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Thrombosis

Venous thrombosis: a multicausal disease

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The risk factors for venous thrombosis differ from those for arterial vascular disease. During the past 5 years, knowledge about the aetiology of venous thrombosis has advanced with the discovery of several factors that contribute to the incidence of thrombosis, particularly the role of coagulation abnormalities. These abnormalities are common in the general population and therefore will be present simultaneously in some individuals. The resultant gene-gene and gene-environment interactions between risk factors are the key to the understanding of why a certain person develops thrombosis at a specific point in time.

Each year venous thrombosis occurs in about one in 1000 people in developed countries [1-3] This disorder commonly manifests as deep-vein thrombosis of the leg, or, if embolisation occurs, as pulmonary embolism Thrombosis may rarely occur in other veins (cerebral sinus, and veins in the arms, retina, and mesentery) [4,5] Major complications of venous thrombosis are a disabling post-thrombotic syndrome and acute death from a pulmonary embolism that occur in 20%[6] and 1–2%[2] of patients, respectively The incidence of thrombosis increases sharply with age, from 1 per 100 000 people per year in childhood to nearly 1% per year in old age [3]

Risk factors

The risk factors for venous thrombosis differ from those for arterial disease—myocardial infarction, stroke, and atherogenic factors such as smoking, hypertension, or hyperlipidaemia do not increase the risk of venous thrombosis Virchow[7] famously postulated three main causes of thrombosis stasis of the blood, changes in the vessel wall, and changes in the composition of the blood The known risk factors for venous thrombosis fall in the first group (stasis) and third group (composition changes), but nowadays a different classification is made into genetic and acquired risk factors

Acquired risk factors for thrombosis include immobilisation (including immobilisation in plaster casts), surgery, trauma, pregnancy, puerperium, lupus anticoagulant, malignant disease, and female hormones The first report of a family with an identified hereditary tendency to thrombosis (a deficiency of antithrombin, previously known as antithrombin III) was made by Egeberg in 1965 [8] In the 1980s, protein C deficiency and protein S deficiency were described in familial thrombophilia[9,10]—thrombophilia being a tendency to venous thrombosis Over the past 5 years, several abnormalities in the clotting system that predispose to venous thrombosis have been discovered Resistance to activated protein C was first described in 1993[11] and subsequently shown to be caused by a mutation in clotting factor V, factor V Leiden [12] Since protein C, protein S, and antithrombin are the main natural inhibitors of the procoagulant system, a heterozygous deficiency of these proteins leads to excessive thrombin formation [13]

When factor V has a mutation at one of the cleavage sites for activated protein C (factor V_{R506Q} or factor V Leiden), it is less sensitive to the natural anticoagulant protein C-protein S system—ie, there is resistance to activated protein C This mutation leads to gain of function rather than loss of function Other abnormalities have been described that lead to gain in function and excesses in the procoagulant system A mutation in the 3'-untranslated region of the prothrombin (factor II) gene (G to A at position 20210, PT20210A) is associated with increased plasma concentrations of prothrombin, and with an increased risk of thrombosis [14] Similarly, high concentrations of clotting factor VIII are related to increased risk of thrombosis [15] Concentrations of factor VIII are determined mostly by blood group, which accounts for the old observation of a relation between non-O blood groups and risk of thrombosis [15,16]

High concentrations of clotting factors are not caused by a mutation that has disrupted the normal sequence of a gene, as is the case with the deficiencies, but are the result of more subtle changes in the regulation of gene activity Several genetic loci influence the concentrations of clotting factors For example, for factor VIII there are at least three sets of genes involved The first are the genes that code for ABO blood group, since people with blood group O have lower concentrations of factor VIII (and von Willebrand factor) than those with non-O blood groups The second are genes for von Willebrand factor, the carrier protein for factor VIII And finally, there is an unknown set of genes because even when blood group and von Willebrand factor are taken into account, there is still a familial tendency to aggregation of factor VIII concentrations,[17] for which no cause has yet been found within the factor VIII gene [18]

