

Factor V Leiden (Resistance to Activated Protein C) Increases the Risk of Myocardial Infarction in Young Women

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Factor V Leiden (factor V Arg506Gln), the genetic defect underlying resistance to activated protein C, is the most common risk factor for venous thrombosis. The relationship between this genetic abnormality and arterial disease is still unresolved. To assess whether factor V Leiden increases the risk of myocardial infarction (MI), we conducted a population-based case-control study among women 18 to 44 years of age in western Washington state. We included 84 women with first MI and 388 control women, ie, women residing in the same area in the same age range without MI (n = 388). The control women were contacted by random digit dialing. Data on risk factor status were collected via personal interview, and data on the factor V genotype via polymerase chain reaction techniques. The factor V Leiden mutation was

found more often in women with MI (10%) than among controls (4%). The odds ratio for MI was 2.4 [95% confidence interval (CI) 1.0 to 5.9]. The risk was increased fourfold (CIs 1.2 to 12.1) when adjusted for major cardiovascular risk factors. Among nonsmokers the factor V Leiden mutation had little effect (odds ratio 1.1, CI 0.1 to 8.5), whereas it had a large effect among smokers (odds ratio 3.6, CI 0.9 to 14.4), which, because smoking was itself a strong risk factor for MI, led to an odds ratio for smoking carriers of the mutation that was 32-fold increased compared with nonsmoking noncarriers. We conclude that factor V Leiden increases the risk of MI in young women. This effect seems to be confined largely to current smokers

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FACTOR V LEIDEN is the most common known hereditary abnormality of the clotting system, with a prevalence of heterozygous carriers of 3% to 5%.¹ Due to a mutation (Arg506Gln or R506Q) at the cleavage site for activated protein C (APC), clotting factor V is inactivated at a reduced rate.^{1,4} This defect leads to a reduced anticoagulant effect of APC (APC resistance), with a less-than-expected prolongation of the activated partial thromboplastin time (APTT).⁵ The reduced anticoagulant effect of the protein C/protein S natural inhibitor system leads to an increased tendency toward thrombosis.^{6,9}

Several studies have demonstrated an association between resistance to activated protein C and venous thrombosis.^{6,7,10,14} Heterozygotes for the abnormality are commonly found among unselected patients with deep-vein thrombosis (20%)⁶ and are even more frequent among referred patients or patients with familial thrombophilia (40-60%).¹⁵ Compared with those without the mutation, heterozygous carriers of the mutation have a sevenfold increased risk of venous thrombosis,^{6,7} homozygous individuals have a risk that is increased up to 100-fold.¹⁶

Whether factor V Leiden influences the risk of arterial disease is arguable, and few studies have investigated the association. Several reports, including a controlled study among patients with coronary stenosis, are suggestive of an association with coronary heart disease,^{17,20} but in several other controlled studies no relationship was observed.^{14,21,23} The reason for the lack of consistency among studies is unclear but may reflect differences in the prevalence of other risk factors that act synergistically with factor V Leiden. When the presence of another factor is important for manifesting the elevated risk, this interaction may go unobserved in studies of selected groups of individuals among whom this other factor is absent or uncommon. The conflicting results also make it unlikely that factor V Leiden is a major risk factor for arterial thrombotic disease as it is of venous thrombosis. Still, even if it had only a moderate effect on the risk of myocardial infarction (MI), or if it imparted a large risk to a subgroup of the population, factor V Leiden would be important because of its high allele frequency and the large burden of arterial disease in many populations.

We studied the effect of factor V Leiden on the occurrence of MI among young women in an ongoing population-based case-control study of myocardial infarction and stroke. Because thrombotic factors are likely to be most important in a group of young individuals among whom atherosclerosis is less prevalent than it is among older subjects, this study provided an excellent opportunity to assess the association of factor V Leiden with arterial vascular disease. Moreover, in this population-based study of young patients, other risk factors, such as smoking, are highly prevalent, enhancing the potential to detect potentially important interactions among risk factors.²⁴

METHODS

General design. We conducted a population-based case control study of MI among women 18 to 44 years of age residing in three contiguous counties of western Washington state. The goal was to include all qualifying patients with a first myocardial infarction during the time frame of the study. Data collection was achieved via personal interview, review of medical records, and analysis of blood samples. This study also includes young women with stroke (n = 105, 40 ischemic strokes), among whom we found no excess number

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of carriers of factor V Leiden and about whom we will not report further here

Recruitment of patients Eligible patients were all 18 to 44-year-old female residents of King, Pierce, or Snohomish Counties, Washington, with no prior history of coronary heart disease or cerebrovascular disease, who were diagnosed between July 1, 1991, and February 28, 1995, with a first acute MI. Cases were identified through the review of hospital discharge diagnoses and incident reports from emergency medical-service systems. Criteria for MI were adapted from the Cardiovascular Health Study²⁵ and were defined by evidence of symptoms, elevated enzymes, and electrocardiographic changes. Using these procedures, we identified 165 eligible patients with MI, of whom 112 could be contacted and were willing to participate in an in-person interview.

