

# Co-segregation of thrombophilic disorders in factor V Leiden carriers; the contributions of factor VIII, factor XI, thrombin activatable fibrinolysis inhibitor and lipoprotein(a) to the absolute risk of venous thromboembolism

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**Background and Objectives.** The clinical expression of factor V Leiden varies widely within and between families and only a minority of carriers will ever develop venous thromboembolism. Co-segregation of thrombophilic disorders is a possible explanation. Our aim was to assess the contributions of high levels of factor VIII:C, factor XI:C, thrombin activatable fibrinolysis inhibitor (TAFI) and lipoprotein (a) (Lp(a)) to the risk of venous thromboembolism in factor V Leiden carriers.

**Design and Methods.** Levels of the four proteins were measured, in addition to tests of deficiencies for antithrombin, protein C and protein S, and the prothrombin G20210A mutation, in 153 factor V Leiden carriers, derived from a family cohort study. The (adjusted) relative risk and absolute risk of venous thromboembolism for high levels of each protein were calculated.

**Results.** Of carriers, 60% had one or more concomitant thrombophilic disorders. Crude odds ratios (95% CI) of venous thromboembolism for high protein levels were: 3.2 (1.1-9.3) (factor VIII:C); 1.7 (0.6-4.9) (factor XI:C); 3.0 (1.1-8.2) (TAFI); and 1.9 (0.7-5.7) (Lp(a)). Adjusted for age, sex, other concomitant thrombophilic disorders and exogenous risk factors, the odds ratio for venous thromboembolism were 2.7 (0.8-8.7) for high factor VIII:C levels and 1.8 (0.6-5.3) for high TAFI levels. Annual incidences in subgroups of carriers were 0.35% (0.09-0.89), 0.44% (0.05-1.57) and 0.94% (0.35-2.05) for concomitance of high levels of factor VIII:C, TAFI and both, respectively, as compared to 0.09% (0.00-0.48) in single factor V Leiden carriers and 1.11% (0.30-2.82) for other concomitant disorders.

**Interpretation and Conclusions.** High levels of factor VIII:C and TAFI, in contrast with factor XI:C and Lp(a), are mild risk factors for venous thromboembolism, and substantially contribute to the risk of venous thromboembolism in

## Thrombosis

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factor V Leiden carriers. Our data support the hypothesis that the clinical expression of factor V Leiden depends on co-segregation of thrombophilic disorders.

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Key words: factor VIII:C; factor XI:C; TAFI, lipoprotein (a); venous thrombosis.

Resistance to activated protein C, due to the factor V:Q506 mutation (factor V Leiden), is the most common known heritable thrombophilic defect.<sup>1</sup> It is found in approximately 5% of Caucasians and, depending on selection, in 20 to 50% of patients with venous thromboembolism.<sup>2,3</sup> Only 20 to 30% of factor V Leiden carriers will ever experience venous thromboembolism during their lifetime.<sup>4,5</sup> Moreover, there is a wide intra- and interfamilial variation in its clinical expression. These observations suggest that the occurrence of venous thromboembolism depends on the concomitance of factor V Leiden with exogenous risk factors or other genetic defects.<sup>6</sup> The contributions of inherited deficiencies of antithrombin, protein C and protein S will be limited because the chance of co-segregation is small, given their low prevalences.<sup>7-9</sup> The prothrombin G20210A mutation, elevated factor VIII activity (factor VIII:C) and mild hyperhomocysteinemia are more prevalent thrombophilic disorders. Previously we demonstrated that the risk of venous thromboembolism in factor V Leiden carriers increased only 1.3 fold if they also carried the prothrombin mutation. The risk was approximately 4-fold higher in homozygous factor V Leiden carriers and 17.5-fold higher if inherited protein C or S defi-

ciency was the concomitant thrombophilic disorder. However, only 20% of symptomatic factor V Leiden carriers showed co-segregation with these thrombophilic disorders.<sup>10</sup>

More recently, high levels of factor XI activity (factor XI:C) and thrombin activatable fibrinolysis inhibitor (TAFI) have been identified as risk factors for venous thromboembolism.<sup>11,12</sup> Elevated levels of lipoprotein a (Lp(a)) may also be involved in the pathogenesis of venous thromboembolism, due to its homology to plasminogen.<sup>13,15</sup>

In the present study we assessed the contributions of factor VIII:C, factor XI:C, TAFI and Lp(a) to the risk of venous thromboembolism in factor V Leiden carriers.

