3.1. Factor V

In the coagulation cascade, factor V acts as cofactor for the serine protease factor Xa. Homozygosity for loss-of function mutations in the factor V gene leads to a bleeding disorder. The most common defect in the factor V gene, however, is a gain-of function mutation which is not associated with bleeding but with venous thrombosis. This mutation is often referred to as factor V-Leiden, and is responsible for a plasmatic abnormality called APC resistance.[35] The genetic basis of factor V-Leiden is mutation of the Arg506 inactivation site in factor Va.[36] As a result, factor Va Leiden is less easily inactivated by APC, whereby the procoagulant properties of factor V are enhanced. The factor V-Leiden abnormality has a prevalence of about 3% in Caucasians, and a great variety of studies have shown factor V-Leiden to be the most common risk factor in venous thrombosis in Caucasians.

Most individuals are heterozygous for factor V-Leiden, but homozygous individuals are also common. Homozygotes have a higher risk of thrombosis than heterozygotes.[37] Please note, however, that the symptoms in these homozygotes are much milder than in those with homozygous protein C or protein S deficiency. In this respect, there is a critical difference between mutations in procoagulant versus anticoagulant proteins.

At least in Caucasian populations, the prevalence of factor V-Leiden is much higher (range 1–10%) than the prevalence of protein C, protein S or antithrombin deficiency. In part, this high prevalence can be understood from the fact that factor V-Leiden homozygotes are mildly affected, so that there is less selective pressure on this abnormality than on mutations responsible for anticoagulant abnormalities. On the other hand, there is evidence that factor V-Leiden carriership has a negative effect on pregnancy outcome, and therefore it is reasonable to assume that there must also be positive selective pressures acting on the prevalence of factor V-Leiden. One possibility is that factor V-Leiden protects against intrapartum bleeding.[38] Although hard evidence is still lacking, another possibility is that enhanced coagulation may improve the innate host response against infection. Improved host defense might contribute to the prevalence of factor V-Leiden by improving survival from severe infection.

The high prevalence of factor V-Leiden did raise the question of whether the point mutation has a single origin (founder) or whether it is a frequent, recurring mutation in the factor V gene. An extensive haplotype analysis using six polymorphic sites in the factor V gene supported a single origin for factor V-Leiden and it was estimated that it occurred around 21,000 years ago.[39]

Two other major cleavage sites for APC exist in factor Va, at Arg306 and Arg679. It is logical to assume, therefore, that mutations at these sites also have occurred and that these too play a key role as risk factors for venous thrombosis. For unknown reasons, this does not seem to be the case. Although two mutations have been found at the 306 position (Arg306Thr and Arg306Gly), only replacement by Thr (factor V-Cambridge) appears to lead to APC resistance in a plasmatic assay.[40,41] However, this mutation appears to be rare in all populations tested, and its significance as a thrombotic risk factor remains disputable at best. The Arg306Gly mutation (factor V-Hong Kong) is prevalent among Hong Kong Chinese, is not associated with plasmatic APC resistance, and, based on currently available data, does not predispose to thrombosis.[41,42]

Another question that is unresolved is why only one mutation at Arg506 has been documented. There are multiple other possibilities whereby single-point mutations could lead to an amino acid replacement and loss of the cleavage site. Part of the explanation may be that the factor V-Leiden mutation obeys the so-called G-to-A rule for mutations at CpG dinucleotides. These CpG dinucleotides are well-known hotspots for mutation, and therefore the factor V-Leiden abnormality is predicted to occur preferably. A second point to consider is that it cannot be excluded that the other possible mutations at the 506 cleavage site also interfere with the procoagulant properties of factor V. If this negative effect on procoagulant properties is not matched or superseded by the effect of loss of APC cleavage, a bleeding tendency, rather than a thrombotic tendency, results.

A number of gene polymorphisms are known for the factor V gene. A particular haplotype (HR2) seems to be associated with partial APC resistance.[43] Several studies have claimed that this haplotype predisposes to venous thrombosis.[44,45] A recent study, however, could

not confirm this relationship.[46] Consequently, the importance of this haplotype remains controversial.