Apart from a genetic make-up, variation may also be explained by acquired factors Obviously, anything that affects the organs where the clotting factors are produced may also affect concentrations of these factors (eg, liver disease, endothelial dysfunction), as may dietary intake of substrates and vitamins (vitamin K deficiency) There are also many other disorder that affect concentrations of clotting factors in more subtle ways,[19,20] and it is plausible that high concentrations of prothrombin and factor VIII reflect a mixture of genetic make-up and acquired factors Acquired factors may contribute to variations within and between individuals, which may underlie differences in

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Risk factor	% general population	% patients with thrombosis
Protein C deficiency	0 2 0 4	3
Protein S deficiency	Not known	1–2
Antithrombin deficiency	0 02	1
Factor V Leiden	5	20
Prothrombin 20210A	2	6
High concentration of factor VIII (>1500 IU/L)	11	25
Hyperhomocysteinaemia (>18 5 μmol/L)	5	10

Table 1 **Prevalence of risk factors for thrombosis**

risk between individuals or situations Even when a mutation is the cause of loss of function of the encoded protein, such as protein C, protein S, and antithrombin, plasma concentrations of these proteins (in heterozygous deficiency) can vary This variation may be due to polymorphisms in the functional allele or to acquired factors such as age and hormones [21 22]

Finally, hyperhomocysteinaemia is an abnormality that has been associated with venous thrombosis in several studies[23 26] and in a meta-analysis [27] This abnormality is found in arterial and venous disease,[28] but its cause is disputed in the absence of biological models Hyperhomocysteinaemia is a good example of abnormal plasma concentrations that result from genetic and acquired factors Mutations of cystathionine β-synthase or methylene tetrahydrofolate reductase (MTHFR) lead to increased concentrations of homocysteine Most individuals with hyperhomocysteinaemia, however, do not carry either genetic variant, but have impaired methionine metabolism, so the hyperhomocysteinaemia[15] is caused by insufficient dietary intake of folic acid and vitamins B6 or B12 [29]

Prevalence and risk estimates

The impact of a risk factor is a function of its prevalence and relative risk Table 1 shows the prevalence of various risk factors among white people in the general population of developed countries and among patients with venous thrombosis [14 30 35] Deficiencies of protein C, protein S, and antithrombin are rare, even among patients with thrombosis Since these deficiencies are rare, the risk is not easy to assess and the risk estimates vary A fair estimate seems to be that all the deficiencies increase the risk of deep-vein thrombosis by about ten-fold [30 36] Four abnormalities that have been discovered or associated with venous thrombosis in the past 5 years are far more common in the general population activated protein C resistance, prothrombin 20210A, high concentrations of factor VIII, and hyperhomocysteinaemia Activated protein resistance, caused by factor V Leiden, occurs in 5% of the population [34 37] The prevalence estimates vary because of regional differences and are high (up to 15%) in southern Sweden and the middle-east Factor V Leiden is restricted to white people [37] Among patients with venous thrombosis, factor V Leiden occurs in 20%[34 38] and seems to be a risk factor of much the same strength as the deficiencies of coagulation inhibitors, increasing the risk by about eight-fold among heterozygous carriers [34]

Prothrombin 20210A is found in 2% of the population worldwide, again with regional differences [39] Among patients with thrombosis, prothrombin 20210A has been found in 6%, and it seems to be a mild risk factor, increasing the risk by two-fold to three-fold [14] Until now, the mutation has been reported mainly in white people [39]

The prevalence of high concentrations of factor VIII and hyperhomocysteinaemia depend on the cut-off values that are applied Factor VIII concentrations that exceed 1500 IU/L (150% of normal) have been found in 11% of the general population, and 25% of patients with thrombosis [15] Such high concentrations compared with those below 1000 IU/L were associated with a six-fold increased risk of thrombosis [15] Because of the high prevalence and relative risk, high concentrations of factor VIII may be responsible for most thrombotic events of the abnormalities listed in table 1 Concentrations of homocysteine greater than 18 5 μmol/L were found in 5% of the general population in the Netherlands, and 10% of the Italian population [25 26] Compared with patients whose concentrations were below 18 5 μmol/L, people with concentrations above 18 5 μmol/L had a 2 5-fold increased risk of thrombosis, for concentrations above 20 μmol/L, the risk increased by three-fold to four-fold

