Resistance to activated protein C and factor V Leiden as risk factors for venous thrombosis

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Resistance to Activated Protein C and Factor V Leiden as Risk Factors for Venous Thrombosis

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Introduction

Venous thromboembolism is a major cause of morbidity and mortality with an incidence of about 1 per 1000 per year. Predisposing factors can be either environmental or genetic. The first include trauma, surgery, immobilization, obesity, oral contraceptives, pregnancy and puerperium. The second included until recently a few rare hereditary disorders of blood coagulation (antithrombin, protein C and protein S deficiency). Together these genetic defects can be found in about 15% of selected patients with a personal and/or family history of thrombo-embolic disease (1–5) and only in a few percent of all patients with venous thrombosis (5,6).

Recently the situation changed dramatically by the discovery of a novel genetic defect associated with thrombophilia (7–10). In 1993 Dahlback et al. reported three families where the laboratory finding of resistance to activated protein C (APC) cosegregates with the thrombophilia (7). They proposed that hereditary APC-resistance is an autosomal dominant disorder. Subsequent studies revealed that APC-resistance is a common finding in patients with venous thrombosis (8,9,11,12) and is almost always caused by one mutation in the factor V gene (1691 G –> A, numbering according to ref.13) which will destroy one of the APC-cleavage sites in factor V by the replacement of Arg-506 by Gln (10,14,15). With the discovery of this new genetic risk factor for thrombosis a major step forward was made in our understanding of the clustering of thrombophilia in selected families (see Table 1). Especially the fact that in contrast to hereditary protein C, protein S and antithrombin deficiency, hereditary APC-resistance is a very common disorder which is caused almost exclusively by one single gene mutation, has contributed to establishing the concept of venous thrombosis as a multifactorial disease.

Table 1. Prevalence of genetic defects in (familial) thrombophilia. Prevalences have been calculated from data reported by Briët et al. (1), Gladson et al. (2), Ben Tal et al. (7), Tabernero et al. (4) and Pabinger et al. (5). The figure for the prevalence of APC resistance refers to our own observations. Three to four percent of the patients have APC-resistance in combination with one of the other genetic defects. The number of different mutations was derived from recent revisions of the respective mutation databases.

Genetic defect	Prevalence (%)	No of different mutations
Dysfibrinogenemia	1.0	>11
Antithrombin deficiency	4.2	>79
Protein C deficiency	4.9	>160
Protein S deficiency	5.1	>13
APC-resistance	46	1
Unknown	42	?

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Laboratory diagnosis of APC-resistance

In their original article Dahlbäck et al described three different tests that can be used for the analysis of the response of a patient's plasma to the anticoagulant action of activated protein C (7). Although the test principle in itself was not new (16–19) and had been used previously for the identification of plasma components that might interfere with the expression of the APC anticoagulant activity (20), the authors were the first to introduce such a test for the study of familial thrombophilia and for the screening of patients with thrombosis. Many modifications of this test or alternative test procedures have been developed (21–27) and used for screening purposes. The question now arises whether all these different tests will identify the same individuals as APC-resistant.

Most tests rely on the measurement of two APTT's, one in the presence of APC and one in its absence, whereas the test result is expressed as an APC sensitivity ratio [APC-SR:APTT (+APC)/APTT(-APC)] or a normalized APC-SR (n-APC-SR:APC-$SR_{patient}$/ APC-SR_{PNP}) (7,21,25). However, even after standardization of the test and analysis of the optimal pre-analytical conditions, tests may differ with respect to their sensitivity and specificity in detecting the factor V mutation. Moreover tests will differ also in their sensitivity for acquired defects like lupus anticoagulant (28,29), reduced prothrombin and factor X (25) and elevated factor VIII (23). Also the performance of the test – like that of the APTT itself – might depend on the actual instrument used (26). Finally, the fact that APC-resistance is a rather common finding in the general population (9,10), will make it necessary to rely on statistical and therefore somewhat arbitrary diagnostic criteria (9,25). All these considerations support the notion that it is not selfevident that the results obtained in one laboratory with one specific test can be reproduced in other laboratories using different tests.

Factor V Leiden

Previously we reported that 80% of the individuals that fulfilled our statistical criteria for APC-resistance (n-APC-SR <0.84

with the local test) were found to be heterozygous or homozygous for the:1691 G ->A substitution in exon 10 of the factor V gene, whereas all carriers of the mutation were APC-resistant (n-APC-SR <0.84) (10). The mutation predicts the replacement of Arg^{506} by Gln in one of the APC-cleavage sites of the heavy chain of factor V. It will reduce the rate of the inactivation of thrombin activated factor V by APC (10,30,31) thereby promoting procoagulant activity of factor V. This might explain why carriers of the mutation have an increased risk for thrombosis (see also below).

