

CHAPTER 6

THE INCIDENCE OF RECURRENT VENOUS THROMBOEMBOLISM IN CARRIERS OF FACTOR V LEIDEN IS RELATED TO CONCOMITANT THROMBOPHILIC DISORDERS

Johan R. Meinardi, Saskia Middeldorp, Pieter J. de Kam,
Maria M.W. Koopman, Elisabeth C.M. van Pampus, Karly Hamulyák,
Martin H. Prins, Harry R. Büller, and Jan van der Meer

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SUMMARY

Background: The duration of anticoagulant treatment after a first episode of venous thromboembolism primarily depends on the risk of recurrence. Variance of recurrence rates in factor V Leiden carriers may be due to concomitant thrombophilic disorders.

Methods: A retrospective study was performed in 329 factor V Leiden carriers with a history of venous thromboembolism (262 probands, 67 relatives). The annual rate of first recurrence was estimated in relatives. The contribution of concomitant thrombophilic disorders to the recurrence rate was evaluated in probands and relatives by a nested case-control analysis in 105 matched pairs of carriers either with or without recurrence.

Results: The overall annual recurrence rate was 2.3 per 100 patient-years. The adjusted risk of recurrence for concomitant thrombophilic disorders was: 9.1 (1.3-62.8) for the factor II mutation; 1.0 (0.2-4.9) for homozygosity for factor V Leiden; 1.5 (0.2-9.5) for inherited deficiencies of protein C or S; 1.8 (0.7-4.9) for factor VIII:C levels >122%; 5.4 (1.6-18.6) for fasting homocysteine levels >15.2 : mol/L and 4.4 (1.0-18.7) for loading homocysteine levels >45.8 : mol/L. The estimated recurrence rate ranged from 0.45 per 100 patient-years after a secondary first event in the absence of concomitant disorders to 4.8 per 100 patient-years when a spontaneous first event was combined with concomitant disorders.

Conclusions: Our study provides supportive evidence that the incidence of recurrent venous thromboembolism in heterozygous factor V Leiden carriers depends on the concomitance of other thrombophilic disorders, as well as to whether the first thrombotic event occurred spontaneously.

6.1 INTRODUCTION

The recurrence rate of venous thromboembolism ranges from 10 to 18% within two years after the first episode and increases to 30% after 8 to 12 years [1-3]. It is higher in patients with persistent risk factors, like inherited thrombophilic defects, than in patients who are exposed to transient risk factors, such as surgery, trauma, immobilization, oral contraception, and pregnancy [1, 2]. Hence, it may be worthwhile to identify thrombophilic disorders in order to adjust the duration of secondary thromboprophylaxis. The factor V:Q⁵⁰⁶ mutation or factor V Leiden is the most common genetic defect associated with venous thromboembolism. Its prevalence in caucasians is approximately 5%, while it was demonstrated in 20-50% of patients with venous thromboembolism [4, 5]. Compared to non-carriers, heterozygous carriers of this mutation showed a three- to sevenfold higher risk of venous thromboembolism, in homozygotes this risk was considered even 80-fold higher [5-8]. Whether factor V Leiden influences the recurrence rate is still controversial. Of five prospective studies on this subject, two reported a two- and fourfold increased risk of recurrence, respectively [9, 10], while the remaining three studies did not show such an association [11-13]. These discrepant findings might partly be due to differences in the distribution of concomitant thrombophilic defects between the studies, as these were not consistently evaluated.

We performed a study to assess the contribution of concomitant thrombotic risk factors, either thrombophilic disorders or exogenous conditions, to the recurrence of venous thromboembolism in a large cohort of factor V Leiden carriers.

