

Venous Thrombotic Risk in Family Members of Unselected Individuals with Factor V Leiden

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Key words

Venous thromboembolism, thrombophilia, Factor V Leiden, hypercoagulability

Summary

The factor V Leiden mutation (FVL) leads to a seven-fold increased risk of venous thromboembolism (VTE). In thrombophilic families, 25% of carriers have experienced thrombosis before the age of 40 years. Aim of our study was to assess the association of FVL with VTE in first-degree family members of unselected symptomatic and asymptomatic carriers of FVL.

We tested 197 relatives of consecutive thrombosis patients with FVL and 36 relatives of asymptomatic carriers on the presence of FVL and the occurrence of VTE.

The incidence of VTE in relatives with FVL of symptomatic carriers was 0.34%/year. This was similar to the incidence in relatives with FVL of asymptomatic carriers. Kaplan Meier analysis in relatives of symptomatic probands showed that at the age of 58 years, thrombosis-free survival was reduced to 75% in carriers and 93% in non-carriers ($P < 0.05$). Carriers of FVL had a three times higher thrombotic risk than non-carriers. In combination with environmental risk factors, FVL clearly adds to the risk of VTE. The thrombotic incidence rate in these unselected relatives with FVL, however, is considerably lower than was seen in carriers of thrombophilic families (1.7%/year). Therefore, special care should be paid to individuals with a positive family history of venous thrombosis while exposed to these risk factors.

Introduction

The annual incidence of venous thromboembolism is about one per 1000 (1, 2). Predisposing risk factors include both genetic and acquired conditions (3). Several studies have demonstrated an increased venous thrombotic risk in individuals with familial resistance to activated protein C (APC-resistance) caused by the factor V Leiden mutation (4–9). This mutation, a 1,691 G to A substitution in the factor V gene, predicts the synthesis of an abnormal factor V molecule (factor V Leiden) that is not properly inactivated by activated protein C (APC) (10–13). This mutation is present in about 5% of healthy Caucasians and has been found in 20% of unselected consecutive patients with deep vein

thrombosis and in 50% of individuals from families referred because of unexplained familial thrombophilia (8, 10, 14, 15).

Important to clinicians is the question what prophylactic policy is advisable for patients and their relatives in families with factor V Leiden. In order to answer this question, the risk of thrombosis in these individuals needs to be assessed. We recently showed the important role of selection of patients and their relatives in these assessments since the mean age at onset is much younger (29 years) in highly selected families with thrombophilia than among unselected consecutive patients (43 years) (16).

In order to further investigate this effect of selection of individuals, we included in the present study first degree relatives of 47 unselected, consecutively diagnosed patients who were identified as carriers of factor V Leiden in the Leiden Thrombophilia (case-control) Study (LETS) (5). We contrasted the thrombotic risk in these families to the thrombotic risk we have estimated in selected thrombophilic families; in addition, we included families of nine asymptomatic carriers of factor V Leiden who were identified in the control group of the LETS study. These can be thought of as mutation carriers found by screening of asymptomatic individuals. Our hypothesis is that thrombosis is a multicausal disease. Thrombophilic families harbour more risk factors than families of unselected patients, and so the risk would be higher. Arguing along the same lines, the asymptomatic carriers harbour few risk factors beyond factor V Leiden – this is why they remained asymptomatic – and so in their families we expected the lowest risk.

Patients, Materials and Methods

Patients and Families

We included patients from a population-based case-control study on venous thrombosis, the Leiden Thrombophilia Study (LETS) (5). Cases were 474 consecutive patients younger than 70 years, with a first, objectively confirmed episode of deep vein thrombosis (no pulmonary embolism or superficial thrombophlebitis) and free of underlying malignancy. Factor V Leiden was detected in 92 cases (84 heterozygotes and eight homozygotes). Of these 92 cases we invited all homozygous patients ($n = 8$) and the heterozygous patients who resided in the area of the University Hospital Leiden ($n = 40$). Controls were 474 unrelated age- and sex-matched acquaintances or spouses: 14 were heterozygous carrier of factor V Leiden whom were all invited to participate. Seven of the eight homozygous patients, all 40 heterozygous patients and nine of the 14 asymptomatic controls were willing to participate in this study. We invited the siblings, parents and children of these 56 probands. Family members under 15 years of age and over 70 years of age were excluded for practical purposes.