### Design and Methods

In a previous family cohort study, designed to estimate the absolute risk of venous thrombosis in factor V Leiden carriers, we enrolled consecutive patients with venous thromboembolism and factor V Leiden (proband) and their first degree relatives older than 15 years.<sup>4</sup> Proband was referred to the out-patient clinics of the three participating university hospitals. Their living first degree relatives were identified through pedigree analysis. After informed consent had been obtained, each relative was interviewed by one of the investigators using a standardized and validated medical history form.<sup>16</sup> Detailed information was collected about previous episodes of venous thromboembolism, surgical interventions, trauma, periods of immobilization and prophylactic or therapeutic use of anticoagulant drugs. For women, the use of oral contraceptives and obstetric history were also documented. At the end of the outpatient visit a blood sample was taken for DNA testing of factor V Leiden.

In the present study, in a single center we performed additional tests on stored plasma and DNA in 153 of the previously enrolled 269 relatives, who carried factor V Leiden. The remaining 116 carrier relatives had to be excluded, because of insufficient amounts of stored plasma. Comparing included and excluded carriers, there were no differences in their clinical characteristics or the prevalence of venous thromboembolism (11.1% versus 10.3%,  $p=1.00$ ), suggesting absence of a selection bias. Carriers were additionally tested for the prothrombin mutation and inherited deficiencies of antithrombin, protein C, and protein S, and plasma levels of factor VIII:C, factor XI:C, TAFI, and Lp(a) were measured. The study was approved by the institutional review board of the hospital and all participants gave informed consent.

### Definitions

A previous episode of deep vein thrombosis or pulmonary embolism was considered to have occurred if confirmed by compression ultrasound, venography, ventilation/perfusion lung scanning, or pulmonary angiography, or if the patient had received full dose heparin and oral anticoagulants for at least 3 months without objective testing at a time when these techniques were not yet available. For this classification the patients' charts were reviewed. Venous thromboembolism was classified secondary if it had occurred within 3 months after exposure to one or more exogenous risk factors including surgery, trauma, immobilization for more than 7 days, oral contraceptive use, pregnancy or malignancy. Venous thromboembolism that occurred in the absence of any exogenous risk factor was considered spontaneous.

### Laboratory studies

Factor V Leiden and the prothrombin mutation were demonstrated by polymerase chain reaction, as described previously.<sup>3,17</sup> Protein C and total protein S antigen levels were measured by ELISA (reagents obtained from DAKO, Glostrup, Denmark), protein C activity (*Berichrom* Protein C, Behring, Marburg, Germany) and antithrombin activity (Coatest<sup>TM</sup>, Chromogenix AB, Mölndal, Sweden) by chromogenic substrate assays.

Inherited deficiencies of antithrombin, protein C or protein S were defined as a plasma level below the lower limit of the normal range in 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. Factor VIII:C and factor XI:C were measured by one-stage clotting assays. TAFI activity was determined with the substrate hippuryl-L-arginine, using HPLC-assisted measurement of the released hippuric acid as described previously.<sup>18</sup> Lp(a) was measured by ELISA (TintElize, Biopool International, US, Denver, Colorado, USA). Levels of factor XI:C, TAFI and Lp(a) above the 75<sup>th</sup> percentile of their distribution in factor V Leiden carrier-relatives were defined as high. As in other studies, we used the upper limit of its normal range, i.e. 150%, as the cut-off point for high levels of factor VIII:C, to enable a comparison of results between our and other studies. Antithrombin, protein C, protein S, factor VIII:C, factor XI:C and TAFI were expressed as percentages of the levels measured in pooled normal plasma set at 100%.