6.2 PATIENTS AND METHODS

6.2.1 Patients

Carriers of factor V Leiden were recruited from a prior family cohort study, that was designed to estimate the risk of venous thromboembolism in factor V Leiden carriers [7]. Briefly, 270 consecutive caucasian patients with venous thromboembolism and factor V Leiden (proband) and their 904 living first degree relatives (parents, siblings and children over the age of 15 years) were enrolled in that study. Detailed information regarding episodes of venous thromboembolism and exposure to exogenous risk factors was retrospectively collected, in relatives this was done prior to DNA testing for factor V Leiden. The present study was restricted to carriers (proband and relatives) who had had at least one episode of deep vein thrombosis or pulmonary embolism. They were additionally tested for: inherited deficiencies of antithrombin, protein C type I or II and protein S type I; the prothrombin G20210A (factor II) mutation; elevated levels of factor VIII coagulant activity (VIII:C); and hyperhomocysteinemia. Inherited deficiencies of antithrombin, protein C or

protein S were defined as plasma levels below the lower limit of the normal ranges at two separate measurements, and in at least two relatives. Protein S deficiency was considered to be acquired due to pregnancy or oral contraceptive use, unless it was established by repeated measurement at least three months after delivery and discontinued oral contraceptive use, respectively. Plasma levels of homocysteine were measured after overnight fasting and in most (71%) subjects also 6 hours after oral loading with 0.1g L-methionine per kg body weight while the patients were on a diet poor in protein. Hyperhomocysteinemia was defined as a fasting level of homocysteine >18.5 : mol/L and/or a loading level >58.8 : mol/L, according to commonly used criteria in the Dutch population [14, 15].

An episode of venous thromboembolism was considered established if demonstrated by objective techniques, such as compression ultrasound, ventilation/perfusion scanning, or pulmonary angiography, and/or if the patient was treated with full dose heparin and oral anticoagulants for at least 3 months. Venous thromboembolism was classified secondary if it had occurred during or less than three months after exposure to one or more concomitant exogenous risk factors like surgery, trauma, immobilization for more than seven days, oral contraceptive use, pregnancy, or malignancy. Venous thromboembolism that occurred in the absence of any exogenous risk factor was considered spontaneous. The study was approved by the institutional review boards of the three participating hospitals and informed consent was obtained from all participants.

6.2.2 Laboratory studies

Factor V Leiden and the factor II mutation were demonstrated by polymerase chain reaction, as described previously [16, 17]. Blood samples for measurements of antithrombin, protein C, protein S, and factor VIII:C were collected by venipuncture on 1/10th volume of 0.109 M trisodium citrate. Platelet-free plasma was prepared by centrifugation at $3200 \times g$ for 10 minutes followed by 5 minutes at $12000 \times g$. Plasma samples were stored at -80 °C until testing. Antithrombin activity (CoatestTM, Chromogenix AB, Mölndal, Sweden) and protein C activity ('Berichrom' Protein C, Behring, Marburg, Germany) were measured by a chromogenic substrate assay. Protein C and total protein S antigen levels were measured by ELISA (with reagents obtained from DAKO, Glostrup, Denmark). Factor VIII:C was measured by an one-stage clotting assay on a KC10A Amelung Coagulometer (Amelung GmbH, Lemgo, Germany). Antithrombin, protein C, protein S, and factor VIII: C were expressed as percentage of the levels measured in pooled normal plasma set at 100%. Normal ranges (mean \pm 2SD) were determined in healthy volunteers, who had no (family) history of venous thromboembolism and were neither pregnant nor had used oral contraceptives during the last three months. For measurement of homocysteine by high performance liquid chromatography [18], EDTA-anticoagulated blood was immediately

centrifugated at 1700 x g for 10 minutes and plasma was stored at -20°C until testing within 4 weeks.

6.2.3 Statistics

Factor V Leiden carriers with recurrent venous thromboembolism were compared to carriers who had had a single thrombotic episode. The annual recurrence rate was computed in relatives by dividing the number of first recurrences by the total number of observation years, counted from the initial thrombotic event until either the date of first recurrence or the date of enrolment in patients without recurrence. A recurrence-free survival curve was constructed according to Kaplan and Meier.