Of the 56 probands with factor V Leiden, 233 eligible first-degree family members (91%) participated in the study while 22 did not, five because they lived abroad and 17 for reasons unknown. These 233 consenting relatives were seen by the same physician (R.P.M.L.) for venipuncture and interview. Risk factor status was assessed by the interviewer before carrier status was deter-

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Table 1 Incidence rates of venous thrombosis in relatives with and without factor V Leiden of symptomatic and asymptomatic probands with factor V Leiden

Probands	Symptomatic probands (n=47)		Asymptomatic probands (n=9)	
	FVL +	FVL -	FVL +	FVL -
n	108	89	14	22
Men (%)	46 (43)	40 (45)	9 (64)	6 (27)
Women (%)	62 (57)	49 (55)	5 (36)	16 (73)
Median age (range)	48 (16-70)	49 (16-70)	45 (25-62)	47 (22-70)
# Events	16	5	2	0
VTE	10	4	1	
STP	6	1	1	
# Personyears	4711	4049	583	1041
Incidence rate all [§]	0.34	0.12	0.34	0
95%CI	0.2-0.5	0-0.2	0-0.8	0-0.4
Incidence rate VTE ^{§§}	0.21	0.10	0.17	0
95%CI	0.1-0.3	0-0.2	0-0.5	0-0.4

[§] Incidence rates % per years, including VTE and STP ^{§§} Incidence rates % per years, STP excluded
FVL= factor V Leiden, VTE= deep venous thromboembolism, STP= superficial thrombophlebitis

mined. A standardized history was taken on the past occurrence of deep venous thrombosis, pulmonary embolism and superficial thrombophlebitis, including the age at each episode, the method of diagnosis, treatment and risk-affecting circumstances. As risk-affecting circumstances we considered: age, sex, surgery, hospital admissions, immobilisation (any period exceeding two weeks), plaster casts, malignancies, oral contraceptives, pregnancies and post-partum periods as well as hormonal replacement therapy. We counted as venous thrombotic events (ie, deep venous thrombosis, pulmonary embolism and superficial thrombophlebitis) only those that were diagnosed by physicians (60% of deep venous thrombotic events were objectively diagnosed; 40% were not because the physicians were convinced by the presentation alone of the symptoms or because the events occurred before objective diagnostic techniques were available; all deep vein thrombotic events and pulmonary embolisms except one were treated with heparin or coumarines when available).

We contrasted these families with 12 thrombophilic families of symptomatic factor V Leiden carriers who were referred to our centre for diagnostic work-up for venous thrombophilia. These probands had a positive family history of venous thrombosis (in addition to the proband at least two symptomatic relatives) and did not have deficiencies of protein C, protein S or antithrombin. We tested 67 first-degree family members of these 12 probands on factor V Leiden and history of venous thrombosis. None of the probands or their relatives of the 12 thrombophilic families was related to or identical with one of the 56 probands from the case control study.

Laboratory Methods

For the detection of the factor V gene mutation, a 220-bp fragment was amplified of exon 10/intron 10 of the factor V gene, followed by digestion with the restriction-enzyme Mnl I, as described previously (10). Normalized APC-sensitivity ratios were measured as described by de Ronde (17). Relatives with a normalized APC-sensitivity ratio <0.84 were considered to be APC-resistant; carriers of factor V Leiden usually have a normalized APC-sensitivity ratio <0.71 (10). We measured normalized APC-sensitivity ratios in all relatives without coumarin treatment and found a clear association of factor V Leiden and an abnormal normalized APC-sensitivity ratio; all tested carriers (n = 116) had either a normalized APC-sensitivity ratio <0.71 (113/116; median normalized APC-sensitivity ratio: 0.56) or borderline results (3/116; normalized APC-sensitivity ratio between 0.70 and 0.84), while all tested non-carriers (n = 106) had either normal (98/106; median normalized APC-sensitivity ratio: 0.96) or borderline results (6/106, normalized APC-sensitivity ratio between 0.70 and 0.84). APC-resistance (normalized APC-sensitivity ratio < 0.71) was

