

MUTATION IN THE GENE CODING FOR COAGULATION FACTOR V AND THE RISK OF MYOCARDIAL INFARCTION, STROKE, AND VENOUS THROMBOSIS IN APPARENTLY HEALTHY MEN

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Abstract *Background.* A specific point mutation in the gene coding for coagulation factor V is associated with resistance to degradation by activated protein C, a recently described abnormality of coagulation that may be associated with an increased risk of venous thrombosis. Whether this mutation also predisposes patients to arterial thrombosis is unknown, as is the value of screening for the mutation in order to define the risk of venous thrombosis among unselected healthy people.

Methods. Among 14,916 apparently healthy men in the Physicians' Health Study who provided base-line blood samples, 374 had myocardial infarctions, 209 had strokes, and 121 had deep venous thrombosis, pulmonary embolism, or both, during a mean follow-up of 8.6 years. We determined whether a mutation at nucleotide position 1691 of the factor V gene was present or absent in these 704 men and in an equal number of matched participants who remained free of vascular disease.

Results. The prevalence of heterozygosity for the mutation among men who had myocardial infarctions (6.1 percent, $P=0.9$) or strokes (4.3 percent, $P=0.4$) was similar to that among men who remained free of vascular disease (6.0 percent). However, the prevalence of the

mutation was significantly higher among men who had venous thrombosis, pulmonary embolism, or both (11.6 percent, $P=0.02$). In adjusted analyses, the relative risk of venous thrombosis among men with the mutation was 2.7 (95 percent confidence interval, 1.3 to 5.6; $P=0.008$). This increased risk was seen with primary venous thrombosis (relative risk, 3.5; 95 percent confidence interval, 1.5 to 8.4; $P=0.004$) but not with secondary venous thrombosis (relative risk, 1.7; 95 percent confidence interval, 0.6 to 5.3; $P=0.3$), and it was most apparent among older men. Specifically, the prevalence of the mutation among men over the age of 60 in whom primary venous thrombosis developed was 25.8 percent (relative risk, 7.0; 95 percent confidence interval, 2.6 to 19.1; $P<0.001$).

Conclusions. In a large cohort of apparently healthy men, the presence of a specific point mutation in the factor V gene was associated with an increased risk of venous thrombosis, particularly primary venous thrombosis. The presence of the mutation was not associated with an increased risk of myocardial infarction or stroke. This mutation appears to be the most common inherited factor thus far recognized that predisposes patients to venous thrombosis. (N Engl J Med 1995;332:912-7.)

A POINT mutation in which adenine is substituted for guanine at nucleotide 1691 in the gene coding for coagulation factor V has recently been reported to be associated with resistance to degradation by activated protein C.¹⁻⁴ In selected referral patients evaluated in retrospective case-control studies, this functional abnormality of hemostasis appears to be associated with an increased risk of venous thromboembolism.⁵⁻⁹ Resistance to activated protein C has been described in 7 percent of a Swedish control group,⁶ whereas the G1691A mutation in the factor V gene is present in 3 to 5 percent of the Dutch population.^{2,7} Among selected young patients with venous thrombosis but no underlying cancer, the prevalence of resistance to activated protein C has ranged from 20 percent to 60 percent.^{2,6,8} Thus, the available data suggest that this newly described genetic abnormality of coagulation may be sub-

stantially more frequent than all other known inherited predispositions to thrombosis.^{10,11}

It is unknown whether the mutation in the factor V gene predisposes healthy people to venous thrombosis. Furthermore, whether resistance to activated protein C is associated with arterial thrombosis is unknown, although several factors associated with coagulation, fibrinolysis, or both, including fibrinogen,¹²⁻¹⁴ factor VII,¹³ fibrin degradation products,^{15,16} tissue plasminogen activator,^{17,18} and plasminogen-activator inhibitor,^{19,20} have all been associated with an increased risk of myocardial infarction, stroke, or both. We therefore investigated whether resistance to activated protein C, as assessed by the presence of the G1691A mutation in the factor V gene, was associated with the occurrence of thrombosis in the coronary and cerebral arterial circulations, as well as in venous vessels.