3.2. Prothrombin

Systematic sequencing of the prothrombin gene in a cohort of probands from thrombophilic families revealed a novel sequence variant.[47] This variant is a G20210A substitution involving the last nucleotide of the transcribed prothrombin RNA. The increased risk conferred by this dimorphism was estimated in the Leiden thrombophilia study to be 2.8. Several other studies have confirmed these findings.[48,49]

The prothrombin 2021 OA allele is associated with elevated prothrombin levels.[47] The mechanism by which prothrombin levels are altered is unknown. May be increased adenylation and improved mRNA stability are involved. The mutation results in loss of a CpG dinucleotide. The cytosine residues in CpG dinucleotides are often methylated in the human genome, and perhaps loss of methylation improves the rate of transcription from the prothrombin gene.

The prothrombin mutation is prevalent only in the Caucasian population (range 1–8%).[50] Haplotype analysis has shown that, like factor V-Leiden, this mutation arose in a founder about 20,000–30,000 years ago.[51] Also, in accordance to the findings with factor V-Leiden, the relatively high prevalence in the population goes with mild symptoms in homozygous subjects.[52–54]

3.3. Factor VII and fibrinogen

Perhaps the first two coagulation genes for which relationships between common DNA polymorphisms and plasma levels have been reported are those encoding factor VII and fibrinogen. The interest in these coagulation proteins has aroused by findings in the Northwick Park Heart Study that elevated levels were prospectively associated with arterial occlusive disease.[55]

The three genes that encode the α-, β-, and γ-chains are closely clustered on chromosome 4q. Multiple variations are known in these genes, but most attention has been focused on two dimorphisms in the β-chain gene: a Bcl I polymorphism in the 3′-region, and a Hae III (G-455A) polymorphism in the 5′-promoter region. The latter polymorphism was particularly attractive in view of nearby interleukin-6 and hepatocyte nuclear factor I responsive promoter elements.

There is general consensus in the literature that these two genetic variations are associated with the plasma level of fibrinogen.[56] From this relationship one can calculate the association between genotype and disease susceptibility. The results from studies that address this issue have been inconsistent at best, and the bottom-line is that there is little evidence that the calculated genotype-disease relationship indeed exists for arterial disease.[57]

With respect to venous thrombosis, there is only one study that investigated plasma level–genotype–disease relationships for fibrinogen.[58] A positive level-related association was indeed found. Subjects with levels above 5g/L had an approximately 4-fold increased risk. The results with respect to genotype, however, were very puzzling: H1/H2 heterozygotes displayed a 40% reduction rather than an increase in risk (the H2 allele contributes to high levels). These findings cannot be easily reconciled with an important role of level determining polymorphisms in the fibrinogen gene. One could even argue that fibrinogen levels are not causally related to thrombotic disease, but secondary to the occurrence of the disease.

The same study also investigated the relationship between coagulation factor VII levels, a Msp I polymorphism in the factor VII gene, and venous thrombosis.[58] The study showed that the plasma factor VII level is unrelated to venous thrombosis. In keeping with this, the allelic distribution of the Msp I polymorphism was the same in patients and controls. This polymorphism shows a strong relationship with factor VII levels.[59]

Consequently, fibrinogen level but not factor VII level is a risk factor for venous thrombosis. The genetic studies however question the cause and effect nature of this risk relationship.

3.4. Factor XIII

Factor XIII has an important role in stabilizing fibrin clots. This is accomplished by a transglutaminase reaction that forms covalent bonds between Lys and Gln side-chain residues near the carboxytermini of γγ chains (within strand bonds) and between γα chains (interstrand bonds) of fibrin.

Recently, a common mutation in the factor XIII gene, Val34Leu, has been implicated as a risk factor for vascular disease. It seems protective against MI, and predisposes to intracerebral hemorrhage.[60,61] Two studies suggest that the Leu allele may also predispose to venous thrombotic disease.[62,63] Again, further studies are needed before a final verdict can be given.