Recruitment of controls We used random digit telephone dialing to identify a sample of women 18 to 44 years of age living in the same area during the time period of the study.²⁶ A household census was completed for 94.9% of the residences contacted. Among the age-eligible (ie, matched to the age distribution of the cases) women identified, we randomly chose 691 (one from each household) to be recruited into the study. Seven of these women were excluded due to a prior history of cardiovascular disease or an inability to speak English. We completed an in-person interview with 525 of the remaining 684 women for an overall response of 72.8% (525/684 × 0.949).

Data collection Participating cases and controls were interviewed in person regarding histories of diabetes, hypertension, and hyperlipidemia, cigarette smoking, height and weight, reproductive and contraceptive histories, and demographic characteristics. All questions elicited information from a time period before each woman's cardiovascular event, or an equivalent date for controls.

In addition, we obtained a 30 mL nonfasting venous blood sample from 84 patients (75%) and 391 (74%) controls who were interviewed. We compared the women who were interviewed and gave blood with those interviewed who declined venipuncture and found no important differences. Blood was drawn from the antecubital vein in EDTA-treated vacutainers and separated by centrifugation at 2,000g for 10 minutes; the buffy coat was resuspended in phosphate-buffered saline and frozen at -70°C. White cell aliquots were shipped to Leiden, The Netherlands, where DNA analysis was performed. DNA was extracted from these samples as described by Millar et al.²⁷ The presence of the factor V mutation (1,691, G → A replacement) was inferred from the loss of an *Mnl* I restriction site as originally described by Bertina et al.¹ The technicians were blinded to whether a specimen was from a case subject or control subject. Analyzable DNA was available for 84 women with MI and 388 control subjects.

Analysis We classified as smokers any woman who reported smoking currently and regularly, and all others as nonsmokers. Women who reported still having menstrual periods were classified as premenopausal, including women who were currently pregnant or nursing. A woman was classified as diabetic, hypertensive, or hypercholesterolemic if she reported that she was currently taking prescription drugs for these conditions, and as obese when her body mass index (BMI) was equal to or exceeded 27.3 kg/m². These latter four variables were grouped together as metabolic risk factors.

The association of carriage of the factor V Leiden mutation with MI was examined by simple cross-tabulation and by the calculation of the odds ratio as a measure for relative risk. Adjustment for age was performed using unconditional logistic regression; in most instances, unadjusted estimates are given because adjustment did not change the estimates. The extent to which the association between factor V Leiden and disease was modified by other characteristics was assessed through stratified analyses. Confidence intervals (CI95)

Table 1 General Characteristics of Patients and Controls

	Patients With MI (n = 84)	Controls (n = 388)
Age (yr)		
Mean	39.6	37.7
Range	23-44	19-44
Premenopausal	57 (68)	343 (88)
Current smokers	62 (74)	87 (22)
Currently treated for		
Hypertension	14 (17)	10 (3)
Hypercholesterolemia	2 (2)	2 (1)
Diabetes mellitus	6 (7)	3 (1)
Obese	51 (62)	104 (27)

Missing are data on BMI in three controls and on hypercholesterolemia in one control. Percentages are in parentheses.

were calculated using standard methods, ie, by Woolf's method or from the logistic regression model.

RESULTS

The majority of the women were 35 to 44 years of age, with a mean age of 38 years (Table 1). Of the 472 women, 72 (15%) had reached menopause (all but four via hysterectomy or bilateral oophorectomy), 47 (10%) used oral contraceptives. Most of the women were white (89%). Table 1 further shows the distribution of several major risk factors, ie, current smoking, pharmacologically treated hypercholesterolemia, hypertension, and diabetes mellitus, and obesity (BMI ≥ 27.3 kg/m²). As expected, these factors were more common in the patients than in the controls.