METHODS

We evaluated the presence of the G1691A mutation in the coagulation factor V gene and the risk of thrombosis by studying prospectively collected DNA samples from a cohort of apparently healthy men participating in the Physicians' Health Study,²¹ a randomized, double-blind, placebo-controlled trial of aspirin and beta carotene for the primary prevention of cardiovascular disease and cancer. The details of the Physicians' Health Study have been presented elsewhere²¹; in brief, 22,071 predominantly white U.S. male physicians 40 to 84 years old who were free of prior myocardial infarction, stroke, transient ischemic attack, and cancer were randomly assigned in a two-by-two factorial design to one of four treatments: 325 mg of aspirin (Bufferin, Bristol-Myers Squibb), given on alternate days; 50

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Supported by grants (HL-26490, HL-34595, HL-14147, CA-34944, CA-42182, and CA-40360) from the National Institutes of Health. Dr. Ridker is the recipient of a Clinician Scientist Award from the American Heart Association, Dallas. Dr. Lindpaintner is the recipient of a Research Career Development Award from the National Heart, Lung, and Blood Institute.

mg of beta carotene (Lurotin, BASF), given on alternate days; both; and neither. Six months after randomization and once a year thereafter, these physicians were sent questionnaires on which they were asked to assess risk factors and disease outcomes; follow-up has been completed for all deaths and for 99.7 percent of all reported morbid events.

At the time of enrollment, all potentially eligible men were assigned to receive aspirin as an active drug and placebo manufactured to resemble beta carotene during an 18-week run-in period in order to identify those with potentially good compliance for long-term follow-up. During the run-in period, all participants were asked to provide base-line EDTA-anticoagulated samples of whole blood and to return them to the laboratory in a cold pack by overnight courier. On arrival in the laboratory, the specimens were divided into aliquots and stored at -80°C . Of the 22,071 physicians randomized, 14,916 returned base-line blood samples (68 percent).

For all reported cases of fatal or nonfatal myocardial infarction, stroke, deep venous thrombosis, or pulmonary embolism occurring after randomization, the hospital records were requested. These records, as well as death certificates and autopsy reports, were reviewed by an end-points committee of physicians who used standardized criteria to confirm or reject the diagnosis of each reported event. A diagnosis of myocardial infarction was considered to be confirmed if the reported event met the World Health Organization criteria for myocardial infarction, which include symptoms plus either elevations of cardiac-enzyme levels or diagnostic changes on the electrocardiogram.²² In the case of a fatal myocardial infarction, we also accepted diagnoses based on autopsy findings and deaths confirmed by the medical records as due to coronary heart disease (codes 411 to 414 of the *International Classification of Diseases*). A diagnosis of stroke was considered to be confirmed if the patient had a new focal neurologic deficit and if the symptoms and signs persisted for more than 24 hours. Computed tomographic scans were available for more than 95 percent of the confirmed strokes. A diagnosis of deep venous thrombosis was considered to be confirmed when there was documentation of a positive venographic study or a positive ultrasonographic study. Reported cases of deep venous thrombosis that were documented by impedance plethysmography or Doppler examination but not by ultrasonography were not considered confirmed. A diagnosis of pulmonary embolism was considered to be confirmed when a positive angiogram or a completed ventilation-perfusion scan showed at least two segmental perfusion defects without ventilation defects. Venous thrombosis associated with cancer or occurring postoperatively was classified as a secondary event. All other venous thromboses were classified as primary events.

According to the study's nested case-control design, each physician who provided an adequate sample of whole blood at base line and had a confirmed myocardial infarction, stroke, deep venous thrombosis, or pulmonary embolism after randomization was matched to one control. The controls were participating physicians, each of whom had provided a base-line sample of whole blood and reported no cardiovascular disease at the time the arterial or venous event occurred in the case patient. The controls were selected at random from among participants who met the matching criteria of age (within one year of the age of the case patient), smoking habits (current smoker, former smoker, or person who never smoked), and time since randomization (in six-month intervals).