### Statistics

The risk of venous thromboembolism was estimated for FVIII:C, FXI:C, TAFI and Lp(a) by univariate analysis. We assessed the influence of age, sex and hormonal state, i.e. premenopause, oral contraceptive use and pregnancy on their plasma levels. The relative risk of venous thromboembolism in subgroups with combined disorders, as compared with in factor V Leiden carriers without concomitant disorders, was calculated from annual incidences. The annual incidences were calculated by dividing the number of persons with venous thromboembolism and the total number of observation years in each subgroup. Observation years were defined as the period from the age of 15 years until the first episode of venous thromboembolism, or until the date of study entry in asymptomatic carriers, considering that venous thrombosis is rarely found before the age of 15 years. Hazard ratios of thrombophilic disorders were calculated using a multivariate Cox proportional hazard model, adjusted for age and sex.

Crude odds ratios were calculated by simple cross tabulation. Continuous variables were analyzed by the Mann-Whitney-U-test and presented as median values and their ranges. Categorical variables were analyzed by Fisher's exact test or the  $\chi^2$  test, as appropriate. A two-tailed *p* value of less than 0.05 was considered to indicate statistical significance. Analyses were performed using SAS software, version 6.12 (SAS-Institute Inc., Cary, North Carolina, USA).

### Results

The characteristics of 153 factor V Leiden carriers in this study are summarized in Table 1. Seventeen carriers (11%) had a history of venous thromboembolism, which occurred at a median age of 33 years (range 17-63). Exposure to exogenous risk factors was similarly distributed among symptomatic and asymptomatic carriers. Venous thromboembolism was classified as spontaneous in 41% of cases. Symptomatic carriers had higher median plasma levels of factor VIII:C, factor XI:C, TAFI and Lp(a) than did asymptomatic carriers. Only differences in levels of factor VIII:C and TAFI were statistically significant (Table 1).

Odds ratios (95% CI) for venous thromboembolism were calculated by comparing carriers with FVIII:C levels  $\geq 150\%$  and  $< 150\%$ , respectively. For FXI:C, TAFI and Lp(a), high levels were defined as values above the 75<sup>th</sup> percentile measured in carriers. Odds ratios were 3.2 (1.1-9.3) in carriers with FVIII:C levels  $\geq 150\%$ ; 1.7 (0.6-4.9) for FXI:C levels

**Table 1. Characteristics of 153 factor V Leiden carriers.**

	VTE (17)	No VTE (136)	<i>p</i>
Women (%)	11 (65)	68 (50)	0.3
Median age at study entry, yr (range)	54 (19-80)	38 (15-81)	0.006
Exposure to exogenous risk factors			
Oral contraception (% women)	5 (45)	47 (69)	0.17
Pregnancy (% women)	10 (91)	41 (60)	0.09
Pregnancies, n	27	127	0.4
Surgery, trauma, immobilization (%)	15 (99)	95 (70)	0.15
First episode of venous thromboembolism			
Median age at onset, yr (range)	33 (17-63)		
Spontaneous (%)	7 (41)		
Secondary to			
Oral contraception (% women)	4 (36)		
Pregnancy (% women)	3 (27)		
Surgery, trauma, immobilization (%)	3 (27)		
Median plasma levels (range)			
FVIII:C, %	166 (47-300)	139 (62-318)	0.04
FXI:C, %	102 (80-152)	98.5 (65-186)	0.12
TAFI, %	115 (87-146)	103 (77-193)	0.009
Lp(a), mg/L	72.5 (6-1321)	58(1-947)	0.20

VTE denotes venous thromboembolism.