An interesting question is whether the factor V mutation will explain all cases of APC-resistance or whether we still should continue our hunt for other mutations. In Table 2 we have summarized the data from a large patient control study (see also Fig. 1). In total 894 individuals (422 patietns and 472 controls) were screened for the presence of APC resistance and factor V mutation. It appears that 95 of the 97 individuals with a n-APC-SR <0.70 (as measured with our local test) are carries of the factor V mutation and none of the 797 individuals with a n-APC-SR >0.70 (see also ref. 32). The latter group includes 57 individuals with a ratio between 0.70 and 0.84, that all fulfill our criterium for APC-resistance (n-APC-SR <0.84). Family studies, that were performed for some of these individuals, did not provide any evidence for an inherited defect (9). Further analysis of these individuals should help us to decide whether we should adjust our criteria for APC-resistance or whether we should consider this group of individuals as carriers of other genetic or acquired defects.

Table 2. Genetic heterogeneity of APC resistance. n-APC-SR and the genotype of nt 1691 in exon 10 of the factor V gene were determined for all patients and controls from the Leiden Thrombophilia Study (cfr. ref. 9) as previously described (9,10,25). Both parameters were available for 422 patients (Np) and 472 controls (Nc).

n-APC-SR	Np	Nc	Genotype (nt 1691)		
			GG	AG	AA
>0.84	298	442	740	—	—
0.70–0.84	41	16	57	—	—
0.50–0.70	74	14	2	86	—
<0.50	9	0	—	1	8

Also data from other laboratories suggest that the large majority of APC-resistant individuals is carrier of the factor V mutation (30,32,33,35,36) but that not all cases of APC-resistance are explained by the mutation (15,33,34). Recently Zöller et al. (37) reported that 29 out of 33 index cases with APC-resistance (APC-SR <2.0) did carry the factor V mutation. However, with the APC-resistance test used in their laboratory, a relatively large percentage of the heterozygous carriers of the factor V mutation, have APC-SR's in the range observed for non carriers of the mutation. Similarly, fifteen percent of the non-carriers have APC-SR's in the range of heterozygous carriers of the mutation. This suggests that their test is less specific in identifying carriers of the factor V mutation, but possibly more sensitive to other as yet unknown acquired or genetic defects. This might explain some of the apparent conflicts recently reported in the literature (see above).

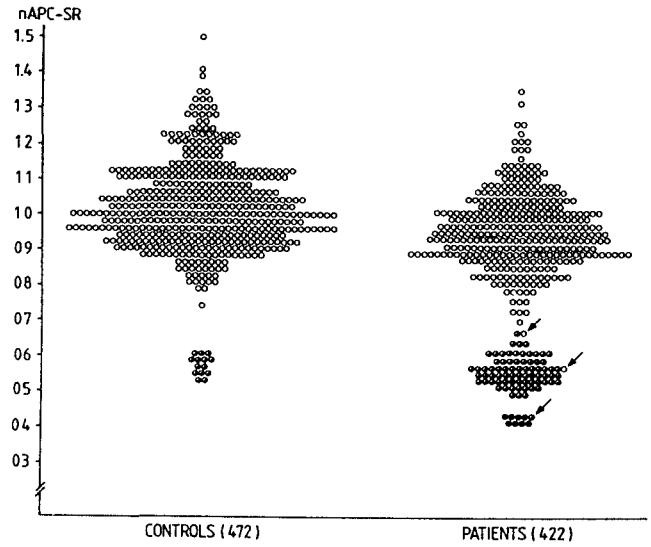

Fig. 1. n-APC-SR and factor V genotype in patients and controls from the Leiden Thrombophilia Study. ⊘heterozygous for the factor V Leiden mutation (1691 AG)● homozygous for the factor V Leiden mutation (1691 AA) ○homozygous normal (1691 GG). Arrows indicate two patients with a n-APC-SR between 0.50 and 0.70 who do not carry the FV mutation and have a lupus anticoagulant and one patient with a n-APC-DR <0.50 who is heterozygous for the FV mutation.