3.5. Tissue factor

The major initiator of the blood coagulation cascade is tissue factor (TF). A recent study asked the question

whether individual differences in TF gene expression would predispose to thrombosis.[64] Sequencing of the promoter region of the TF gene yielded 6 novel polymorphisms that were distributed over two haplotypes with equal frequencies (designated I208 D and 1208 I). In a case-control study of venous thromboembolism, the odds ratio associated with the presence of at least one D allele was 0.72. Interestingly, the protective D allele was associated with lower levels of circulating tissue factor than the I allele. Therefore polymorphisms in the TF gene might well be weak but significant genetic risk factors for venous thrombosis.

3.6. Emerging risk factors

The LETS (Leiden Thrombophilia Study) recently has yielded potentially relevant novel associations between the levels of coagulation factors and thrombotic risk. In particular, levels of factor VIII, factor XI, and TAFI (thrombin activatable fibrinolysis inhibitor) were positively associated with risk.[65–67] Despite searches, however, genetic markers in these genes, that rival the importance of the factor V and prothrombin markers, have not been found. Maybe in the near future such genetic markers will be identified that further extend the collection of useful predictors of thromboembolic disease.

4. CONCLUSIONS

Mutations in both prothrombotic and antithrombotic genes predispose to thrombotic disease. The evidence is extensive and convincing for mutations in the protein C, protein S, antithrombin, factor V and prothrombin genes. In particular, the mutations in the latter two genes have become very popular targets for testing in individuals with venous thrombosis. This is not surprising in view of the ease with which genetic testing can be performed for factor V-Leiden or prothrombin 2021 OA. Genetic testing for the whole spectrum of mutations underlying anticoagulant deficiency states remains expensive and time consuming, and in these diseases the laboratory analysis will rely on plasmatic assays for some time to come.

The jury is still out for the other anticoagulant and procoagulant candidate genes reviewed here. Also, there are several additional procoagulant genes, such as factor VIII and von Willebrand factor, that may become important risk factors once the genetic markers that determine plasma levels become known.

ΠΕΡΙΛΗΨΗ

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Η φλεβική θρομβοεμβολή είναι συχνή πολυπαραγοντική νόσος. Ποικίλοι συνδυασμοί παραγόντων κινδύνου διαδραματίζουν ρόλο στην έναρξη της νόσου. Οι παράγοντες κινδύνου αυτοί συχνά είναι περιβαλλοντικοί, αλλά βασικής σημασίας είναι και γενετικοί παράγοντες. Στους γενετικούς αυτούς παράγοντες περιλαμβάνονται κοινές μεταλλάξεις αύξησης της λειτουργίας (gain-of function) των προπηκτικών πρωτεϊνών παράγοντα V και προθρομβίνης, οι οποίες συμβαίνουν με σχετικά μεγάλη συχνότητα σε πληθυσμούς της Καυκάσιας φυλής. Σημαντικοί είναι επίσης διάφοροι αντιπηκτικοί παράγοντες. Οι μεταλλάξεις απώλειας λειτουργίας (loss-of function) χαρακτηρίζονται από τεράστια ποικιλότητα, αλλά χαμηλή συχνότητα στον πληθυσμό. Ο μικρός αυτός επιπολασμός στον πληθυσμό πιθανώς οφείλεται στην απώλεια μεταλλαγμένων αλληλίων από τη δεξαμενή των γονιδίων μέσω των βαριά πασχόντων ομοζυγωτών. Οι μεταλλάξεις αύξησης της λειτουργίας των προπηκτικών πρωτεϊνών διαφέρουν από τις μεταλλάξεις απώλειας λειτουργίας, καθώς τα ομοζυγωτικά άτομα πάσχουν σχετικώς ελαφρά.