$\geq 111\%$ ; 3.0 (1.1-8.2) for TAFI levels  $\geq 116\%$ , and 1.9 (0.7-5.7) for Lp(a) levels  $\geq 216$  mg/L.

Levels of FVIII:C ( $p < 0.001$ ), TAFI ( $p = 0.02$ ), FXI:C ( $p = 0.001$ ) and Lp(a) ( $p = 0.06$ ) increased with age (*data not shown*). There were no differences between premenopausal women and men younger than 50 years of age, and between postmenopausal women and men of the same age. Women on oral contraceptives ( $n = 25$ ) had higher levels of FXI:C (median 102% versus 94%,  $p = 0.03$ ) and TAFI (111% versus 101%,  $p = 0.02$ ) than non-users younger than 50 years of age ( $n = 20$ ), whereas observed differences in levels of FVIII:C (120% versus 146%,  $p = 0.13$ ) and Lp(a) (median 72 mg/L versus 50 mg/L,  $p = 0.40$ ) were not significant.

Factor V Leiden carriers were divided into subgroups in accordance with concomitance of the thrombophilic disorders that were identified as risk factors for venous thromboembolism (Table 2).

Group 1 ( $n = 62$ ) contained carriers without a concomitant disorder; group 2 ( $n = 41$ ), carriers with FVIII:C levels  $\geq 150\%$ ; group 3 ( $n = 17$ ), carriers with TAFI levels  $\geq 116\%$ ; and group 4 ( $n = 20$ ), carriers with levels of FVIII:C  $\geq 150\%$  and TAFI  $\geq 116\%$ . The remaining factor V Leiden carriers (group 5,  $n = 13$ ) had any of the following less frequently found concomitant disorders: the prothrombin gene mutation ( $n = 3$ ); the prothrombin gene mutation and a

**Table 2. Frequency of a first episode of venous thromboembolism in 153 factor V Leiden carriers related with concomitance of high levels of factor VIII:C and/or TAFI, and other combinations of thrombophilic disorders.**

	Group 1 FVL only (n=62)	Group 2 FVL+FVIII (n=41)	Group 3 FVL+TAFI (n=17)	Group 4 FVL+FVIII + TAFI (n=20)	Group 5 Other comb. (n=13)
Patients with VTE, (n)	1	4	2	6	4
Observation period, (yr)	1150	1149	459	635	360
Annual incidence (95%CI)	0.09 (0.00-0.48)	0.35 (0.09-0.89)	0.44 (0.05-1.57)	0.94 (0.35-2.05)	1.11 (0.30-2.82)
Relative risk (95% CI)	1.0 (reference)	4.0 (0.5-30.4)	5.0 (0.6-43.6)	10.9 (2.0-59.1)	12.8 (2.3-70.1)

VTE, venous thromboembolism; FVL, heterozygous factor V Leiden; FVIII, factor VIII:C  $\geq 150\%$ ; TAFI,  $\geq 116\%$ ; other combinations included the prothrombin gene mutation, homozygous factor V Leiden, and protein S deficiency.

TAFI level  $\geq 116\%$  (n=1); the prothrombin gene mutation and a FVIII:C level  $\geq 150\%$  (n=4); the prothrombin gene mutation, a TAFI level  $\geq 116\%$  and a FVIII:C level  $\geq 150\%$  (n=1); protein S deficiency (n=1); homozygous factor V Leiden (n=2); and homozygous factor V Leiden and a FVIII:C level  $\geq 150\%$  (n=1).