For each case patient and each control, the whole blood collected and stored at base line was thawed and underwent DNA extraction. A commercially available process was used that was based on the absorption of DNA to a silica membrane after lysis with a proprietary agent (Diagen) and proteinase K in the presence of a high salt concentration and 33 percent isopropanol. Genotype assays using the polymerase-chain-reaction (PCR) technique were performed in a second laboratory, where the investigators and laboratory personnel were unaware of each subject's status as a case patient or control. Extracted DNA samples from the case patients and controls were analyzed in pairs, with the sequence varied at random within the pairs to avoid systematic bias.

The samples in each pair were handled together and in an identical manner throughout the processing and analysis. In brief, the

G1691A mutation in exon 10 (nucleotides 1487 to 1701) was detected by the loss of a cleavage site for *MnlI*. A 267-base-pair (bp) fragment spanning the exon-intron junction was amplified from genomic DNA as described by Bertina et al.²³; an additional sequence from intron 10 was obtained by sequencing the product from 24 samples. Subsequently, an optimized 5' primer (5'ACCCACAGAAAATGATGCCAG3', nucleotides 1566 to 1587) and a new 3' primer (5'TGCCCCATTATTTAGCCAGGAG3', nucleotides -66 to -87 of intron 10) were used to amplify a 223-bp fragment from genomic DNA samples. The PCR conditions were as follows: 75 to 150 ng of DNA in 15 μl of TRIS-EDTA buffer (10 mM TRIS and 0.1 mM EDTA; pH 8.0) under 50 μl of mineral oil was heated in a Hybaid thermal cycler to 96°C for 60 seconds and 95°C for 180 seconds, and then held at 80°C for 10 minutes while 35 μl of amplification solution was added that contained 5 μl of 20 mM solution of each of the deoxynucleoside triphosphates (adenosine triphosphate, cytidine triphosphate, thymidine triphosphate, and guanosine triphosphate), 200 ng of each primer, 0.1 μl of 1.0 M magnesium chloride, and 0.5 U of *Taq* polymerase (Promega). Thirty cycles of 93°C for 60 seconds, 50°C for 30 seconds, and 72°C for 90 seconds were followed by a final 10 minutes at 72°C . Aliquots (10 μl) were digested for 12 hours at 37°C with 1 U *MnlI* (New England Biolabs) after the addition of 2.5 μl of 5 \times digest buffer (1 \times digest buffer is 50 mmol sodium chloride, 10 mmol TRIS-hydrochloric acid, 10 mmol magnesium chloride, and 1 mmol dithiothreitol; pH 7.9). The fragments (measuring 37, 82, and 104 bp for the *1691G* allele, and 82 and 141 bp for *1691A*) were then separated on 2 percent agarose gels and visualized with ethidium bromide. The presence of the G1691A mutation was confirmed in each instance by a second amplification and restriction-enzyme digestion.

In addition to providing blood samples before randomization, the participating physicians also reported their base-line cardiovascular risk factors, which included the matching variables of age and smoking status, as well as their height, weight, systolic and diastolic blood pressure, history of any hypercholesterolemia, parental history of any myocardial infarction before the age of 60, the presence of any diabetes mellitus, and the frequency of vigorous exercise. The body-mass index was calculated as the weight in kilograms divided by the square of the height in meters.

Statistical Analysis

Means and proportions for base-line cardiovascular risk factors and for the presence of the factor V mutation were computed for the case patients and controls. The significance of any difference in means was tested by the paired Student t-test, whereas the significance of any difference in proportions was tested by the chi-square statistic. Logistic-regression analyses were performed to estimate relative risks and 95 percent confidence intervals. Adjusted relative risks were calculated further by logistic-regression models that controlled for the matching variables of age and smoking status as well as for the presence of hypertension, diabetes, hypercholesterolemia, any family history of myocardial infarction before the age of 60, body-mass index, and frequency of exercise. All regression analyses controlled for the treatment assignments, and all P values are two-tailed.

RESULTS

The base-line characteristics of men in whom myocardial infarction, stroke, or venous thrombosis developed during follow-up and those who remained free of cardiovascular disease are shown in Table 1. As expected, the subjects who had myocardial infarction or stroke during follow-up had a higher prevalence of conventional atherosclerotic risk factors at base line than did the control subjects.