The effects of concomitant thrombophilic disorders on the recurrence rate were assessed by a nested case-control analysis in 105 matched pairs of factor V Leiden carriers (proband and relatives) with either a recurrence (cases) or a single episode of venous thromboembolism (controls). They were matched for sex, age at enrolment, and observation time. Multiple logistic regression analysis was carried out to estimate the contribution to recurrence of a concomitant factor II mutation, homozygosity for factor V Leiden, inherited deficiencies of antithrombin, protein C or protein S, increased levels of factor VIII:C, and hyperhomocysteinemia. Results were expressed as adjusted odds ratios and their 95% confidence intervals (CI). To assess a dose-response relationship between the factor VIII:C and homocysteine levels, respectively, and the recurrence risk, odds ratios for quartiles of factor VIII:C and homocysteine levels (fasting and loading levels separately) were calculated in the model. Differences in continuous variables between patients with and without recurrences were analyzed by Wilcoxon's two-sample test and presented as median values and their ranges. Differences in categorical variables were analyzed by Fisher's exact test or the chi-square test, when appropriate. A two-tailed p-value of less than 0.05 was considered to indicate statistical significance. Analysis was performed using SAS software, version 6.12 (SAS-Institute Inc., Cary, North Carolina).

6.3 RESULTS

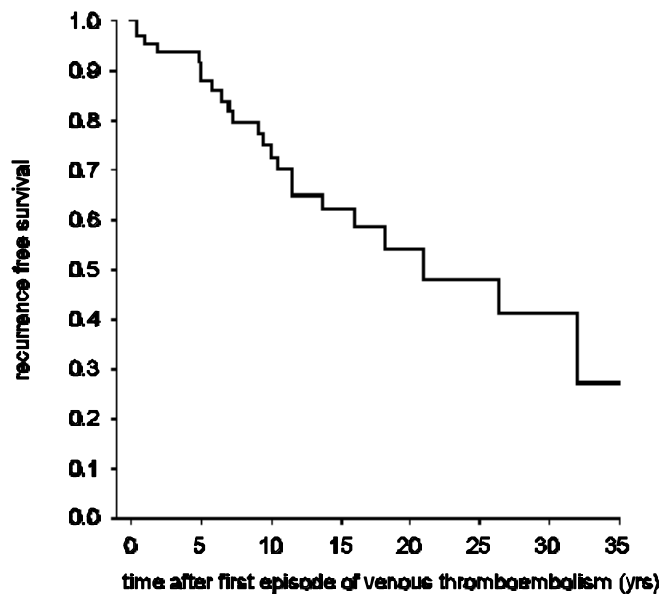
Of 793 factor V Leiden carriers who enrolled the original study, 337 had a history of venous thromboembolism. Eight patients (2%) were excluded since their first thrombotic event was located at unusual sites; retinal vein (four), caval vein (two), mesenteric vein

Table B.1 Characteristics of 329 factor V Liden carriers with a history of venous thrombembolism

	probands			relatives		
	recurrence (n=91)	no- recurrence (n=171)	P	recurrence (n=24)	no- recurrence (n=43)	P
patients:						
women, %	56	71	0.02	50	65	0.30
median age at enrolment (range), yr	49 (19-81)	36 (8-88)	< 0.001	57 (31-80)	53 (20-79)	0.42
median age at first event (range), yr	36 (12-77)	31 (8-88)	0.27	31 (16-63)	38 (15-79)	0.02
first thrombotic event:						
deep vein thrombosis, %	81	77	0.53	83	83	1.00
exogenous risk factors, %:						
none	53	40	0.07	42	47	0.80
surgery, trauma or immobilization	20	15	0.39	21	19	1.00
pregnancy/puerperium	15	9	0.15	25	16	0.52
oral contraceptives	11	36	< 0.001	13	19	0.73
malignancy	2.2	0	0.12	0	0	-
anticoagulant therapy, yr	0.28 (0-9)	0.33 (0-18)	0.22	0.50 (0-32)	0.26 (0-10)	0.39

(one) and cerebral sinus (one). The remaining 329 carriers (262 probands, 67 relatives) were analyzed in the present study; of these, 115 (35%) had recurrent deep vein thrombosis or pulmonary embolism. Their characteristics are summarized in Table 6.1. Probands and relatives showed a similar distribution of characteristics among groups with and without recurrences. Women were predominant in the groups without recurrences. Median age at enrolment was higher in relatives than probands, and in the groups with recurrences as compared to the groups without recurrences. Of 329 first thrombotic events, 80% presented as deep vein thrombosis and 45% occurred spontaneously. A majority (72%) of these events was diagnosed by objective tests. In women with recurrent venous thromboembolism, 21% of the first episodes were associated with the use of oral contraceptives, as compared to 47% in women without recurrences. An association of the first episode with pregnancy or puerperium was observed in 32% and 15% of women, respectively. Only two patients, both probands with recurrences, had a first event that was related to malignancy.