Λέξεις ευρετηρίου: Ανεπάρκεια αντιθρομβίνης, Ανεπάρκεια πρωτεΐνης C, Ανεπάρκεια πρωτεΐνης S, Factor V-Leiden, Προθρομβίνη 2021 OA

References

1. CHOWDBURY V, LANE DA, MILLE B, AUBERGER K, GANDENBERGER-BACHEM S, ET AL. Homozygous antithrombin deficiency: Report of two new cases (99 Leu to Phe) associated with arterial and venous thrombosis. *Thromb Haemost* 1994, 72:198–202
2. LANE DA, BAYSTON T, OLDS RJ, FITCHES AC, COOPER DN, MILLAR DS ET AL. Antithrombin mutation database: 2nd (1997) update. For the Plasma Coagulation Inhibitors Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost* 1997, 77:197–211
3. PROCHOWNIK EV, ANTONARAKIS S, BAUER KA, ROSENBERG RD, FEARON ER, ORKIN SH. Molecular beterogeneity of inherited antithrombin III deficiency. *N Engl J Med* 1983, 308:1549–1552
4. KONDO S, TOKUNAGA F, KARIO K, MATSUO T, KOIDE T. Molecular and cellular basis for type I heparin cofactor II deficiency (heparin cofactor II Awaji). *Blood* 1996, 87:1006–1012
5. BERNARDI F, LEGNANI C, MICBELETTI F, LUNGBI B, FERRARESI P, PALARETI G, BIAGI R, MARCHETTI G. A heparin cofactor II mutation (HCII Rimini) combined with factor V Leiden or type I protein C deficiency in two unrelated thrombophilic subjects. *Thromb Haemost* 1996, 76:505–509
6. ESMON CT: Molecular events that control the protein C anticoagulant pathway. *Thromb Haemost* 1994, 70:29–35
7. GRIFFN JH, EVATT B, ZIMMERMAN TS, KLEISS AJ, WIDEMAN C. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest* 1981, 68:1370–1373
8. BRANSON HE, KATZ J, MARBLE R, GRIFFIN JH. Inherited protein C deficiency and coumarin-responsive chronic relapsing purpura fulminans in a newborn infant. *Lancet* 1983, 19:1165–1168
9. GRUNDY CB, MELISSARI E, LINDO V, SCULLY MF, KAKKAR VV, COOPER DN. Late-onset homozygous protein C deficiency. *Lancet* 1991, 338:575–576
10. REITSMA PH, BERNARDI F, DOIG RG, GANDRILLE S, GREENGARD JS, IRELAND H ET AL. Protein C deficiency: a database of mutations,1995 update. On behalf of the Subcommittee on Plasma Coagulation Inhibitors of the Scientific and Standardization Committee of the ISTH. *Thromb Haemost* 1995, 73:876–889
11. KRAWCZAK M, REITSMA PH, COOPER DN. the mutational demography of protein C deficiency. *Hum Genet* 1995, 96:142–146
12. REITSMA PH, POORT SR, ALLAART CF, BRIET E, BERTINA RM. The spectrum of genetic defects in a panel of 40 Dutc families with symptomatic protein C deficiency type I: heterogeneity and founder effects. *Blood* 1991, 78:890–894
13. PLOOS VAN AMSTEL JK, VAN DER ZANDEN AL, BAKKER E, REITSMA PH, BERTINA RM. Two genes homologous with human protein S cDNA are located on chromosome 3. *Thromb Haemost* 1987, 58:982–987
14. COMP PC, NIGON RR, COOPER MR, ESMON CT. Familial protein S deficiency is associated with recurrent thrombosis. *J Clin Invest* 1984, 74:2082–2088