Among the 704 men who remained free of cardiovascular disease during follow-up, 662 (94.0 percent) were found to be homozygous for the *1691G* allele, whereas 42

(6.0 percent) were heterozygous for both the *1691G* and the *1691A* alleles. No *1691A* homozygotes were observed. Thus, the observed frequency of the *1691G* allele was 97.0 percent (95 percent confidence interval, 96.0 to 97.8) and that of the *1691A* allele 3.0 percent (95 percent confidence interval, 2.2 to 4.1). The observed distribution of genotypes was consistent with that predicted by the Hardy–Weinberg equilibrium.

No statistically significant differences in the prevalence of the factor V mutation were found between the men who had myocardial infarction (6.1 percent, $P=0.9$) or stroke (4.3 percent, $P=0.4$) during follow-up and the men who remained free of cardiovascular disease (6.0 percent). In contrast, the mutation was significantly more prevalent among men in whom deep venous thrombosis, pulmonary embolism, or both developed during follow-up (11.6 percent, $P=0.02$) (Fig. 1).

Table 2 shows the crude and adjusted relative risks of myocardial infarction, stroke, and venous thrombosis that were associated with the presence of the factor V mutation. The adjusted relative risk of myocardial infarction or stroke among heterozygous subjects was 1.3 (95 percent confidence interval, 0.7 to 2.2; $P=0.4$). Restricting this analysis to subjects with stroke of clear thromboembolic origin did not materially alter the results. Spe-

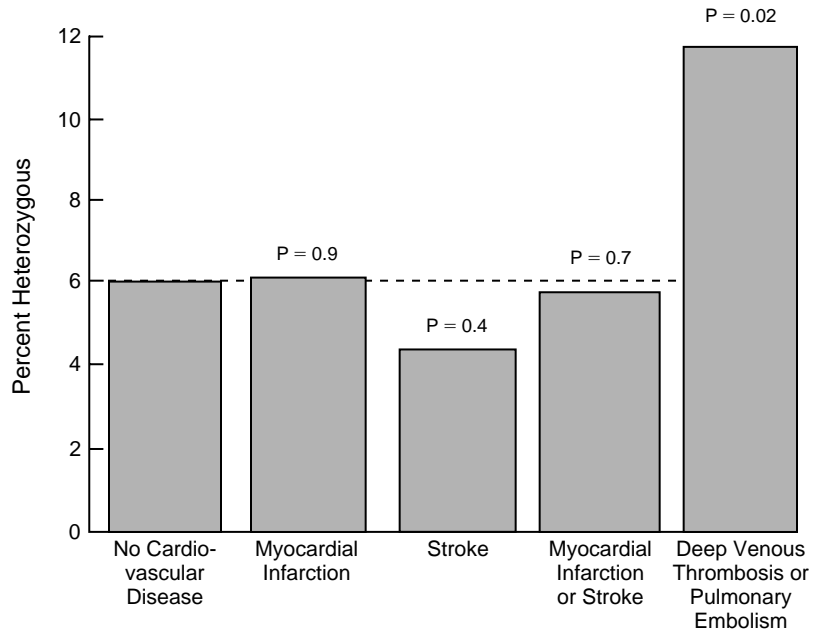


Figure 1. Prevalence of Heterozygosity for the Factor V Mutation among 1408 Apparently Healthy Men in the Physicians' Health Study.

Data shown are for cardiovascular disease occurring during follow-up. No subject was homozygous for the mutated *1691A* allele. P values shown are for the comparison with the reference value (dashed line).

Table 1. Base-Line Clinical Characteristics of 1408 Apparently Healthy Men Participating in the Physicians' Health Study, According to the Development of Cardiovascular Disease during Follow-up.