Figure 6.1 Cumulative rate of a first recurrence of venous thromboembolism in 67 relatives with factor V Leiden



There were no differences in duration of anticoagulant therapy after the first event. Of 115 first recurrences, 71% occurred spontaneously and 83% were located in the limbs. Of recurrences, 70% were demonstrated by objective tests. In one case, initial leg vein thrombosis recurred in the caval vein. Of 80 patients, in whom both first and recurrent event were located

in the limbs, 44% had a contralateral recurrence. The median (range) interval between first event and recurrence was 4.2 (0.1-35.1) years in probands and 9.3 (0.4-42.8) in relatives (p=0.01).

The annual recurrence rate in relatives was 2.3 per 100 patient-years (24 first recurrences/1062 observation years). The recurrence-free survival curve is shown in Figure 6.1. The 1-, 5-, 10- and 15 year cumulative recurrence rates were 5, 10, 28 and 38%, respectively.

The influence of concomitant thrombophilic disorders on the recurrence rate was analyzed in 105 pairs of cases (factor V Leiden carriers with recurrences) and controls (carriers without recurrences) (Table 6.2). For 10 of 115 factor V Leiden carriers with recurrences no control was available that fulfilled all matching criteria.

Table 6.2 Results of univariate analysis of concomitant thrombophilic disorders in 105 matched pairs of heterozygous factor V Leiden carriers either with (cases) or without (controls) recurrent venous thromboembolism

	cases (n=105)	controls (n=105)	P
women, n (%)	61 (58)	62 (59)	1.00
median age at enrolment, yr (range)	48 (19-80)	47 (16-81)	0.74
median observation time, yr (range)	4 (0-42)	4 (1-47)	0.56
spontaneous first thrombotic event, n (%)	52 (50)	49 (47)	0.78
concomitant thrombophilic disorders, n (%)			
factor II mutation	10 (10)	4 (4)	0.0002
homozygosity for factor V Leiden	9 (9)	5 (5)	0.41
deficiencies of antithrombin, protein C or protein S*	6 (6)	3 (3)	0.50
factor VIII:C > 150%	48 (55)	37 (40)	0.06
hyperhomocysteinemia**	16 (19)	11 (12)	0.22

* including 3 patients with protein C deficiency and 6 with protein S deficiency

** homocysteine fasting levels >18.5 µmol/L and/or loading levels > 58.8 µmol/L

Concomitant thrombophilic disorders were observed in 75% of cases as compared to 63% of controls (p=0.05). Established genetic defects, i.e. heterozygous factor II mutation, homozygosity for factor V Leiden, and inherited deficiencies of protein C or protein S (antithrombin deficiency was not observed), were demonstrated approximately twice as

Table 6.3 Influence of concomitant thrombophilic disorders on the risk of recurrent venous thromboembolism in heterozygous factor V Leiden carriers

	adjusted odds ratio*	(95% CI)
factor II mutation	9.1	1.3-62.6
homozygosity for factor Leiden	1.0	0.2-4.9
deficiencies of protein C or protein S	1.5	0.2-9.5
factor VIII:C, %		
first quartile < 122	1.0	reference
second quartile 122-144	2.0	0.6-6.6
third quartile 145-179	1.6	0.5-5.4
fourth quartile >179	1.8	0.5-6.3
fasting homocysteine, µmol/L		
first quartile < 9.4	1.0	reference
second quartile 9.5-11.2	1.0	0.3-3.5
third quartile 11.3-15.2	3.8	1.1-13.0
fourth quartile > 15.2	5.4	1.6-18.6
loading homocysteine, µmol/L		
first quartile < 28.8	1.0	reference
second quartile 29-36.5	1.5	0.4-5.6
third quartile 37-45.8	1.5	0.4-6.3
fourth quartile > 45.8	4.4	1.0-18.7