15. MAHASANDANA C, SUVATTE V, MARLAR RA, MANCO JOBNSON MJ, JACOBSON LJ, HATHAWAY WE. Neonatal purpura fulminans associated with homozygous protein S deficiency. *Lancet* 1990, 335: 61–62
16. GANDRILLE S, BORGEL D, IRELAND H, LANE DA, SIMMONDS R, REITSMA PH ET AL. Protein S deficiency: a database of mutations. For the Plasma Coagulation Inhibitors Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost* 1997, 77:1201–1214
17. BERTINA RM, PLOOS VAN AMSTEL HK, VAN WIJNGAARDEN A, COENEN J, LEEMHUIS MP, DEUTZ TERLOUW PP ET AL. Heerlen polymorphism of protein S, an immunologic polymorphism due to dimorphism of residue 460. *Blood* 1990, 76:538–548
18. ESPINOSA-PARRILLA Y, MORELL M, SOUTO JC, BORRELL M, HEINE-SUNER D, TIRADO I ET AL. Absence of linkage between type III protein S deficiency and the PROSl and C4BP genes in families carrying the protein S Heerlen allele. *Blood* 1997, 89:2799–2806
19. DUCHEMIN J, GANDRILLE S, BORGEL D, FEURGARD P, ALHENC-GELAS M, MATHERON C ET AL. The Ser 460 to Pro substitution of the protein S alpha (PROS1) gene is a frequent mutation associated with free protein S (type IIa) deficiency. *Blood* 1995, 86:3436–3443
20. NORLUND L, ZOLLER B, OHLIN AK. A novel thrombomodulin gene mutation in a patient suafering from sagittal sinus thrombosis. *Thromb Haemost* 1997, 78:1164–1166
21. OHLIN AK, NORLUND L, MARLAR RA. Thrombomodulin gene variations and thromboembolic disease. *Thromb Haemost* 1997, 78: 396–400
22. NAKAZAWA F, KOYAMA T, SAITO T, SHIBAKURA M, YOSHINAGA H, CHUNG DH ET AL. Thrombomodulin with the Asp468Tyr mutation is expressed on the cell surface with normal cofactor activity for protein C activation. *Br J Haematol* 1999, 106:416–420
23. VAN DER VELDEN PA, KROMMENHOEK-VAN ES T, ALLAART CF, BERTINA RM, REITSMA PH. A frequent thrombomodulin amino acid dimorphism is not associated with thrombophilia. *Thromb Haemost* 1991, 65:511–513
24. NORLUND L, HOLM J, ZOLLER B, OHLIN AK. A common thrombomodulin amino acid dimorphism is associated with myocardial infarction. *Thromb Haemost* 1997, 77:248–251
25. IRELAND H, KUNZ G, KYRIAKOULIS K, STUBBS PJ, LANE DA. Thrombomodulin gene mutations associated with myocardial infarction. *Circulation* 1997, 96:15–18
26. KUNZ G, IRELAND HA, STUBBS PJ, KAHAN M, COULTON GC, LANE DA. Identification and characterization of a thrombomodulin gene mutation coding for an elongated protein with reduced expression in a kindred with myocardial infarction. *Blood* 2000, 95:569–576
27. STEARNS-KUROSAWA DJ, KUROSAWA S, MOLLICA JS, FERRELL GL, ESMON CT. The endothelial cell protein C receptor augments protein C activation by the thrombin-thrombomodulin compleg. *Proc Natl Acad Sci USA* 1996, 93:10212–10216
28. SIMMONDS RE, LANE DA. Structural and functional implications of the intron/egon organization of the buman endothelial cell protein C/activated protein C receptor (EPCR) gene. comparison

