

Factor V Leiden Is not a Risk Factor for Arterial Vascular Disease in the Elderly: Results from the Cardiovascular Health Study

Mary Cushman¹, Frits R. Rosendaal², Bruce M. Psaty³, E. Francis Cook⁴, J. Valliere⁵, Lewis H. Kuller⁶, Russell P. Tracy⁵

From the ¹University of Vermont, Departments of Medicine and Pathology, Burlington, VT, USA;

²University Hospital, Departments of Clinical Epidemiology and Hematology, Leiden, The Netherlands;

³University of Washington, Departments of Medicine, Epidemiology, and Health Services, Seattle, WA, USA;

⁴Harvard University, Departments of Medicine and Preventive Health, Boston, MA, USA;

⁵University of Vermont, Department of Pathology, Burlington, VT, USA;

⁶University of Pittsburgh, Department of Epidemiology, Pittsburgh, PA, USA

Summary

Coagulation factor V Leiden is a risk marker for venous thrombosis. For arterial thrombosis no large study to date has included population-based elderly patients. The Cardiovascular Health Study is a longitudinal study of 5,201 men and women over age 65. With 3.4-year follow-up, we studied 373 incident cases of myocardial infarction (MI), angina, stroke, or transient ischemic attack (TIA), and 482 controls. The odds ratios for each event with heterozygous factor V Leiden were: MI, 0.46 (95% CI 0.17 to 1.25); angina, 1.0 (95% CI 0.45 to 2.23); stroke, 0.77 (95% CI 0.35 to 1.70); TIA, 1.33 (95% CI 0.5 to 3.55); any outcome, 0.83 (95% CI 0.48 to 1.44). Adjustment for cardiovascular risk factors did not change relationships. In older adults factor V Leiden is not a risk factor for future arterial thrombosis.

Introduction

Activated protein C resistance is a risk factor for venous thrombosis (1-3), and is usually inherited as heterozygous factor V Leiden (4). Factor V Leiden is characterized by a point mutation in the coagulation factor V gene, causing arginine 506 → glutamine substitution (4), rendering an activated factor V molecule that is relatively resistant to inactivation by activated protein C (5). The mutation is associated with a 3-7 fold increased risk of venous thrombosis, and the prevalence of heterozygotes is 3-7% (1, 6).

A relationship between factor V Leiden and risk of cardiovascular disease may be hypothesized. About 20% of factor V is found in platelets (7), and platelets are intimately involved in pathogenesis of atherosclerotic lesions. While normal and mutant platelet and plasma factor Va are completely inactivated by activated protein C, in both cases the mutant is inactivated more slowly (5, 8). So, part of the procoagulant effect of platelets may be related to residual factor Va, which is present for more time in the mutant.

While most studies have found no relationship between factor V Leiden and arterial thrombosis (9), in a recent study the mutation predicted myocardial infarction (MI) in young women with other cardiovascular risk factors (10). While these findings are of great interest, the majority of deaths from cardiovascular disease are in

those over age 65 (11), and this group is underrepresented in the existing literature.

Since factor V is a key procoagulant, and thrombin activity increases with age (12), we predicted a relationship between the mutation and cardiovascular events in older persons. Since subclinical cardiovascular disease predicts clinical cardiovascular events in the elderly (13), factor V Leiden might be especially relevant in persons with subclinical disease. To address these hypotheses, we report a nested case-control study of the risk of incident MI, angina, stroke, and transient ischemic attack (TIA) related to factor V Leiden over 3.4 years follow-up, in men and women 65 years of age and older.

Methods

Setting

The Cardiovascular Health Study is a longitudinal, population-based study of 5,201 men and women 65 years of age and older at enrollment (14). Subjects were recruited from random samples of Medicare eligibility lists in 1989-90 at four field centers: Forsyth County, North Carolina; Washington County, Maryland; Sacramento County, California; and Pittsburgh, Pennsylvania. Informed consent was obtained with protocols approved by institutional review committees at each center. The baseline examination consisted of interview, physical examination, assessment of medication use, phlebotomy and blood tests, and assessment of clinical and subclinical vascular disease status (14, 15).