* **Adjusted for sex, age, observation time, proband state, spontaneous first event and any other of the listed thrombophilic disorders**

much in cases than controls. Only for the combination of factor V Leiden and the factor II mutation, the difference was statistically significant (p=0.0002). Elevated factor VIII:C levels (>150%) were found in 55% of cases versus 40% of controls (p=0.06). Median levels were 158% versus 140% (p=0.008). Hyperhomocysteinemia (fasting level >18.5 : mol/L

and/or loading level >58.8 : mol/L) was more common in cases than controls (19% versus 12%, $p=0.22$). Of cases, 11% had elevated fasting levels, 15% had elevated loading levels and in 7% both levels were elevated, as compared to 7, 9 and 3% of controls, respectively. Median homocysteine levels were higher in cases than controls, fasting 12 versus 11 : mol/L ($p=0.008$) and after loading 40 versus 36 : mol/L ($p=0.09$). The results of the multivariate analysis are shown in Table 6.3.

Table 6.4 Effects of interactions between endogenous and exogenous thrombotic risk factors on the rates of first recurrence of venous thromboembolism in heterozygous carriers of factor V Leiden

concomitant thrombotic disorder*	spontaneous first event	adjusted odds ratio** (95% CI)	annual recurrence rate***
-	-	1.0 (reference)	0.45
-	+	4.3 (0.7-25.0)	1.9
+	-	4.5 (1.1-18.6)	2.0
+	+	10.6 (1.9-58.5)	4.8

* including factor II mutation, homozygosity for factor V Leiden, deficiencies of protein C or protein S, factor VIII:C $>150\%$, and fasting homocysteine >11.2 $\mu\text{mol/L}$

** adjusted for sex, age, observation time and proband state.

*** per 100 patient-years

Of established genetic defects, the factor II mutation showed to be an independent contributor to recurrent venous thromboembolism in factor V Leiden carriers (adjusted odds ratio 9.1; 95% CI 1.3-62.8), by contrast with a homozygous factor V Leiden carrier state (1.0, 0.2-4.9) and deficiencies of protein C or protein S (1.5, 0.2-9.5). The risk of recurrence tended to be higher at factor VIII:C levels between 122 and 144%, but did not increase further at higher levels. Overall, the risk of recurrence at factor VIII:C levels $>122\%$ was approximately two-fold higher than at lower levels (adjusted odds ratio 1.8, 95% CI 0.7-4.9). The homocysteine related risk showed a proceeding incline at fasting levels >11.2 : mol/L [(5.4, 1.6-18.6) for levels >15.2 : mol/L]. A significant incline was demonstrated at loading levels >45.8 : mol/L (4.4, 1.0-18.7).

To estimate the effect of interactions between endogenous and exogenous thrombotic risk factors on the recurrence rate, we stratified cases and controls according to the presence or absence of concomitant thrombotic risk factors (Table 6.4). In this analysis, fasting homocysteine levels >11.2 : mol/L were considered as a risk factor for recurrence as demonstrated by multivariate analysis. The lowest recurrence rate was observed in heterozygous factor V Leiden carriers who had a secondary first event without concomitant

thrombophilic disorders. The risk of recurrence was approximately four-fold higher after either a spontaneous first event without a concomitant thrombophilic disorder or a secondary first event in the presence of any of these disorders. It was almost 11-fold higher when a spontaneous first event was associated with a concomitant disorder. Estimated annual recurrence rates ranged from 0.45 to 4.8 per 100 patient-years.

6.4 DISCUSSION

This study shows that concomitance of other thrombophilic disorders and a spontaneous first episode of venous thromboembolism are determinants of the risk of recurrence in heterozygous carriers of factor V Leiden. The estimated annual recurrence rate ranged from 0.45 per 100 patient-years in heterozygous factor V Leiden carriers who experienced a secondary first thromboembolic episode in the absence of other thrombophilic disorders to 4.8 per 100 patient-years in those with a spontaneous first event in combination with a concomitant disorder.