CHARACTERISTIC	CARDIOVASCULAR DISEASE DURING FOLLOW-UP			
	NONE (N = 704)	MYOCARDIAL INFARCTION (N = 374)	STROKE (N = 209)	VENOUS THROMBOSIS OR PULMONARY EMBOLISM (N = 121)
Age (yr)*	60.3	59.5	62.9	58.9
Body-mass index	24.9	25.5	25.5	25.9
Smoking status (%)*				
Never smoked	42.5	42.6	40.4	46.3
Past smoker	41.7	41.3	39.9	46.3
Current smoker	15.8	16.1	19.7	7.4
Diabetes (%)	3.9	6.5	12.5	2.5
Parental history of coronary artery disease (%)	11.1	15.6	10.8	8.3
Weekly exercise (%)	70.9	65.3	67.0	75.2
History of high cholesterol (%)	8.6	16.0	11.5	5.5
History of hypertension (%)	17.4	29.0	36.2	20.3
Blood pressure (mm Hg)				
Systolic	128.5	130.6	135.3	127.4
Diastolic	79.3	81.1	82.5	80.2

*This variable was used as one of the matching criteria.

cifically, the prevalence of the mutation among men with either myocardial infarction or thromboembolic stroke was 5.4 percent (adjusted relative risk, 1.0; 95 percent confidence interval, 0.6 to 1.7; $P=0.9$). No association was found between the presence of the factor V mutation and the risk of myocardial infarction or stroke in analyses of subgroups restricted to men 60 years of age or less. Similarly, no association was found among nonsmokers, those with no family history of coronary disease, those with no evidence of hypercholesterolemia, those free of hypertension, or those with all these characteristics (Table 3).

The adjusted relative risk of deep venous thrombosis, pulmonary embolism, or both among heterozygous subjects was 2.7 (95 percent confidence interval, 1.3 to 5.6; $P=0.008$) (Table 2). To explore further the relation between the factor V mutation and the risk of future venous thromboembolism, we repeated these analyses in the 63 subjects with primary venous thrombosis and in the 58 subjects in whom the venous thrombosis was determined to be secondary. In this analysis, the increase in the risk of deep venous thrombosis, pulmonary embolism, or both that was associated with the presence of the mutation appeared to be due principally to primary venous thrombosis (adjusted relative risk, 3.5; 95 percent confidence interval 1.5 to 8.4; $P=0.004$) rather than secondary venous thrombosis (adjusted relative risk, 1.7; 95 percent confidence interval, 0.6 to 5.3; $P=0.3$) (Fig. 2). These effects were most apparent among older men. As shown in Table 4, the prevalence of the factor V mutation among men over the age of 60

Table 2. Relative Risk of Myocardial Infarction, Stroke, and Venous Thrombosis Associated with the Presence of the Factor V Mutation.

VARIABLE	CARDIOVASCULAR DISEASE DURING FOLLOW-UP				
	NONE (N = 704)	MYOCARDIAL INFARCTION (N = 374)	STROKE (N = 209)	MYOCARDIAL INFARCTION OR STROKE (N = 583)	VENOUS THROMBOSIS OR PULMONARY EMBOLISM (N = 121)
Crude analysis*					
Relative risk	1.0	1.1	0.7	0.9	2.1
95% CI†	—	0.6–1.8	0.3–1.4	0.6–1.5	1.1–4.0
P value	—	0.8	0.3	0.7	0.02
Adjusted analysis‡					
Relative risk	1.0	1.5	1.0	1.3	2.7
95% CI†	—	0.8–2.7	0.4–2.2	0.7–2.2	1.3–5.6
P value	—	0.2	0.9	0.4	0.008

*Adjusted for treatment assignment, age, and smoking status.

†CI denotes confidence interval.

‡Adjusted for treatment assignment, age, smoking status, history of hypertension, history of elevated cholesterol, family history of myocardial infarction before the age of 60, body-mass index, presence of diabetes, and frequency of exercise.

years in this cohort who subsequently had any venous thrombosis was 17.9 percent (relative risk, 3.3; 95 percent confidence interval, 1.5 to 7.5; $P=0.004$). Among men over the age of 60 years who had primary venous thrombosis, the prevalence of the mutation was 25.8 percent (relative risk, 5.3; 95 percent confidence interval, 2.1 to 13.3; $P<0.001$). In analyses of heterozygous men older than 60 that were adjusted for smoking status, history of hypertension, hypercholesterolemia, body-mass index, diabetes, and family history of coronary disease, the relative risk of any venous thrombosis was 4.0 (95 percent confidence interval, 1.6 to 9.7; $P=0.003$) and that of primary deep venous thrombosis was 7.0 (95 percent confidence interval, 2.6 to 19.1; $P<0.001$).