Thrombosis as a multicausal disease

Thrombosis manifests itself as a multicausal disease most clearly in children In the rare event of thrombosis in children, several acquired and genetic risk factors are usually present simultaneously Not only is it rare to find children with thrombosis without any risk factor, but many have three or four risk factors In 25–30% of children with thrombosis, deficiencies of protein C, protein S, or antithrombin have been reported, but thrombosis did not develop until other risk factors were present, such as intravenous lines or major illness [40 41]

Thrombosis is also a multicausal disease in adults since many risk factors are common in the general population, such as factor V Leiden, prothrombin 20210A, high concentrations of factor VIII, and hyperhomocysteinaemia, which frequently occur together in one individual The acquired risk factors, such as pregnancy, puerperium, use of oral contraceptives, and immobilisation, also affect many people, so a combination of risk factors in one person is common Indeed, multiple risk factors are a prerequisite for thrombosis to develop

Selection and interaction

Among families with a tendency to thrombosis, the prevalence of thrombogenic abnormalities is much higher than among the unselected "average" patient with thrombosis who is described in table 1 In thrombophilic families, deficiencies of the main coagulation inhibitors occur in 15%, prothrombin 20210A in nearly 20%, and factor V Leiden in 40–60% The risk of thrombosis is also higher in members of these families than among other individuals with similar defects [12 33 42–47]

Comparison of patients with the same defect shows that the way in which they were identified seems to be the most important determinant of their individual thrombotic risk The mean age at first thrombosis for patients from thrombophilic families is much younger than for consecutive patients with thrombosis, irrespective of the underlying defect (table 2) [18] Remarkably, thrombosis even occurs at an early age in thrombophilic families with no identifiable defect [48]

Risk factor	Age (years) at first thrombosis	
	Patients from thrombophilic families (n=78)	Consecutive unselected patients (n=105)
Protein C deficiency	31	47
Factor V Leiden	29	43
No defect found	34	46

Data are from Lensen and colleagues [48]

Table 2 **Age at first thrombosis by origin of patient**

The most likely explanation for this observation is that, although thrombosis is multicausal, familial thrombophilia is multigenic—ie, in each of the families there are several genetic defects For example, with the protein C families registered at the Leiden clinic, a high risk of thrombosis was reported among individuals with protein C deficiency in these families, compared with relatives without the deficiency (figure 1) [16] For relatives with protein C deficiency, 50% had thrombosis at age 50 years, which suggested a very high risk of thrombosis associated with protein C deficiency Several years later, these families were investigated for factor V Leiden, which turned out to be common in the families The risk of thrombosis was much higher for those family members who carried both defects than for those who carried protein C deficiency or factor V Leiden (figure 1) [49] With this knowledge of the additional risk factor in these families, it became clear that the risk of thrombosis associated with protein C deficiency had initially been overestimated

Gene-gene interaction

The high risk of thrombosis associated with the combination of protein C deficiency and factor V Leiden is an example of gene-gene interaction Similar findings have been documented for families with protein S deficiency,[50] antithrombin deficiency,[2] and prothrombin 20210A,[5] factor V Leiden is common in these families, and those with a combined defect have a high risk of thrombosis These findings all suggest that the risk of abnormalities will be an overestimate if it is derived from family studies additional defects explain individual variation within and between families