APC-resistance as risk factor for thrombosis

The first indication that APC-resistance might be associated with an increased risk for thrombosis came from observations in families with thrombophilia (7,12) and groups of selected patients with idiopathic or familial thrombosis (8). Formal evidence for this association came from the analysis of the data obtained in a large population based patient-control study:the Leiden Thrombophilia Study (LETS) (9). In this study 474 consecutive patients with a first episode of objectively confirmed deep vein thrombosis and 474 age and sex matched healthy controls were included. From the analysis of the results of the APC-SR measurements in the first 301 patients and controls it was concluded that 21% of the patients and 5% of the healthy controls had an APC-SR ≤2.17 with the local test. This leads to an estimated seven fold increase in risk of deep-vein thrombosis (matched odds ratio 6.6; 95% (CI 3.6–12.0). In the meantime APC-SR's and the factor V genotype have been measured in all patients and controls. Both parameters were available for 472 controls and 422 patients (49 patients were on oral anticoagulants and for five individuals one of the parameters was missing). The data are shown in Fig. 1. Twenty eight percent of the patients and only 5.7% of the controls have a n-APC-SR ≤0.84, confirming that having an APC-SR <0.84 is a risk factor for thrombosis (9). From the 97 individuals with an n-APC-SR ≤0.70, 95 were carrier of the FV mutation while 2 had a low n-APC-SR associated with the presence of lupus anticoagulant (see also 28,29). It is interesting to note that even when we omit all carriers of the factor V mutation the mean n-APC-SR for patients is lower than for the controls. The reason for this is not known. It might reflect a post-thrombotic effect on the n-APC-SR or point to a second

unidentified risk factor for thrombosis which affects the response of plasma to activated protein C.

Frequencies of APC resistance in cohorts of thrombosis patientsdepending on patients selectivity criteria, range from 17.5% to 64% (8?9,11,12,21,38–40). Prevalence of APC resistance in controls was reported to be from 2% to 7% (9,11,35). One should keep in mind that in these studies different tests and criteria for APC resistance have been used.

Factor V Leiden as risk factor for thrombosis

The evidence that factor V Leiden is a risk factor for thrombosis again comes from the aforementioned patient–control study (41). After genotyping of all patients and controls it was calculated that the relative risk of thrombosis is 7.9 (CI95:4.1–13.0) in heterozygous carriers of the defect. In homozygous carriers the relative risk was estimated to be 91 (CI95:26–322) using the assumption of complete Hardy Weinberg equilibrium for the Factor V mutation in the control group. The median age of onset of symptoms was 44 (range 17–69) in heterozygotes and 31 (range 22–55) in homozygotes (41). In general the relative risks were found to be independent of age. Given that the risk of thrombosis increases with age this indicates a higher incidence of thrombosis in carriers of the mutation in the older age groups.

Additional information on the risk of thrombosis in carriers of the factor V mutation comes from the study of relatives of symptomatic patients who carry the mutation (37). Zöller et al. (37) studied 50 Swedish thrombophilic families with inherited APC resistance. In the 47 families, where the factor V mutation was segregating, they observed that the cumulative penetrance of thrombosis was 44% in homozygotes for the factor V mutation, 30% in heterozygotes and 10% in homozygous normals. These figures clearly indicate that in these families the factor V Leiden mutation is a risk factor for thrombosis. Whether similar results will be obtained in families identified through an asymptomatic carrier of the factor V mutation needs to be established in the future.

Interaction of factor V Leiden with other genetic risk factors for thrombosis

During the past twenty years the study of familial thrombophilia has been focussed mainly on the identification of single genetic defects that could explain the segregation of the thrombophilia in affected families. The concept underlying this approach is that familial thrombophilia is a single gene disorder. The notion that this view might be too simple has been expressed repeatedly during the last years especially in relation to the observations in protein C deficient families (42,43). Data coming from patient control studies clearly indicate that protein C deficiency is a risk factor for thrombosis (6,44). So it is not surprising that in families of symptomatic probands a significant association between thrombophilia and protein C deficiency is observed (45–47), although the penetrance of the disease is variable and incomplete (clinically dominant protein C deficiency). In contrast, it was surprising that the prevalence of protein C deficiency among healthy blood donors is much higher than expected (48,49) and that thrombosis is a very rare observation in these protein C deficient families (clinically recessive protein C deficiency). Together these observations have led to the hypothesis that in clinically dominant protein C deficient families the segregation of other genetic risk factors might contribute to the phenotype of thrombophilia (42,43).

Support for this hypothesis, however, was difficult to find. Because the prevalence of protein C, protein S and antithrombin deficiency is rather low, families where two or more of these genetic defects segregate are extremely rare (5). This situation changed with the recent discovery of factor V Leiden as risk factor for thrombosis. The factor V mutation is found in 3–4% of the population and ten times more common than all the other genetic risk factors together (14,36,41). This finding made it possible for the first time to test the concept that familial thrombophilia might be a multiple gene disorder.