The annual incidence of venous thromboembolism was 0.09 in group 1; 0.44 in group 2; 0.35 in group 3; and 0.94 in group 4 (Table 2). In group 5 it was 1.11. The additional risks of separate concomitant disorders are presented in Table 3. Because interactions between exogenous risk factors and thrombophilic disorders were not demonstrated, the former were considered as a composite variable in the multivariate model. Adjusted hazard ratios were 2.7 (0.8-8.7) for FVIII:C  $\geq 150\%$ ; 0.8 (0.3-2.5) for FXI:C  $\geq 111\%$ ; 1.8 (0.6-5.3) for TAFI  $\geq 116\%$ ; 1.3 (0.4-3.8) for Lp(a)  $\geq 216$  mg/L; and 1.5 (0.3-7.6) for exposure to any of the mentioned exogenous risk factors.

## Discussion

We found that high levels of factor VIII:C ( $\geq 150\%$ ) or TAFI ( $\geq 116\%$ , the 75<sup>th</sup> percentile of the distribution of values in factor V Leiden carrier-relatives) were associated with an increased risk of venous thromboembolism in factor V Leiden carriers. High levels of factor XI:C ( $\geq 111\%$ , the 75<sup>th</sup> percentile) and Lp(a) ( $\geq 216$  mg/L, the 75<sup>th</sup> percentile) were not identified as risk factors. Using the cur-

**Table 3. The influence of concomitant thrombophilic disorders and exogenous factors on the risk of venous thromboembolism in 153 factor V Leiden carriers.**

Concomitant risk factors	Crude OR (95% C.I.)	Hazard ratio (95% C.I.)*
FVIII:C $\geq 150\%$	3.2 (1.1-9.3)	2.7 (0.8-8.7)
FXI:C $\geq 111\%$	1.7 (0.6-4.9)	0.8 (0.3-2.5)
TAFI $\geq 116\%$	3.0 (1.1-8.2)	1.8 (0.6-5.3)
Lp(a) $\geq 216$ mg/L	1.9 (0.7-5.7)	1.3 (0.4-3.8)
Any exogenous factor <sup>#</sup>	3.1 (0.7-13.3)	1.5 (0.3-7.6)

OR denotes odds ratio. \*Also adjusted for age, sex and other concomitant thrombophilic disorders and exogenous risk factors. <sup>#</sup>Ever exposed versus never exposed to risk factors, including surgery, trauma, or immobilization, oral contraceptive use and pregnancy.

rent definitions, one or more concomitant thrombophilic disorders were demonstrated in approximately 60% of heterozygous factor V Leiden carriers. These were high levels of factor VIII:C and TAFI, deficiencies of antithrombin, protein C and protein S, the prothrombin G20210A mutation and homozygosity for factor V Leiden. Venous thromboembolism occurred in 17.6% of carriers with concomitant disorders, compared with in 1.6% of single factor V Leiden carriers ( $p=0.001$ ). Our data support the hypothesis that the wide spectrum of clinical expression of factor V Leiden depends on clustering of thrombophilic disorders.

The estimated risk of venous thromboembolism at high levels of factor VIII:C and TAFI, respectively, is in line with the results of previous studies. A recent study in unselected families showed an absolute annual risk (0.27%) in factor V Leiden carriers who also had factor VIII:C levels  $\geq 150\%$ , corresponding with the here reported risk (0.35%).<sup>19</sup> The relative risk of venous thromboembolism associated with high levels of TAFI (3.0, 1.1-8.2) is comparable with the finding of van Tilburg *et al.*, but they did not demonstrate an additional risk of high TAFI levels in factor V Leiden carriers.<sup>12</sup>

Although high levels of factor XI:C were associated with an increased risk of venous thromboembolism, comparable to the relative risk reported from a recent case-control study,<sup>11</sup> we did not demonstrate statistical significance (odds ratio 1.7, 95% CI 0.6-4.9). This might be explained by the

smaller size of our study group.