A special type of gene-gene interaction is present in homozygous disease Homozygous protein C and protein S deficiency are rare but devastating disorders, with severe thrombosis (purpura fulminans) occurring shortly after birth [3,54] Homozygous antithrombin deficiency may not even be compatible with life [5] Because of the low prevalence of these deficiencies, homozygous patients are rare and are commonly the result of consanguinity Homozygous carriers of factor V Leiden are more common (1 per 5000 people) [31] The thrombotic risk for individuals homozygous for factor V Leiden is high (80-fold increased vs non carriers), but not as high as for people with homozygous deficiencies of the coagulation inhibitors most patients do not develop thrombosis until adulthood and may remain symptomfree until old age [3,4,5] This situation also seems to be the case for homozygous carriers of prothrombin 20210A,[57] the explanation for the absence of a greatly increased risk, as compared with that for homozygous deficiencies of the coagulation inhibitors, is that these are mutations that lead to gain rather than loss of function

Gene-environment interaction

Since some of the recently discovered genetic abnormalities are common, as are several acquired risk factors, the joint effects of such factors on risk of thrombosis warrants investigation Clear indications of synergistic effects come from studies in thrombophilic families, where high risks were found in pregnancy and the puerperium, and during use of oral contraceptives, for women with deficiencies of protein C, protein S, or antithrombin [56,58,6] In several series of unselected women

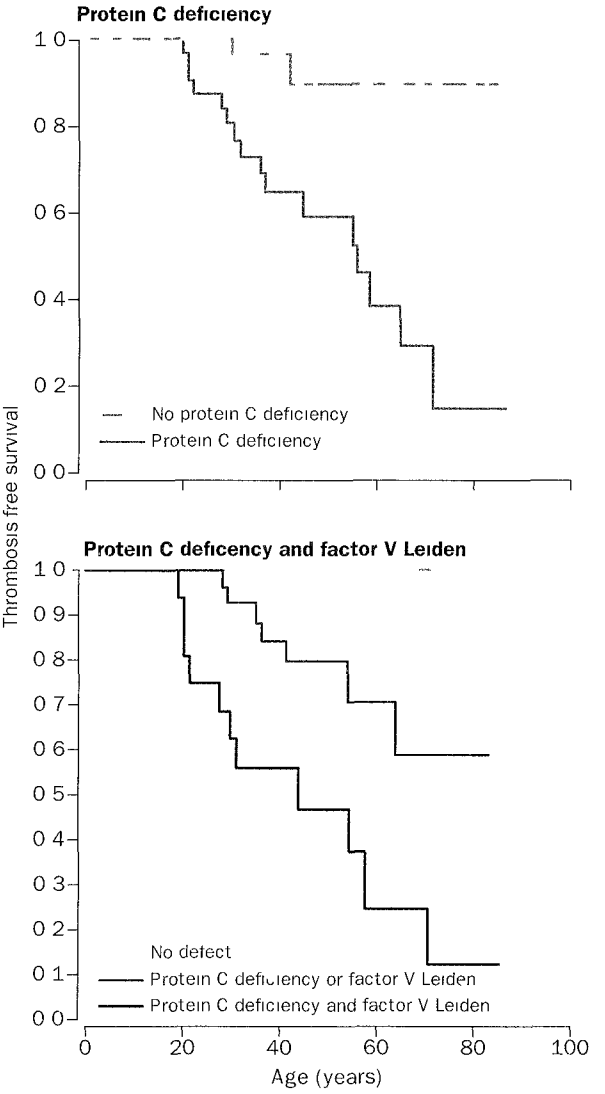

Figure 1 **Thrombosis-free survival in families with thrombophilia due to protein C deficiency (proband excluded)**

with thombosis during pregnancy, factor V Leiden was more common than in the general population [3] The frequency of factor V Leiden among these women varied widely between studies, from 8% in Scotland[4] to 50 60% in Sweden, [3,1] which partly reflects geographical differences in the population prevalence of factor V Leiden These data suggest that a substantial part of pregnancy-related thrombosis results from concomitant abnormalities in the haemostatic system