Ten percent of the women who had sustained MI carried the factor V Leiden mutation (8 of 84, 9.5%), compared with 4.1% (16 of 388) of the controls (Table 2). The odds ratio associated with factor V Leiden for MI was 2.4 (CI95 1.0 to 5.9). Adjustment for age yielded a similar result (odds ratio 2.4, CI95 1.0 to 5.8). This association changed only in trivial ways when the analysis was restricted to the white women (OR 2.1), the premenopausal women (OR 2.5), or the women not using oral contraceptives (OR 2.5). The odds ratio adjusted for age and major cardiovascular risk factors (smoking, diabetes, hypercholesterolemia, hypertension and obesity) was 4.0 (CI95 1.2 to 12.1).

Current smoking and the presence of metabolic risk factors were strong risk factors for MI. Of the patients with MI, 62 (74%) were smokers, compared with 87 (22%) of the controls; smoking was associated with a nearly tenfold increased risk (OR 9.8, CI95 5.7 to 16.8, age-adjusted OR 8.6, CI95 5.4 to 13.7). One or more of the metabolic risk factors (hypertension, diabetes mellitus, hypercholesterolemia, and obesity) were present in 59 (70%) of the patients with MI and 110 (29%) of the control subjects, which indicates a sixfold increased risk for women with one or more of these risk factors as compared with women with none of these factors (OR 5.9, CI95 3.5 to 9.9).

Further analyses explored whether the risk associated with factor V Leiden was different for women with other major risk factors compared with women without other major risk factors. Odds ratios were calculated for each combination of

Table 2 Factor V Leiden Among Patients With MI and Controls

	Patients With MI (n = 84)	Controls (n = 388)
Factor V Leiden (AG)	8 (9.5)	16 (4.1)
Factor V wild type (GG)	76 (90.5)	372 (95.9)

Values are the number of patients with percentages in parentheses

risk factors (Tables 3 and 4). The simultaneous presence of factor V Leiden and either smoking or one or more of the metabolic risk factors led to a 25- to 32-fold increased risk compared with those without factor V Leiden and smoking (Table 3) or without factor V Leiden and one or more metabolic risk factors (Table 4). When we compared the risk of individuals who either smoked or had one of the metabolic risk factors and carried the factor V Leiden mutant gene (eight cases, five controls), relative to those who did not smoke, had none of the metabolic risk factors, and did not carry the factor V Leiden gene (3 cases, 202 controls), the odds ratio increased markedly to 108 (CI95 22 to 532).

The results shown in Tables 3 and 4 indicate that factor V Leiden carriership leads to the highest risk when other risk factors are also present, although the small number of mutant gene carriers do not allow formal statistical tests for interaction to reach significance. The presence of one or more of the metabolic risk factors and factor V Leiden (Table 4) each increase the risk, which is highest when both are present, factor V Leiden carriership increases the risk among those with one or more metabolic risk factors (OR 3.9, CI95 0.7 to 22.1) as well as among those without one of these four metabolic risk factors (OR 3.6, CI95 1.1 to 11.7). For smoking the results are different: the combined effect of smoking and factor V Leiden, which has a high odds ratio of 32, much exceeds the separate effects of these two factors because smoking appears to be a prerequisite for the risk-increasing effect of factor V Leiden; carriership of factor V Leiden increases the risk among smokers (OR 3.6, CI95 0.9 to 14.4) but not among nonsmokers (OR 1.1, CI95 0.1 to 8.5). In the most extensive model, with adjustment for age and the presence of one or more metabolic risk factors, the risk was increased more than 50-fold in women who smoked and carried the mutation as compared with nonsmoking non-carriers (OR 52.5, CI95 11.2 to 247).

DISCUSSION

MI is a rare event in young women. Several well-known risk factors (hypercholesterolemia, hypertension) are also

Table 3 Factor V Leiden and Current Smoking Separate and Combined Effects on MI

Current Smoker	Factor V Genotype	Patients	Controls	OR*	CI95
No	Wild type	21	288	1	
No	Leiden	1	13	1.1	0.1-8.5
Yes	Wild type	55	84	9.0	5.1-15.7
Yes	Leiden	7	3	32.0	7.7-133

* All odds ratios are relative to the reference category, ie those who did not smoke and did not carry the mutation. Age adjusted logistic regression led to similar odds ratios.

Table 4 Factor V Leiden and Metabolic Risk Factors Separate and Combined Effects on MI

Risk Factors*	Factor V Genotype	Patients	Controls	OR†	CI95
None	Wild type	21	260	1	
None	Leiden	4	14	3.5	1.1-11.7
One or more	Wild type	55	108	6.3	3.6-10.9
One or more	Leiden	4	2	24.8	4.5-194

* Obesity (BMI > 27.3), treated hypertension, diabetes, or hypercholesterolemia, or combinations of same (these data were missing in four controls).