Ascertainment of Events

Patients were evaluated twice annually, alternating between a clinic visit and a telephone call. They were questioned about new diagnoses, hospitalizations, and procedures. There were four endpoints studied: angina, MI, ischemic stroke, and TIA. Events were adjudicated by committee using published criteria (16). Incident angina was the development of probable or definite angina during follow-up, in participants free of baseline history of angina, MI, coronary artery bypass surgery or coronary angioplasty. Incident MI was the development of probable or definite MI or definite fatal coronary heart disease, in participants free of baseline history of MI. Incident stroke and TIA were the development of probable or definite stroke or TIA in participants free of baseline history of stroke or TIA.

Two controls for each MI and angina case were selected, matched on field center and length of follow-up. The MI controls were free of baseline or incident MI. Angina controls were free of baseline or incident angina, MI, coronary artery bypass surgery, and coronary angioplasty. Since the decision to study cerebrovascular events was made after initial case-control selection, the combined control group for angina and MI cases (with exclusion of those with baseline stroke or TIA) was used as the control group for stroke and TIA cases.

Correspondence to: Dr. Russell P. Tracy, Laboratory for Clinical Biochemistry Research, Department of Pathology, University of Vermont, 55A South Park Drive, Colchester, VT 05446, USA - Tel.: +1 802 656 8961; FAX Number: +1 802 656 8965; E-mail: rtracy@salus.uvm.edu

Table 1 Baseline characteristics among cases and controls

Variable, mean (SD) or frequency	Angina (n=140)	MI (n=149)	Stroke (n=159)	TIA (n=57)	Any CVD (n=373)†	All Controls (n=482)
Age, years	72.8 (5.3)	74.3 (5.8)	75.6 (5.9)	74.6 (5.8)	74.2 (5.8)	72.3 (5.8)
Gender, male	55%	62%	48%	44%	52%	37%
Hypertension, %	50%	50%	60%	51%	51%	34%
Diabetes, %	24%	30%	39%	19%	28%	16%
Smoking, % ever	58%	61%	45%	58%	54%	50%
Overweight, %	26%	26%	32%	19%	26%	29%
Cholesterol, mmol/L	5.78 (1.14)	5.44 (0.98)	5.62 (0.91)	5.72 (1.01)	5.62 (1.04)	5.72 (1.04)
Fibrinogen, g/L	3.21 (0.7)	3.25 (0.59)	3.29 (0.79)	3.32 (0.89)	3.22 (0.67)	3.18 (0.68)
Baseline MI, %	0	0	10%	9%	0	0
Baseline Angina, %	0	9%	15%	25%	0	0
Baseline Stroke, %	3%	3%	0	0	0	0
Baseline TIA, %	2%	4%	0	0	0	0

MI, myocardial infarction; TIA, transient ischemic attack; CVD, cardiovascular disease

† The sample size of the any CVD group is less than the sum of the four case groups since some subjects had more than one event type, and those with any type of baseline CVD (MI, angina, stroke, TIA) were excluded

Definition of Variables

Hypertension was systolic blood pressure ≥ 160 mmHg or diastolic pressure ≥ 95 mmHg, or self-reported hypertension and use of anti-hypertensive medications. Diabetes was categorized as none, impaired glucose tolerance, or diabetes. Obesity was body-mass index greater than 27.3 in women and 29.6 in men (over 130% ideal body mass). Smoking was categorized as ever or never use, since there were few current smokers. Subclinical cardiovascular disease was defined using a modification of previously published criteria (13). It was classified as the presence of any one of the following: the mean of multiple measurements of maximal intimal-medial thickness of the internal or common carotid artery (17) over the 80th percentile or stenosis greater than 25%, ankle-brachial index less than 0.9, presence of major electrocardiogram abnormalities (18), or left ventricular wall motion abnormality or low ejection fraction by echocardiogram (19).

Laboratory Methods

The DNA samples were obtained at baseline or the year three exam if no baseline DNA was available. The DNA was prepared from peripheral blood leukocytes using a modification of the salt precipitation method (20). There were 14 cases without available DNA, and they and their matched controls were excluded. There were 10 controls without a DNA sample and they were replaced with new controls using the same selection criteria. Factor V Leiden status was determined as previously reported (4). Analysis and data entry were completed by one technician, blind to case-control status.