Among unselected patients, a synergistic effect has been shown for factor V Leiden and use of oral contraceptives the estimated baseline risk of thrombosis for non-carriers who do not use oral contraceptives was 0 8 per 10 000 people per year The annual risk for women with factor V Leiden who did not use oral contraceptives was 5 7 per 10 000 people (relative risk 6 9), that for women who used oral contraceptives but did not carry factor V Leiden was 3 0 per 10 000 women (relative risk 3 7), and that for women with factor V Leiden who used oral contraceptives was 28 5 per 10 000 people (relative risk 34 7) [7]

For cerebral sinus thrombosis, increased risk of thrombosis has been reported for thrombophilic defects The combination of protein C deficiency, factor V

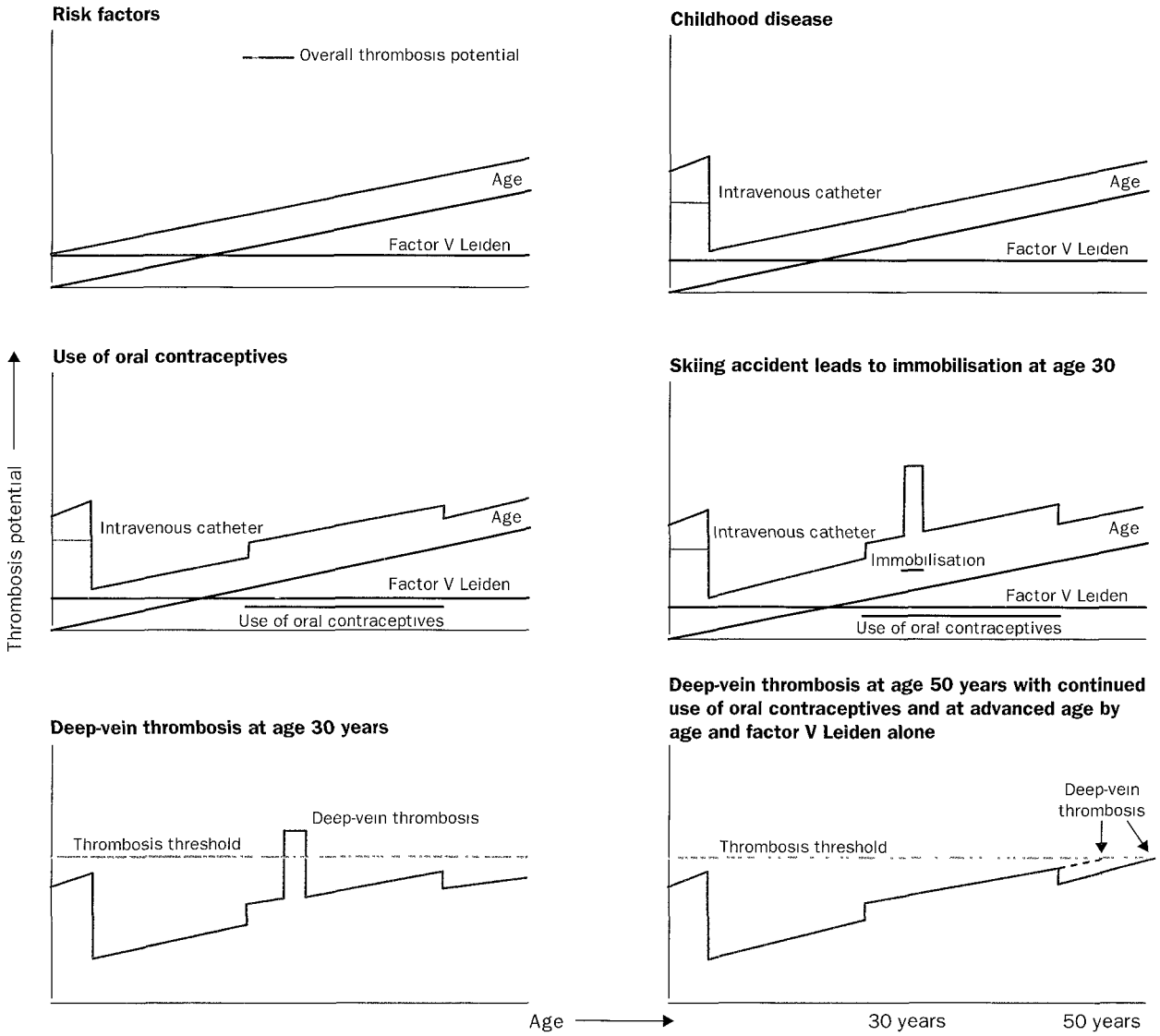

Figure 2: **Models of thrombosis risk**
In each panel, the figure shows the thrombosis (black) potential of each risk factor present during an individual's life and the resultant thrombosis potential (red).

Leiden, or prothrombin 20210A, and use of oral contraceptive led to a 30-fold to 150-fold increased risk, compared with that for women who did not use oral contraceptives and did not have such a defect.[68,69] These combined risks are much higher than the individual risk conferred by use of oral contraceptives or a thrombophilic defect.[68,69]

For many combinations of risk factors, there are no reliable estimates of risk and conclusions are made on the basis of only one or a few studies. For the more common clotting abnormalities and for combined acquired risk factors (environment-environment interaction), results are likely to be forthcoming.

Models of thrombosis risk