High Lp(a) levels were not associated with an increased risk of venous thromboembolism (odds ratio 1.93, 95% CI 0.7-5.7). In a previous analysis of a larger number (392) of factor V Leiden carriers and non-carriers, that included the present study population, we found no differences in Lp(a) levels comparing subjects with (median 80 mg/L) and those without (69 mg/L) venous thromboembolism ( $p=0.24$ ).<sup>20</sup> Although these findings are in agreement with the results of two case-control studies,<sup>21,22</sup> two other studies demonstrated an increased risk of venous thromboembolism at Lp(a) levels >300 mg/L.<sup>14,15</sup>

To estimate the contributions of high levels of factor VIII:C, factor XI:C, TAFI and Lp(a), separately, relative risks were adjusted for age, sex, other concomitant disorders and exposure to exogenous risk factors. Adjusted hazard ratios, though not statistically significant, suggested a 2.7 and 1.8-fold higher risk in carriers who had high levels of factor VIII:C and TAFI, respectively. High levels of factor XI:C and Lp(a) did not influence the risk of venous thromboembolism in carriers of factor V Leiden.

The clinical implications of concomitant risk factors depend on the absolute rather than the relative risk of venous thromboembolism. The absolute annual risk, as we have reported from the original family cohort study, was 0.45% in relatives who carried factor V Leiden, as compared with 0.10% in non-carrier relatives.<sup>4</sup> The here presented results show annual incidences in carriers ranging from 0.35% to 1.11% for various concomitant disorders. Single factor V Leiden carriers will consequently have a lower absolute risk. The annual incidence in this subgroup which contained 40% carriers, was 0.09% and hence comparable to the absolute annual risk in non-carriers<sup>4</sup> and to the risk in the general population.<sup>23</sup> This finding may explain why many factor V Leiden carriers will never develop venous thromboembolism. It emphasizes the need for risk stratification and, accordingly, recommendations concerning prophylaxis and treatment of venous thromboembolism in factor V Leiden carriers.

Our study has obvious limitations. First, we were able to analyze only some of the factor V Leiden carriers who enrolled in the original study, but overt selection bias seems unlikely. Second, as non-carriers were excluded from additional testing, we missed the opportunity to compare the distribution of concomitant thrombophilic disorders in carriers and non-carriers and to assess clustering in non-carriers. Considering the low absolute annual risk of venous thromboembolism in single factor V

Leiden carriers, and the high prevalences and relative risks of high factor VIII:C and TAFI levels, the question is raised as to whether the latter disorders are less frequently found in non-carriers.

It will be clear that the presented results must be interpreted cautiously. However, they support the supposed co-segregation and interactions of an increasing number of prevalent, mild risk factors in symptomatic factor V Leiden carriers. Further studies are warranted to establish our findings. Extensive testing of concomitant disorders in carriers will become important in clinical practice if it enables us to identify carriers who are at either low or high risk of venous thromboembolism.

In conclusion, high levels of factor VIII:C and TAFI are mild risk factors for venous thromboembolism that are frequently found in factor V Leiden carriers and substantially contribute to their risk of venous thromboembolism. This could not be demonstrated for high levels of factor XI:C and Lp(a).

#### *Contributions and Acknowledgments*

*EJL drafted the article. KH, HB and JvdM conceived and designed the study. CPB-V and MHP performed the statistical analyses. EJL, IB and JRM collected and validated all data. SM, MMWK and ECM vP critically revised the manuscript. All authors read and approved the final version to be published. EJL: responsible for the publication and all tables and figures.*

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#### *Disclosures*

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

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## PEER REVIEW OUTCOMES

### *Manuscript processing*

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Vicente and the Editors. Manuscript received May 29, 2002; accepted August 1, 2002.

### *What is already known on this topic*

There are a wide intra- and interfamilial variations in clinical expression in factor V Leiden carriers.

### *What this study adds*

This study shows that high plasma levels of factor VIII:C and TAFI contribute to the risk of venous thromboembolism in factor V Leiden carriers.

### *Potential implications for clinical practice*

These results support the belief that the clinical expression of factor V Leiden depends on co-segregation of other thrombophilic anomalies.

*Vicente Vicente, Deputy Editor (Murcia, Spain)*