detected in two non-carriers; they were related to the same asymptomatic proband with factor V Leiden. All individuals were screened for deficiencies of protein C, protein S and antithrombin, which were found in none. These laboratory measurements were done by one laboratory technician (H.R.) without any knowledge of the medical history.

Statistics

We constructed thrombosis-free survival curves by the Kaplan-Meier method and compared them by a logrank test (chi-square distribution, one degree of freedom).

Incidence rates in different groups were calculated by dividing the number of first events by the total of observation-years (follow-up time). Follow-up for symptomatic individuals started at birth and ended at the date of the first venous thrombosis. Follow-up for asymptomatic individuals started at birth and ended at the date of the interview.

With Poisson regression analysis we studied the effect of risk-affecting circumstances: age (seven ten-years age groups ranging from age 0-10 to age 61-70), sex, factor V Leiden carrier status, surgery (exposition window: one year per operation), immobilisation longer than two weeks (including hospital admissions and plaster casts; exposition window: one year), obesity (body mass index > 25 kg/m²), smoking (ever/never), pregnancy/post partum period (exposition window set at one year) and use of oral contraceptives (only for time periods lasting at least one month).

Results

Clinical Data

Of a total of 233 relatives of 56 probands, 197 were related to 47 symptomatic probands with factor V Leiden, and 36 to nine asymptomatic probands with factor V Leiden. The 197 relatives of symptomatic probands consisted of 111 women and 86 men. The overall median age was 49 years (range 16 to 70). Total follow up time was 8760 years and 21 individuals had experienced venous thromboembolism (median age of onset was 38 years, range 15 to 66 years). First events were deep vein thrombosis (n = 13), superficial thrombophlebitis (n = 7) and pulmonary embolism (n = 1). Including recurrences, 15 of the 21 patients had experienced deep venous thrombosis or pulmonary embolism. Of these 197 individuals, 108 (55%) carried the factor V Leiden mutation, 105 were heterozygous and three were homozygous (Table 1). The 21 patients came from 16 unrelated families and clustering of deep venous thrombotic events was clear in one family (the proband was homozygous and three of the four relatives had experienced a thrombotic event).

We included 36 relatives of nine asymptomatic heterozygous carriers, 21 women and 15 men, overall median age: 47 years (range 22 to 70). Total follow up time was 1624 years and two of these relatives had experienced a venous thromboembolism (at age 25 and 58). One relative had experienced a pulmonary embolism and the other a superficial thrombophlebitis. Both symptomatic relatives were carrier of factor V Leiden and experienced recurrences. Of the 36 relatives, 14 (39%) carried the factor V Leiden mutation (all were heterozygous).

Risk of Venous Thrombosis

For all 233 relatives of symptomatic and asymptomatic affected probands, the annual incidence rate in carriers was 0.34% (18 events on 5294 person-years, 95% CI 0.2-0.5%). The annual incidence rate in non-carriers was 0.10% (5 events on 5090 person-years, 95% CI 0.01-0.2%). Therefore, the overall risk ratio for venous thrombosis (deep

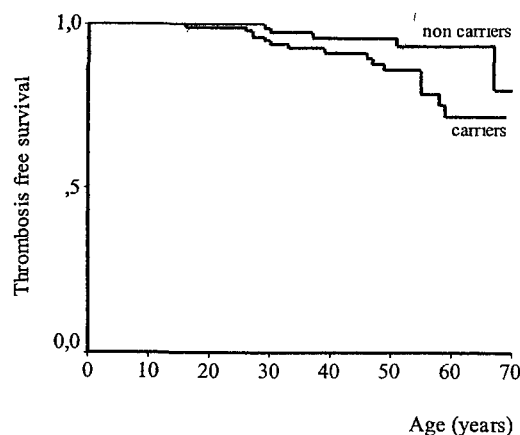


Fig. 1A Thrombosis free survival curves in carriers (lower line, probandi excluded) and non-carriers (upper line) among relatives of symptomatic probandi. In carriers, thrombosis-free survival was lower than in non-carriers ($p < 0.05$)