When the first coagulation defects that underlie thrombophilia were discovered, such as, deficiencies of antithrombin, protein C, and protein S, thrombosis was considered a single-gene defect.[70,71] Since the first families studied were those with the most pronounced thrombophilia, for reasons explained above, it is understandable that for some time one defect was thought sufficient for thrombosis. Of course, the risk in these families was so high because they harboured several defects. Since not every person with a deficiency developed thrombosis, this single-gene model was abandoned.[72,73] A subsequent model was that of familial thrombophilia as a multiple-gene disorder that is analogous to the multistage or multiple-hit theories for cancer.[74] Support for this view can be found in families with thrombophilia who frequently harbour several known defects, and possibly, and most likely, unidentified defects as well. However, although this model is an improvement on the single-gene concept, it is not all encompassing and makes an artificial difference between thrombophilia and thrombosis. Moreover, this model ignores acquired risk factors. The multiple-gene model seems appropriate only for homozygous deficiencies of protein C and protein S, in which thrombosis occurs immediately after birth. In all other instances, even when the risk of thrombosis is high, as in individuals with homozygous factor V Leiden or with both factor V

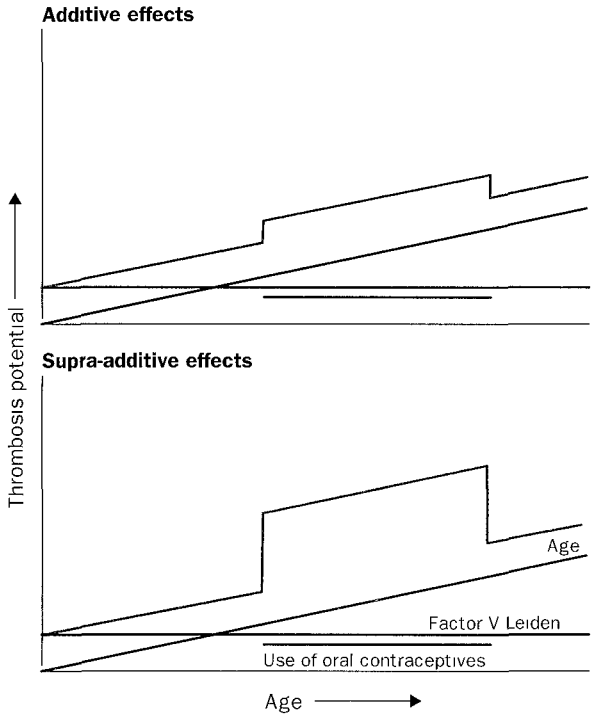

Figure 3 **Models of thrombosis risk with different interactions between factor V Leiden and use of oral contraceptive**
Top the thrombosis potentials add to form the resultant potential Bottom effect of the combination is supra additive