† All odds are relative to the reference category ie those who did not have any of these four risk factors and did not carry the mutation. Age adjusted logistic regression yielded similar odds ratios.

uncommon in the young, which makes this particular group well suited to investigate new causes of MI. This is especially the case for thrombotic risk factors, which may have a striking effect in a population in which atherosclerosis has had less time to progress. Therefore, young women are an excellent group to investigate whether factor V Leiden increases the risk of MI.

Factor V Leiden has been shown to be a strong and common risk factor for venous thrombosis.^{6,7,16,28} From the present study it appears that it is also a determinant of myocardial infarction in young women, increasing the risk about four-fold when other major risk factors for MI are taken into account.

Although we could not include women who did not survive the MI or women who refused the interview or the venipuncture, it is unlikely that this led to biased results. It is inconceivable that nonresponse would be determined by factor V genotype, whereas it is also not likely that the women who had died (a much smaller number) would have had an over- or under-representation of the mutant gene.

The overall increase in the risk for MI was confined to women who were current smokers, and the interaction with smoking appeared strong. Smoking women who carried the factor V Leiden mutation had a 32-fold increased risk of MI (50-fold in the most extensive model), whereas the risk appeared not to be increased at all in nonsmoking gene carriers. These associations are strong but must be interpreted with some caution. First, the incidence of MI in young women is low, therefore, even a small increase in the number of events on an absolute scale leads to large relative risks. The findings thus are unlikely to be directly applicable to populations with a higher overall baseline risk, such as older women and men. Second, our estimates are based on small numbers that are subject to considerable statistical uncertainty. Nevertheless, one may speculate why factor V Leiden has a synergistic effect with cigarette smoking in young women. Both are either fully or partially prothrombotic factors, whereas the metabolic factors we investigated are mainly atherogenic. Since by its chronicity atherosclerosis takes more time, it is conceivable that the combined effect of prothrombotic factors stands out most sharply in young individuals.

Several studies have addressed the effect of factor V

Leiden on coronary artery disease. Holm et al reported two women who had MI at the uncommonly young age of 33 and 34 years and who both were homozygous for the factor V mutation.¹⁸ In another case series²⁹ of 60 patients, and several controlled studies,^{14,21,23} no excess of carriers of factor V Leiden were found among patients with MI. In another large study, however, among 224 patients with angiographically demonstrated coronary artery disease, the factor V mutation was found more often in the patients than in 196 controls, with an odds ratio of 2.4.¹⁹ In a recent study from Finland, the factor V Leiden gene also was found more often in patients with MI (5.7%) than in controls (2.9%).²⁰

The discrepancies among studies may well be the result of differences in study populations. Most of the previous studies included exclusively or predominantly men. The etiology of MI may in part differ between men and women. At the biochemical level, it has been demonstrated that estrogens, endogenous as well as exogenous, increase the resistance to APC in women who carry the mutation as well as in women who do not. In other words, it seems that estrogens further lower the inactivation rate of factor Va by APC.³⁰ It is therefore possible that factor V Leiden is mainly a risk factor in women. The effect of oral contraceptives in increasing the risk of MI is enhanced by smoking.²⁴ Previously we described an interaction between factor V Leiden and the use of oral contraceptives on the risk of developing deep-vein thrombosis.³¹ The small number of oral contraceptive users did not allow us to investigate this issue here for MI, and additional studies will be needed to clarify the role and possible interplay of factor V Leiden, smoking, and estrogens in MI.

If the effect of factor V Leiden on MI is mainly brought about by an interaction with current smoking, this finding also may explain the conflicting results reported so far. The Physicians' Health Study¹⁴ was conducted as a nested case-control analysis among male U.S. physicians who consented to participate in a randomized trial of primary prevention and who were followed prospectively. The number of current smokers among subjects who developed MI was only 16%, which is far lower even than the prevalence in the general population of men in the United States. This extremely low prevalence of smoking may explain why no association between factor V Leiden and MI was observed in that highly selected cohort, whereas we did find a relationship in our population-based study of young women among whom smoking was prevalent (74% of the women with MI).

In conclusion, factor V Leiden is a risk factor for MI in young women. Because of its high prevalence compared with other genetic mutations relevant to thrombosis, the effect of factor V Leiden in populations of other age and sex, in association with other risk factors, needs to be further determined.

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