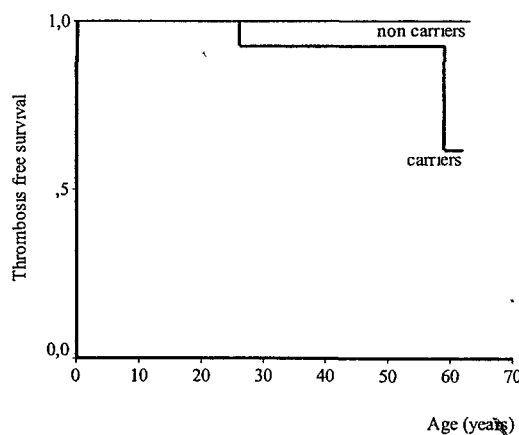


Fig. 1B Thrombosis free survival curves in carriers (lower line, probandi excluded) and non-carriers (upper line) among relatives of asymptomatic probandi

vein thrombosis and superficial thrombophlebitis) in carriers versus non-carriers was 3.4 (95% CI 1.3-9.1).

For relatives of symptomatic affected probands, the annual incidence rate in carriers was 0.34% (16 events on 4711 person-years, 95% CI 0.2-0.5%), which was 2.8-fold (95% CI 1.0-7.7) higher than the annual incidence rate of 0.12%/year among non-carriers in this group (5 events on 4049 person-years, 95% CI 0.01-0.23%) (Table 1).

For relatives of asymptomatic affected probands the annual incidence rate in carriers was also 0.34%/year (two events on 583 person-years, 95% CI 0-0.8%). None of the 22 non-carriers had experienced a venous thrombotic event (on 1041 person-years).

Fig. 1A shows the thrombosis-free survival for the 197 relatives of the 47 symptomatic probandi. At the age of 58 years, the probability to be still free of venous thrombosis was reduced to 75% for the 108 carriers, while 93% of the 89 non-carriers was still free of thrombosis at this age ($p = 0.03$). Overall relative risk of carriers versus non-carriers, as calculated by Cox proportional hazards model, was 2.9 (95% CI 1.1-7.9). Fig. 1B shows the thrombosis-free survival for the 36 relatives of the nine asymptomatic probands. Two of the 14 carriers had experienced a thrombotic event ($p = 0.06$).

Eight of the 16 symptomatic carriers and two of the five symptomatic non-carriers, related to the symptomatic probands, had experienced a recurrent event. Both symptomatic carriers related to asymptomatic probands had experienced a recurrence. None of the 23 symptomatic relatives were treated with coumarines longer than three months after the first thrombotic event; five of these 12 relatives with recurrences were treated with coumarines during three months after their first event (at least one year before the second event occurred).

The annual incidence rate of venous thrombosis in the 12 thrombophilic families was 1.7% in carriers of factor V Leiden, nearly six times (95% CI: 2.0-16.8) the incidence rate in non-carriers (0.3%). At the age of 62 years, the probability to be free of venous thrombosis was reduced to 50% for carriers and to 84% for non-carriers ($p < 0.001$).

Effect of Other Risk Factors

Among the thrombosis patients related to the symptomatic probands, precipitating risk factors during or shortly before the first event were present in 12 of the 16 factor V Leiden carriers (75%) and in three of the

five non-carriers (60%). Most important environmental risk factors were pregnancy (including puerperium) and surgery. When we regard all relatives ($n = 233$), 1.6% of all episodes of surgery, immobilisation and pregnancy were complicated by a venous thrombotic event in carriers while this was the case in 0.4% of the non-carriers.