Leiden and protein C deficiency, the question arises of why one person develops thrombosis while another does not, and why an individual develops thrombosis at a certain age and not before

Even a multicausal model that incorporates genetic and acquired risk factors does not readily explain why the same number of risk factors causes thrombosis in one person and not in another, or even why the same set of risk factors does not cause thrombosis in children but causes this disorder in older people Thus, there is a need for a dynamic age-dependent model that allows for various forms of interaction of risk factors (eg, additive effects or synergism) Figure 2 shows such a model Here, the assumption is that each risk factor adds to the risk, which can be called an individual's thrombosis potential For each part of figure 2, the black lines show the thrombosis potential of each individual risk factor for a particular woman, and the red line her total thrombosis potential The horizontal axis shows time (the age of the individual) The model is dynamic, because we assume that the thrombosis potential is age-dependent, which is based on the observation that more risk factors are required in children than in adults and that the incidence of thrombosis increases with age So, age itself seems to increase the thrombosis potential, probably as the result of wear-and-tear on the vessels and their valves, or of such factors as decreased mobility Figure 2 follows an individual with factor V Leiden through her life The figure starts with the risk factors age and factor V Leiden and in red the thrombosis potential when no other risk factors are encountered throughout life Then a major disease during childhood is introduced that required an intravenous line, which is a factor with a high thrombosis potential, and so there is a short period with a high thrombosis potential, after which the thrombosis potential reverts to its previous level Then, between age 20–40 years she started to take oral contraceptives, which have an intermediate thrombosis potential and alter the overall thrombosis potential for 20 years Finally, at age 30, she had a plaster cast after a skiing injury, which conferred a high thrombosis potential for a short time The overall lifetime thrombosis potential now has a complicated form and will, because of the specific set of risk factors at a certain age, exceed the thrombosis threshold (in green), and lead to deep-vein thrombosis at age 30 The same set of risk factors (factor V Leiden, oral contraceptives, plaster cast) would not have caused thrombosis at age 20 Similarly, if she had not had the plaster casts, but continued to use the pill, thrombosis would have occurred around age 50 And at an older age, the mere effect of age and factor V Leiden would have been sufficient to lead to thrombosis

Interaction

Interaction occurs when two risk factors in combination produce an effect that exceeds the sum of their separate effects It has been shown that different hypothetical biological mechanisms may lead to diverse risk profiles [75] Interaction is, therefore, defined in numerical terms, and not in terms of biological mechanism, and the presence or absence of interaction does not allow conclusions about biological mechanisms, even though the finding of interaction may prompt research into mechanisms [76] It is sufficient to define interaction on an additive scale—ie, whether the combination of risk factors has greater effect than the sum of the effects of separate risk factors—as opposed to a definition on a multiplicative scale, in which the relative risk of the combination needs to exceed the product of the separate relative risks [31] However, since interaction is only a numerical concept, it is more relevant to estimate the magnitude of the combined effect of two factors, rather than to attempt to decide whether two factors display synergism or not, which is a theoretical issue without clear biological meaning

The model outlined in figure 2 can be extended to allow for a specific set of risk factors that yield higher thrombosis potentials together than separately, or in the presence of other factors Figure 2 shows models where all factors are assumed to behave in an additive manner—ie, without interaction Figure 3 shows that when factors have supra-additive effects, for example, factor V Leiden and use of oral contraceptives, this effect can be built into the model, and results in much higher thrombosis potential

Conclusion

Thrombosis is a disease in which genetic and acquired risk factors interact dynamically A time-dependent model that incorporates interaction of risk factors is valuable to explain why thrombosis occurs in one person at a specific time Such a model will primarily be useful to shape thinking about the aetiology of thrombosis Theoretically, this model will guide us to be able to provide individual risk estimates and set guidelines for prevention and prophylaxis This process will require much additional knowledge first, of the combined effect of all possible combinations of risk factors, and, second, of the dynamic interplay between genes and environment in determining concentrations of clotting factors and coagulation homoeostasis

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