DISCUSSION

Recent investigations into the protein C system have led to major new insights into the process of thrombus formation. In 1993 Dahlback and colleagues⁵ described resistance to activated protein C, which appeared to be a characteristic of selected patients with venous thromboembolism, particularly those who had positive family histories and were relatively young at presentation.²⁻⁹ In rapid succession, studies directed by Bertina^{2,7} and Dahlback,^{5,6} as well as the Leiden Thrombophilia Study,⁷ found that the cofactor responsible for resistance to activated protein C was a previously unrecognized form of coagulation factor V that had normal procoagulant activity but was resistant to degradation by activated protein C. Almost immediately thereafter, these and other researchers reported that resistance to activated protein C is almost always associated with the substitution of a single base at nucleotide 1691 of the gene

coding for coagulation factor V, which leads to the replacement of arginine by glutamine.^{2,4} The presence of arginine in this position is essential for the proteolytic inactivation of factor V by activated protein C.

We have described a large-scale epidemiologic assessment of the factor V mutation in a healthy population of predominantly white men followed prospectively for the occurrence of cardiovascular disease, in particular arterial and venous thrombosis. With regard to arterial thrombosis, there appeared to be no association between the presence of the factor V mutation and the risk of myocardial infarction, stroke from all causes, or thromboembolic stroke over a follow-up period of 8.6 years. Furthermore, there was no association between the factor V mutation and myocardial infarction among younger men or men without risk factors for cardiovascular disease. In contrast, the adjusted relative risk of deep venous thrombosis, pulmonary embolism, or both among men with the mutation was 2.7 times that among men without the mutation (95 percent confidence interval, 1.3 to 5.6; $P=0.008$). For events unrelated to recent surgery or cancer, the presence of the mutant allele was associated with a three-fold increase in risk (adjusted relative risk, 3.5; 95 percent confidence interval, 1.5 to 8.4; $P=0.004$). Among older men in whom primary venous thrombosis developed, the prevalence of the mutation exceeded 25 percent, conferring an adjusted risk of primary venous thromboembolism that was 7.0 times higher than that of men homozygous for the wild-type allele (95 percent confidence interval, 2.6 to 19.1; $P<0.001$).

Unlike previous studies of resistance to activated protein C, which relied on limited kindreds, patients known to have recurrent idiopathic thromboembolism, or patients referred for the evaluation and treatment of venous thromboembolism,³⁻⁹ the current study was based on a large cohort of apparently healthy men who were prospectively followed for the development of cardiovascular disease. Therefore, these findings are not subject to many types of epidemiologic bias that can influence analyses based on case reports, case series, cross-sectional surveys, and retrospective studies of se-

Table 3. Relative Risk of Myocardial Infarction and Stroke Associated with the Presence of the Factor V Mutation among Subjects without the Usual Coronary Risk Factors.*

VARIABLE	MYOCARDIAL INFARCTION			MYOCARDIAL INFARCTION OR STROKE		
	NO. OF SUBJECTS	RELATIVE RISK (95% CI)	P VALUE	NO. OF SUBJECTS	RELATIVE RISK (95% CI)	P VALUE
Age \leq 60 yr	208	1.1 (0.5–2.3)	0.8	285	1.1 (0.6–2.2)	0.8
Nonsmoker	313	1.1 (0.6–2.0)	0.7	480	1.0 (0.6–1.6)	0.9
No parental history of coronary disease	309	1.2 (0.7–2.1)	0.5	491	1.0 (0.6–1.7)	0.9
No history of hypercholesterolemia	289	1.2 (0.6–2.2)	0.6	459	1.0 (0.6–1.7)	0.9
No history of hypertension	265	0.8 (0.4–1.6)	0.6	397	0.8 (0.4–1.4)	0.4
Nonsmoker, no parental history of coronary disease, no history of hypertension or hypercholesterolemia	137	1.0 (0.4–2.4)	0.9	216	0.8 (0.4–1.8)	0.6