In Table 2, the rate ratios are presented when the main risk factors were entered in the Poisson regression model. In this multivariate-adjusted model, the strongest risk factor was pregnancy (rate ratio 24.6) followed by surgery (rate ratio 16.6), immobilisation (rate ratio 8.4), use of oral contraceptives (rate ratio 4.9) and factor V Leiden (rate ratio 3.0). Neither smoking, nor overweight was associated with an increased risk of venous thrombosis. We explored interaction between these risk factors and factor V Leiden. By estimating incidences of venous thrombosis for different combinations of risk factors, we found a higher risk for the combination of factor V Leiden with immobilisation, surgery, pill use and pregnancy/puerperium (Table 3).

Discussion

To explore the role of selection in the assessment of thrombotic risk in factor V Leiden carriers, we performed a study on families of 47 symptomatic and nine asymptomatic unselected consecutive probandi

Table 2 Adjusted rate ratios (95% CI) for the main potential risk factors on venous thrombotic events for the 197 relatives

Potential risk factor	Rate ratio (95% CI)
Pregnancy and puerperium	24.7 (6.1 - 98.1)
Surgery	16.6 (5.8 - 47.7)
Pill use	4.9 (1.4 - 17.3)
Immobilisation	8.4 (1.8 - 39.5)
Factor V Leiden	3.0 (1.1 - 8.1)
Age	1.7 (1.2 - 2.3)

The rate ratios are the risks of thrombosis in the presence of the risk factor as compared to its absence, adjusted for the other factors in the model. All variables are coded as yes/no, age is coded in 10 years intervals

Table 3 Incidence rates of venous thrombosis in the absence or presence of environmental risk factors without and with factor V Leiden

Potential risk factors		Incidence	rate ratio
Surgery -	factor V Leiden -	0.1	1*
Surgery -	factor V Leiden +	0.3	3
Surgery +	factor V Leiden -	1.7	17
Surgery +	factor V Leiden +	2.9	29
Oral contraceptives [‡] -	factor V Leiden -	0.1	1*
Oral contraceptives -	factor V Leiden +	0.4	4
Oral contraceptives +	factor V Leiden -	0.3	3
Oral contraceptives +	factor V Leiden +	0.7	7
Pregnancy [§] -	factor V Leiden -	0.2	1*
Pregnancy -	factor V Leiden +	0.3	1.5
Pregnancy +	factor V Leiden -	0	-
Pregnancy +	factor V Leiden +	3.3	16.5
Immobilisation -	factor V Leiden -	0.1	1*
Immobilisation -	factor V Leiden +	0.3	3
Immobilisation +	factor V Leiden -	0	-
Immobilisation +	factor V Leiden +	2.4	24

Incidence rates are in %/year. # Reference category. [‡] Only women aged < 51 years included. [§] Pregnancy and puerperium.

with factor V Leiden. In the present study, the incidence of thrombosis was clearly higher among carriers of factor V Leiden (0.34%/year) than among non-carriers (0.10%/year). At age 58, the thrombosis-free survival was reduced to 75% in carriers and to 93% in non-carriers. Important environmental risk factors were pregnancy, surgery, immobilisation and use of oral contraceptives. The incidence of venous thrombosis among carriers of factor V Leiden was two times the estimated incidence rate in the Dutch population (approximately 0.11%/year, as reported by the National Medical Registration of Dutch Hospitals, excluding superficial thrombophlebitis) (18) while the incidence among non-carriers was very similar to this figure.

We recently demonstrated a higher thrombotic tendency as assessed by age of onset, in selected patients from 12 thrombophilic families with factor V Leiden than in unselected consecutive patients with a first venous thrombosis who carried the factor V Leiden mutation (16). The annual incidence rate of venous thrombosis in these thrombophilic families was 1.7% in carriers, about five times the annual incidence rate in factor V Leiden carrying relatives of unselected probands reported here (0.34%). These figures suggest the presence of additional defects in thrombophilic families, as was illustrated by the identification of the prothrombin 20210 A allele in two of these families (17%) (vs. two of the 47 families [4%] of unselected patients reported here, both families included one symptomatic relative), about two years after the onset of the study (19). The higher thrombotic risk we found in thrombophilic families is probably caused, although not highly plausible, by stochastic phenomena, ie, families with, by chance, many thromboses, become diagnosed as thrombophilic families, and subsequently appear at

high risk in retrospective. To settle the issue of prognosis, therefore, prospective studies are necessary, as are currently underway (20).