Statistical Analysis

The Statistical Package for Social Sciences was used for data analysis with EGRET used for conditional logistic regression. The Cardiovascular Health Study database used was an updated database with minor corrections made through September, 1995. Means and proportions for baseline characteristics were computed for each case and control group. The measure of association between factor V Leiden and incident cardiovascular events was the odds ratio (OR), to represent relative risk (RR). For MI and angina, conditional logistic regression was used to compute OR with 95% CI. For case groups without matched controls (stroke, TIA) unconditional logistic regression was used. Risk estimates were adjusted for cardiovascular risk factors by multivariable logistic regression, with independent variables simultaneously entered into the models. In crude analysis, using the observed prevalence of the mutation in the control

group, the power of the study was 85% to detect a 2-fold increased risk of any cardiovascular event and 58% to detect a 50% reduced risk. To explore combinations of factor V Leiden and presence of other cardiovascular risk factors, the four case groups were combined, excluding participants with a baseline history of any of the four endpoints. Significance of any difference in ORs with stratification was determined using interaction terms in multivariable models.

Results

With median follow-up of 3.4 years (17,839 person-years), 149 MI, 140 angina, 159 stroke, and 57 TIA cases occurred. There were 961 individuals studied, with 63/961 (6.6%) factor V Leiden heterozygotes (95% CI 5.0% to 8.2%), and no homozygotes. Of 34 non-Caucasians

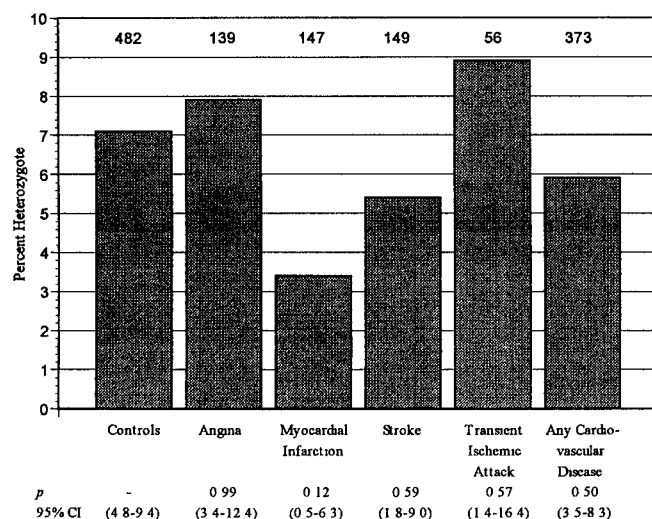


Fig. 1 Prevalence of factor V Leiden heterozygotes in cases and controls. Numbers across the top indicate the number of patients in each group. The P values are for comparison of each case group with the control group by Chi-square analysis. Confidence intervals are for the proportion of heterozygotes in each group. CI, confidence interval

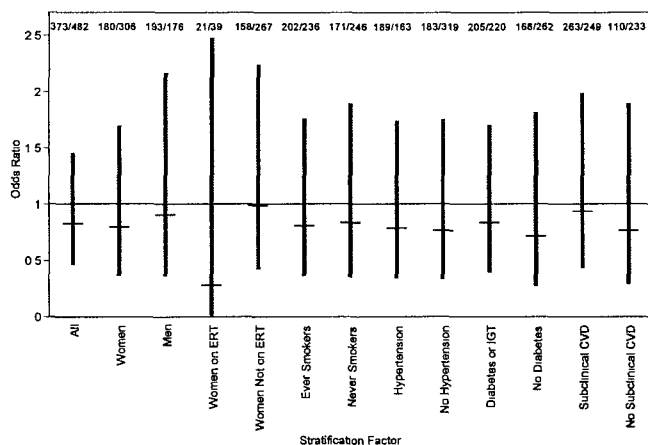


Fig. 2 Effect of cardiovascular risk factors on the relationship between factor V genotype and incident cardiovascular disease. Risks were calculated after stratification by risk factors shown. Vertical bars represent 95% confidence intervals. Numbers across the top indicate the number of cases and controls for each subgroup (case/control). ERT, estrogen replacement therapy; IGT, impaired glucose tolerance; CVD, cardiovascular disease

(31 African-American; 16 cases, 18 controls), there were no heterozygotes.

Baseline characteristics of each case group and all controls are shown in Table 1. Cases were more likely than controls to be male, have hypertension, and have diabetes. For the 373 individuals developing any study event, 263/373 (71%) had subclinical cardiovascular disease at baseline, while for controls 249/482 (52%) had subclinical disease.