with the structure of CD1/major histocompatibility compleg alpha1 and alpha2 domains. *Blood* 1999, 94:632–641

29. MERATI GB, BIGUZZI F, OGANESYAN N, FETIVEAU D, QU P, BUCCIARELLI DJ ET AL. A 23 bp insertion in the endothelial protein C receptor (EPCR) gene in patients with myocardial infarction and deep vein thrombosis. *Thromb Haemost* 1999, 82:507
30. BROZE GJ Jr, GIRARD TJ, NOVOTNY WF. Lipoprotein-associated coagulation inhibitor. *Current Studies in Hematology & Blood Transfusion* 1991:22–25
31. KLEESIEK K, SCHMIDT M, GOTTING C, BRINKMANN T, PROHASKA W. A first mutation in the human tissue factor pathway inhibitor gene encoding (P151L) TFPI (Letter). *Blood* 1998, 92:3976–3977
32. MOATTI D, SEKNADJI P, GALAND C, POIRIER O, FUMERON F, DESPREZ S, GARBARZ M ET AL. Polymorphisms of the tissue factor pathway inhibitor (TFPI) gene in patients with acute coronary syndromes and in healthy subjects: impact of the V264M substitution on plasma levels of TFPI. *Arterioscler Thromb Vasc Biol* 1999, 19: 862–869
33. KLEESIEK K, SCHMIDT M, GOTTING C, SCHWENZ B, LANGE S, MULLER-BERGHAUS G ET AL. The 536C→T transition in the human tissue factor pathway inhibitor (TFPI) gene is statistically associated with a higher risk for venous thrombosis. *Thromb Haemost* 1999, 82: 1–5
34. GONZALEZ-CONEJERO R, LOZANO ML, CORRAL J, MARTINEZ C, VICENTE V. The TFPI 536C→T mutation is not associated with increased risk for venous or arterial thrombosis [(Letter) In Process Citation] *Thromb Haemost* 2000, 83:787–788
35. DAHLBACK B, CARLSSON M, SVENSSON PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci USA* 1993, 90: 1004–1008
36. BERTINA RM, KOELEMAN BPC, KOSTER T, ROSENDAAL FR, DIRVEN RJ, DE RONDE H ET AL. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994, 369:64–67
37. ROSENDAAL FR, KOSTER T, VANDENBROUCKE JP, REITSMA PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood* 1995, 85:1504–1508
38. LINDQVIST PG, SVENSSON PJ, DAHLBACK B, MARSAL K. Factor V Q506 mutation (activated protein C resistance) associated with reduced intrapartum blood loss-a possible evolutionary selection mechanism. *Thromb Haemost* 1998, 79:69–73
39. ZIVELIN A, GRIFFN JH, XU X, PABINGER I, SAMAMA M, CONARD J ET AL. A single genetic origin for a common Caucasian risk factor for venous thrombosis. *Blood* 1997, 89:397–402
40. WILLIAMSON D, BROWN K, LUDDINGTON R, BAGLIN C, BAGLIN T. Factor V Cambridge–a New Mutation (Arg306Thr) Associated with Resistance to Activated Protein C. *Blood* 1998, 91:1140–1144
41. CHAN WP, LEE CK, KWONG YL, LAM CK, LIANG R. A Novel Mutation of Arg306 of Factor V Gene in Hong Kong Chinese. *Blood* 1998, 91:1135–1139
42. LIANG R, LEE CK, WAT MS, KWONG YL, LAM CK, LIU HW. Clinical significance of Arg306 mutations of factor V gene (Letter). *Blood* 1998, 92:2599–2600
43. BERNARDI F, FAIONI EM, CASTOLDI E, LUNGHI B, CASTAMAN G, SACCHI E, MANNUCCI PM. A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. *Blood* 1997, 90:1552–1557
44. FAIONI EM, FRANCHI F, BUCCIARELLI P, MARGAGLIONE M, DE STEFANO V, CASTAMAN G ET AL. Coinheritance of the HR_2 haplotype in the factor V gene confers an increased risk of venous thromboembolism to carriers of factor V R506Q (factor V Leiden). *Blood* 1999, 94:3062–3066
45. ALHENC-GELAS M, NICAUD V, GANDRILLE S, VAN DREDEN P, AMIRAL J, AUBRY ML ET AL. The factor V gene A4070G mutation and the risk of venous thrombosis. *Thromb Haemost* 1999, 81:193–197
46. LUDDINGTON R, JACKSON A, PANNERSELVAM S, BROWN K, BAGLIN T. The factor V R2 allele: risk of venous thromboembolism, factor V levels and resistance to activated protein C. *Thromb Haemost* 2000, 83:204–208
47. POORT SR, ROSENDAAL FR, REITSMA PH, BERTINA RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996, 88:3698–3703
48. CUMMING AM, KEENEY S, SALDEN A, BHAVNANI M, SBWE KH, HAY CR. The prothrombin gene G20210A variant: prevalence in a U.K. anticoagulant clinic population. *Br J Haematol* 1997, 98:353–355
49. SOUTO JC, COLL I, LLOBET D, DEL RIO E, OLIVER A, MATEO J, BORRELL M, FONTCUBERTA J. The prothrombin 2021 0A allele is the most prevalent genetic risk factor for venous thromboembolism in the Spanish population. *Thromb Haemost* 1998, 80:366–369
50. ROSENDAAL FR, DOGGEN CJ, ZIVELIN A, ARRUDA VR, AIACB M, SISCOVICK DS ET AL. Geographic distribution of the 20210 G to A prothrombin variant. *Thromb Haemost* 1998, 79:706–708
51. ZIVELIN A, ROSENBERG N, FAIER S, KORNBROT N, PERETZ H, MANNHALTER C, HORELLOU MH, SELIGSOHN U. A single genetic origin for the common prothrombotic G20210A polymorphism in the prothrombin gene. *Blood* 1998, 92:1119–1124
52. HOWARD TE, MARUSA M, CBANNELL C, DUNCAN A. A patient homozygous for a mutation in the prothrombin gene 3′-untranslated region associated with massive thrombosis [see comments). *Blood Coagul Fibrinolysis* 1997, 8:316–319
53. GIORDANO P, DE LUCIA D, COPPOLA B, IOLASCON A. Homozygous prothrombin gene mutation and ischemic cerebrovascular disease: a case report. *Acta Haematol* 1999, 102:101–103
54. SOUTO JC, MATEO J, SORIA JM, LLOBET D, COLL I, BORRELL M ET AL. Homozygotes for prothrombin gene 2021 0A allele in a thrombophilic family without clinical manifestations of venous thromboembolism. *Haematologica* 1999, 84:627–632
55. MEADE TW, MELLOWS S, BROZOVIC M, MILLER GJ, CHAKRABARTI RR, NORTH WR, HAINES AP ET AL. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986, 2:533–537
56. HUMPHRIES SE, COOK M, DUBOWITZ M, STIRLING Y, MEADE TW. Role of genetic variation at the fibrinogen tocus in determination of plasma fibrinogen concentrations. *Lancet* 1987, 1:1452–1454