Despite the small number of symptomatic relatives with factor V Leiden of asymptomatic probands, the thrombotic tendency appeared to be similar to the thrombotic tendency in relatives of symptomatic probands (both 0.34%/year). Therefore, it seems that our hypothesis was confirmed, regarding the difference in thrombotic tendency in thrombophilic families and thrombotic tendency in relatives of unselected consecutive probands. However, we could not establish the expected difference in risk between relatives of symptomatic and those of asymptomatic probands. Such a difference has been suggested in protein C deficiency (Miletić et al.) (21) and antithrombin deficiency (Tait et al.) (22). Two explanations are possible: one, in the other studies, the comparison was (at least in part) between relatives from probands from thrombophilic families and of asymptomatic screenees, rather than between relatives of unselected patients and of screenees. Second, this difference in thrombotic tendency might be less pronounced in thrombophilia, caused by factor V Leiden than in the other forms of thrombophilia.

When we directly compare patients with healthy controls in the LETS case-control study, we find a seven-fold increased thrombotic risk associated with factor V Leiden (5). In the families of these probands (only patients with deep vein thrombosis in the lower extremities), we now found a slightly weaker association, with a relative risk of 2.8 (2.6 after exclusion of patients with superficial thrombophlebitis or pulmonary embolism, 95% CI 1.0-9.6). This may have several explanations, ie, the low number of symptomatic individuals in the present study and difference in study design (concurrent case-control study versus retrospective cohort study). At the time that patients were included in the case control study, it was unknown how many of their relatives had experienced a thrombotic event.

Our data revealed that 15% (16/108, Table 1) of factor V Leiden carriers experienced a venous thrombotic event and this was equal to the results Martinelli et al. found in carriers in families with factor V Leiden (30/200, despite the fact that they included also second-degree relatives) (23). After exclusion of superficial thrombophlebitis, we found a symptomatic fraction of 10% among factor V Leiden carriers (10/102, Table 1). This was very similar to the symptomatic fraction in factor V Leiden carriers, found in a retrospective family study by Middeldorp et al. (12%, 29/236) and a retrospective family study by Simioni et al. (8%, 17/224) (24, 25).

Part of our results is based on physician's diagnosis of thrombosis, which was common practice in the past. If this biased the results, it is likely that it would have led to an overestimate of the risk rather than an underestimate. For our previous study in thrombophilic families, we used the same methodology, and so this comparison is likely to be fair (16). To solve this problem, an analysis confined only to individuals with an objective diagnosis should have been done but for that, a larger sample size is required.

In conclusion, the thrombotic tendency in family members with factor V Leiden of unselected symptomatic carriers appears to be similar to the thrombotic tendency in family members with factor V Leiden of asymptomatic carriers of the factor V Leiden mutation. The thrombotic risk in thrombophilic families with factor V Leiden is twice as high as the thrombotic risk in families of unselected probands. So, the origin of family members of symptomatic carriers can be viewed as a separate risk factor for venous thrombosis and this urges to cautious interpretation of results derived from different study populations. In 70% of carriers, the occurrence of venous thrombosis events was associated with a precipitating condition (most common: pregnancy and surgery) and this

supports the conclusion of other studies that, considering its high prevalence, factor V Leiden in itself is a moderate cause of venous thrombosis. This justifies a policy of anticoagulant treatment only during exposure to these predisposing conditions. Finally, in clinical practice special attention should be paid to individuals with a positive family history of venous thromboembolism who are at risk.

Acknowledgements

This study was supported by a grant of the Netherlands Heart Association (grand no 95.026).

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Received March 22, 1999 Accepted after resubmission June 18, 1999