All case groups except TIA had a similar or lower prevalence of factor V Leiden heterozygotes compared to the control group (Fig. 1). MI cases had the lowest prevalence of the mutation, 3.4%, and there were 5.4% heterozygotes in the stroke patients. In the control group there were 7.1% heterozygotes, while the prevalence was 8.9% and 7.9% in TIA and angina patients respectively. Crude and adjusted RR for each outcome are shown in Table 2. Estimates of RR were all 1.0 or less, except for the TIA group, with an RR of 1.33. A nonsignificant protective effect of the mutation on risk of MI and stroke was suggested. Combining all study groups, excluding patients with any type of baseline clinical cardiovascular disease, in 373 cases and 482 controls the RR was 0.83 (0.48-1.44). Adjustment for cardiovascular risk factors did not change any of the relationships observed (Table 2). Since factor V Leiden is rare in non-Caucasians (21), we excluded them, resulting in an RR for any outcome of 0.82 (95% CI 0.47 to 1.43).

Effects of combination of factor V Leiden with other risk factors are shown in Fig 2. For all the risk factors, gender, estrogen replacement use, smoking, hypertension, diabetes, and subclinical disease, no subgroup had increased risk of disease related to the mutation. For the 569 individuals possessing any single risk factor (current smoking, diabetes, hypertension, or LDL-C over 4.14 mmol/l) there was also no increased risk related to the mutation (RR, 0.78, 95% CI 0.41-1.49).

Discussion

In this population-based study of Americans over age 65, the factor V Leiden mutation was not a risk marker for first MI, angina, stroke, or TIA after 3.4 years of follow-up. Results agree with the majority of other studies concerning middle-aged persons, and extend previous findings by including older patients. Results also illustrate differences in mechanisms of venous and arterial thrombosis, suggesting less importance of factor Va inactivation in plaque development, rupture and thrombotic response.

The observed nonsignificant protective effect of the mutation on risk of MI and stroke merits cautious interpretation since statistical power for this analysis was insufficient, and a biological explanation is not plausible.

A weak relationship between factor V Leiden and TIA cannot be excluded; we observed a nonsignificant 30% increased risk in heterozygotes. Three other studies have suggested that activated protein C resistance predicts TIA, and in 2 studies this was independent of factor V genotype (22-24). The results suggest that the activated protein C ratio measures other elements of hemostasis with pathogenic importance.

The hypothesis that factor V Leiden might be a better predictor of cardiovascular disease in the elderly because of increased subclinical disease was not proven true. Our sample size was too small to exclude a modest interaction, and since subclinical disease is a risk factor for later clinical events (13), this question remains important.

One study reported increased risk of MI with factor V Leiden in young female smokers (10), while a smoking effect was not observed in the Physicians' Health Study (6) or our study (both studies having few current smokers). The association in young women may be related to interaction between genotype, endogenous estrogen and smoking (10). Additional studies of the effect of smoking with factor V Leiden are needed.

Our study illustrates an important point related to study of genetic risk markers; confounding by other risk factors was not apparent. There is little possibility of confounding by other risk factors in the relationship between a genotype and event. Confounding can only exist if the confounder is genetically linked to the mutation being studied.

Cardiovascular Disease During Follow-up						
	None (n=576)	Angina (139 cases, 278 controls)	MI (147 cases, 294 controls)	Stroke (149 cases, 495 controls)	TIA (56 cases, 495 controls)	Any CVD (373 cases, 482 controls)
RR (95% CI)	1.0 (Reference)	1.0 (0.45-2.23)	0.46 (0.17-1.25)	0.77 (0.35-1.70)	1.33 (0.50-3.55)	0.83 (0.48-1.44)
RR _{adj} * (95% CI)	1.0 (Reference)	0.88 (0.38-2.04)	0.42 (0.14-1.28)	0.76 (0.32-1.81)	1.30 (0.46-3.49)	0.86 (0.48-1.54)

MI, myocardial infarction; TIA, transient ischemic attack; CVD, cardiovascular disease

* Adjusted for age, gender, race, smoking status, systolic blood pressure, diabetes, cholesterol, and body-mass index

Table 2 Risk of incident cardiovascular disease in factor V Leiden heterozygotes after 3.4 years of follow-up

Our results argue against a relationship between factor V Leiden and cardiovascular events in persons over age 65. Further research might address the effects of smoking, estrogen use, and subclinical cardiovascular disease on the relationship between the mutation and arterial occlusion. Study of other components of the protein C system which are more likely involved in local plaque evolution, such as thrombomodulin expression, is needed (25).

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