57. LANE DA, GRANT PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* 2000, 95:1517–1532

58. KOSTER T, ROSENDAAL FR, REITSMA PH, VAN DER VELDEN PA, BRIET E, VANDENBROUCKE JP. Factor VII and fbrinogen levels as risk factors for venous thrombosis. A case-control study of plasma levels and DNA polymorphisms-the Leiden Thrombophilia Study (LETS). *Thromb Haemost* 1994, 71:719–722

59. GREEN F, KELLEHER C, WILKES H, TEMPLE A, MEADE T, HUMPHRIES S. A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. *Arteriosclerosis* 1991, 11:540–546

60. KOHLER HP, STICKLAND MH, OSSEI-GERNING N, CARTER A, MIKKOLA H, GRANT PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thromb Haemost* 1998, 79:8–13

61. CATTO AJ, KOHLER HP, BANNAN S, STICKLAND M, CARTER A, GRANT PJ. Factor XIII Val 34 Leu: a novel association with primary intracerebral hemorrhage. *Stroke* 1998, 29:813–816

62. CATTO AJ, KOHLER HP, COORE J, MANSFIELD MW, STICKLAND MH, GRANT PJ. Association of a common polymorphism in the factor XIII gene with venous thrombosis. *Blood* 1999, 93:906–913

63. FRANCO RF, REITSMA PH, LOURENCO D, MAFFEI FH, MORELLI V, TAVELLA MC ET AL. Factor XIII Val34Leu is a genetic factor involved in the etiology of venous thrombosis. *Thromb Haemost* 1999, 81: 676–679

64. ARNAUD E, BARBALAT V, NICAUD V, CAMBIEN F, EVANS A, MORRISON C, ET AL. Polymorphisms in the 5′ regulatory region of the tissue factor gene and the risk of myocardial infarction and venous thromboembolism: the ECTIM and PATHROS studies. Etude Cas-Temoins de l'Infarctus du Myocarde. Paris Thrombosis case-control Study. *Arterioscler Thromb Vasc Biol* 2000, 20:892–898

65. KOSTER T, BLANN AD, BRIET E, VANDENBROUCKE JP, ROSENDAAL FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *Lancet* 1995, 345:152–155

66. MEIJERS JC, TEKELENBURG WL, BOUMA BN, BERTINA RM, ROSENDAAL FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. *N Engl J Med* 2000, 342:696–701

67. VAN TILBURG NH, ROSENDAAL FR, BERTINA RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. *Blood* 2000, 95:2855–2859

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