*Relative risks are adjusted for treatment assignment. CI denotes confidence interval.

lected referral populations.²³ Furthermore, the current study assessed the factor V mutation directly by molecular genetic analysis rather than by relying on functional assays to determine the presence of resistance to activated protein C. Thus, in addition to permitting a prospective evaluation of the relation between the factor V mutation and the risk of venous and arterial thrombosis, these data allowed the frequencies of the wild-type and mutant alleles in a predominantly white North American male population to be estimated precisely. On the basis of the observed data, the allelic frequency of the factor V mutation is 3.0 percent (95 percent confidence interval, 2.2 to 4.1 percent), and the prevalence of the heterozygous state in the population is 6.0 percent (95 percent confidence interval, 4.3 to 8.0 percent). These data provide strong evidence that genetic resistance to activated protein C is the most common inherited predisposition to thrombosis recognized to date.^{10,11}

It is important that the factor V mutation is prevalent among men over the age of 60 who have a first episode of venous thrombosis, because most earlier studies have suggested that thrombosis associated with inherited abnormalities of hemostasis typically presents at a young age (<40 years). Although the association between inherited coagulation defects and thrombosis may be modified by age, it is also possible that age-specific differences between the subjects in our study and those in most previous studies reflect the fact that the referral populations examined in most earlier retro-

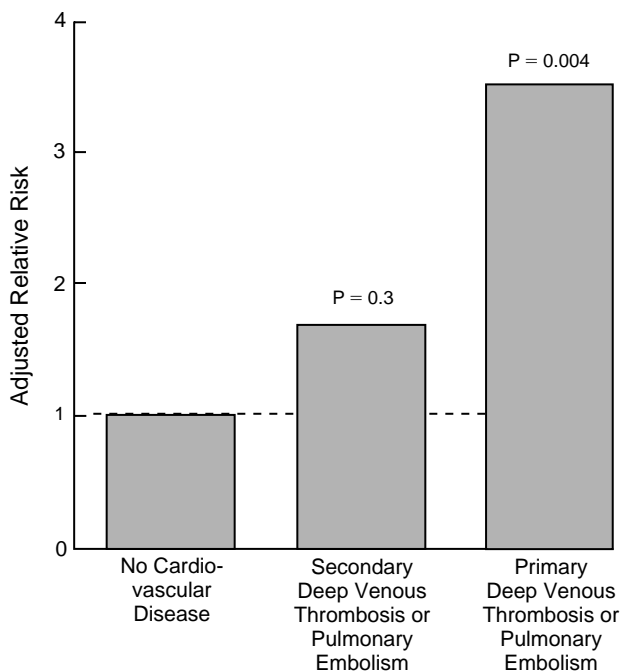


Figure 2. Adjusted Relative Risk Associated with Primary and Secondary Deep Venous Thrombosis or Pulmonary Embolism in Study Subjects with the Factor V Mutation.

Data shown are for cardiovascular disease occurring during follow-up. P values are for the comparison with the reference value (dashed line).

Table 4. Relative Risk of Venous Thrombosis or Pulmonary Embolism Associated with the Presence of the Factor V Mutation among Subjects over the Age of 60.

VARIABLE*	CARDIOVASCULAR DISEASE DURING FOLLOW-UP		
	NONE (N = 355)	ANY VENOUS THROMBOSIS OR PULMONARY EMBOLISM (N = 56)	PRIMARY VENOUS THROMBOSIS OR PULMONARY EMBOLISM (N = 31)
Prevalence of mutation (%)	6.5	17.9	25.8
Crude analysis*			
Relative risk	1.0	3.3	5.3
95% CI†	—	1.5–7.5	2.1–13.3
P value	—	0.004	<0.001
Adjusted analysis‡			
Relative risk	1.0	4.0	7.0
95% CI†	—	1.6–9.7	2.6–19.1
P value	—	0.003	<0.001

*Adjusted for treatment assignment and smoking status.