The factor II mutation increased the risk of recurrence nine-fold. Considering that previous studies did not demonstrate an increased risk of recurrence in single carriers of the factor II mutation [12, 13, 19], the genetic interaction between factor V Leiden and the factor II mutation apparently results in a synergistically increased risk. Our finding is in agreement with two recent reports that showed a two- to threefold higher risk of recurrence in double-heterozygous carriers of factor V Leiden and the factor II mutation as compared to single carriers of factor V Leiden [20, 21]. Of established genetic defects, deficiencies of protein C or protein S, and homozygosity for factor V Leiden were not identified as independent predictors of recurrence in our study, although these were found more frequently in the recurrence group. This finding is not plausible and can be explained by the small numbers of patients with these combined defects.

Elevated levels of factor VIII:C and hyperhomocysteinemia have been recognized more recently as possibly inherited risk factors for (recurrent) venous thromboembolism [14, 15, 22-24]. Both disorders were frequently observed in our patients and did increase the risk of recurrence. Actually, the effects of factor VIII:C and homocysteine were already demonstrated at levels within the defined normal ranges, suggesting an interaction with factor V Leiden. Levels of factor VIII:C >122% and fasting homocysteine >11.2 : mol/L were associated with an approximately two- and five-fold increased risk of recurrence, respectively. Although these risk estimates are modest and not statistically significant for factor VIII:C, it should be noticed that these levels were found in 75% and 50% of our patients, respectively, and may consequently account for a substantial proportion of recurrences in factor V Leiden carriers.

In the absence of any concomitant thrombophilic disorder, the risk of recurrence was still four times higher in factor V Leiden carriers who experienced a spontaneous first

thrombotic event as compared to a secondary first event. This finding suggests the presence of (an)other, thusfar not recognized thrombophilic disorder(s). On the other hand, the lower risk of recurrence in patients with a secondary first event may partly be attributed to discontinuation of oral contraceptives after the first event and thromboprophylaxis at renewed exposure to exogenous risk factors. It also explains why women had less recurrences than men, as well as the large proportion of spontaneous recurrences.

The survival curve suggests a steadily continuing risk of recurrence over a period of 35 years. Beyond a period of eight years, this finding may be flawed because less carriers without recurrences had a follow-up longer than eight years. In addition, the diagnosis of the first event in patients with a recurrence after more than 20 years was made before the introduction of objective tests and hence maybe less reliable than in patients who had their first event less than 20 years ago.

So far, two of five prospective studies reported an increased recurrence risk in factor V Leiden carriers [9-13]. Noteworthy, one of both studies was addressed to men and spontaneous first events [9], clinical variables that were related to the risk of recurrence in the present and previous studies [1, 2, 13]. Moreover, recurrence rates in prior studies were not adjusted for the influence of the factor II mutation and elevated factor VIII:C or homocysteine levels.

This retrospective study has limitations. Firstly, referral bias might have occurred easily, as it is likely that especially patients with recurrences will be referred. This is supported by the longer observation time in our patients with recurrences. However, the proportion of patients with recurrences did not differ between probands and relatives, while the annual incidence rate was estimated in relatives. Secondly, postphlebotic signs and symptoms might have been wrongly interpreted as recurrence. We found an ipsilateral recurrence in about half of the patients with deep vein thrombosis, similarly to previously reported rates in venographically controlled studies [1, 2]. Moreover, the postphlebotic syndrome as reason for misclassification can be ruled out in the remaining half of patients who experienced contralateral recurrences.

A possible clinical implication of our findings is the need for risk stratification in heterozygous factor V Leiden carriers who experience a first episode of venous thromboembolism since their risk of recurrence may range from 0.45 to 4.8 per 100 patient-years. It should be noticed, however, that though concomitant disorders were demonstrated in a majority (75%) of factor V Leiden carriers with recurrent venous thromboembolism, these were also frequently (63%) found in carriers who had experienced only a single episode of venous thromboembolism. The optimal duration of anticoagulant treatment after a first event primarily depends on the risk of recurrence over time, as well as the risk of major bleeding. As a consequence of an individual risk assessment, especially heterozygous carriers of factor V Leiden with a first spontaneous episode of venous thromboembolism may have to be tested for all known thrombophilic disorders.

In conclusion, our study provides supportive evidence that the risk of recurrent venous thromboembolism in heterozygous factor V Leiden carriers depends on the concomitance of

other thrombophilic disorders, as well as to whether the first thrombotic event occurred spontaneously.

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