The first support for this concept came from a study of Koeleman et al. (50). They reported that 19% of 48 unrelated symptomatic protein C deficient probands from thrombophilic families is also carrier of the factor V Leiden mutation. In 6 families where both the factor V mutation and a protein C gene mutation are segregating, the penetrance of thrombophilia is significantly higher in carriers of both gene defects (73%) than in carriers of a single gene defect (36% and 10%). This is also illustrated in Fig. 2 where the thrombosis free survival curves have been plotted for those carrying two gene defects, only one gene defect or no gene defect. These results indicate that in these families carriers of both gene defects have an increased risk for thrombosis compared with siblings with a single defective gene. The high prevalence of the factor V mutation among symptomatic

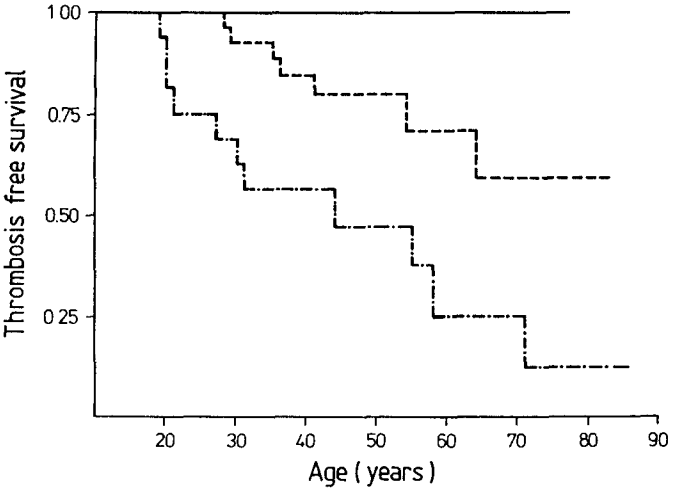

Fig. 2. Thrombosis free survival curves for family members carrying both the factor V mutation and a protein C gene mutation (- · - · -), for family members carrying only one gene mutation (- - -) and for those relatives who carry no gene mutation (—).

protein C deficient probands might be related to the fact that most of these families have been selected because of clustering of thrombophilia. When we repeat the analysis in a panel of unselected symptomatic protein C deficient patients from the aforementioned patient control study (44), the frequency of the factor V mutation is much lower (0/13). So selection for familial thrombophilia

is important for finding these combined deficiencies. This might explain some of the apparently conflicting data in the literature (8,51).

We have found that the factor V mutation can also be found frequently in families with familial thrombophilia and other genetic defects: 1/12 patients with dysfibrinogenaemia, 3/11 patients with antithrombin deficiency and 6/16 patients with protein S deficiency (unpublished observations).

All these findings support the concept that familial thrombophilia is a multiple gene disorder The observation that the penetrance of thrombosis in patients with two gene defects is higher than in those with a single gene defect will have important implications for the clinical management of these individuals in the future.

Interaction of factor V Leiden with environmental risk factors for thrombosis

The high prevalence of the factor V mutation among patients with deep vein thrombosis, also makes it possible to study its interaction with common environmental risk factors for thrombosis. For instance the use of oral contraceptives is a well known, and much debated risk factor for venous thrombosis The question arises whether the occurrence of thrombosis in oral contraceptive users might be explained by the factor V Leiden mutation. This question was recently addressed by Vandenbroucke et al (52) in a reanalysis of data from 155 consecutive premenopausal women who had developed venous thrombosis and 169 population controls (data derived from the larger case-control study). They found that the risk of thrombosis increased 3.8 fold among oral contraceptive users (when compared to non users) and 8 fold among factor V Leiden carriers (when compared to non carriers). However in those who used oral contraceptives and were carrier of the factor V mutation, the risk of thrombosis was 35 fold higher than in those not using oral contraceptives and not carrying the factor V mutation By backcalculation of their data to population incidence rates, the authors demonstrated that the combination of the factor V mutation and oral contraceptive use gives a larger risk difference than the sum of the individual effects (see Table 2 in ref. 52). The risk of thrombosis among women who have both the factor V mutation and use oral contraceptives, might indicate that both factors act on the same physiological process. In this respect it is of interest that also in healthy women, the use of oral contraceptives leads to a significant decrease in APC-SR (53).

Future studies should define whether the presence of the factor V mutation also will increase the sensitivity of individuals for other established environmental risk factors for thrombosis like trauma, immobilization, pregnancy and puerperium.

Summary

The recent discovery of the factor V Leiden mutation as the molecular defect in the large majority of APC-resistant individuals, has drastically changed our view on familial thrombophilia and it has contributed to a better understanding of the interaction of genetic and environmental risk factors. It has offered firm support for the view that venous thrombosis is a multifactorial disease and that the risk of thrombosis will increase as the number of genetic and/or environmental risk factors increases

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