†CI denotes confidence interval.

‡Adjusted for treatment assignment, smoking status, history of hypertension, history of elevated cholesterol, family history of myocardial infarction before the age of 60, body-mass index, presence of diabetes, and frequency of exercise.

spective analyses did not include adequate numbers of older patients. For example, the mean age of patients with deep venous thrombosis in earlier reports of resistance to activated protein C ranged from 34 to 46 years — far lower than the mean age of 65 reported in community-based surveys of patients presenting with first venous thromboemboli.²⁴ In our prospective study of apparently healthy men, the mean age at the time of the first deep venous thrombosis, pulmonary embolism, or both was 63.2 years. Our finding that the mutation in the factor V gene is an important risk factor for venous thrombosis in older subjects is consistent with observations from the Leiden Thrombophilia Study⁷ about the prevalence of resistance to activated protein C among patients as old as 70.

From a clinical and public health perspective, these data have important implications. It has been estimated that pulmonary embolism is the primary cause of 50,000 to 100,000 deaths annually in the United States and that it is a contributing cause in tens of thousands of other deaths.^{25–27} Among older persons hospitalized with pulmonary embolism, 21 percent die during that hospitalization and another 39 percent die over the next 12 months.^{24,28} Among older patients presenting with deep venous thrombosis, recurrence rates approach 30 percent and one-year mortality exceeds 20 percent.^{28,29} Thus, the knowledge that 6 percent of the U.S. male population is genetically at increased risk for venous thromboembolism raises the possibility that these people should be identified and treated differently from people without the factor V mutation. For example, it is not known whether patients with the mutation should be treated with more prolonged or more intense anticoagulation after a thrombotic episode or whether they require more aggressive prophylactic regimens in high-risk clinical situations, such as elective hip surgery. Thus, randomized trials to determine whether different anticoagulant regimens are needed by patients with the factor V mutation should be con-

sidered seriously. Such studies should ideally include enough women and members of minority groups for associations between the factor V mutation and thrombosis to be evaluated in these populations.

We are indebted to Kirsten Williams and Robert Cannon for their technical assistance.

REFERENCES

1. Dahlback B, Hildebrand B. Inherited resistance to activated protein C is corrected by anticoagulant cofactor activity found to be a property of factor V. *Proc Natl Acad Sci U S A* 1994;91:1396-400.
2. Bertina RM, Koelman BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994;369:64-7.
3. Voorberg J, Roelse J, Koopman R, et al. Association of idiopathic venous thromboembolism with single point-mutation at Arg⁵⁰⁶ of factor V. *Lancet* 1994;343:1535-6.
4. Zoller B, Dahlback B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. *Lancet* 1994;343:1536-8.
5. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci U S A* 1993;90:1004-8.
6. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med* 1994;330:517-22.
7. Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet* 1993;342:1503-6.
8. Griffin JH, Evatt B, Wideman C, Fernandez JA. Anticoagulant protein C pathway defective in majority of thrombophilic patients. *Blood* 1993;82:1989-93.
9. Halbmayer WM, Haushofer A, Schon R, Fischer M. The prevalence of poor anticoagulant response to activated protein C (APC resistance) among patients suffering from stroke or venous thrombosis and among healthy subjects. *Blood Coagul Fibrinolysis* 1994;5:51-7.
10. Miletich JP, Prescott SM, White R, Majerus PW, Bovill EG. Inherited predisposition to thrombosis. *Cell* 1993;72:477-80.
11. Heijboer H, Brandjes DPM, Büller HR, Sturk A, ten Cate JW. Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatients with deep-vein thrombosis. *N Engl J Med* 1990;323:1512-6.
12. Wilhelmssen L, Svärdsudd K, Korsan-Bengtson K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. *N Engl J Med* 1984;311:501-5.
13. Meade TW, Mellows S, Brozovic M, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. *Lancet* 1986;2:533-7.
14. Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease: the Framingham Study. *JAMA* 1987;258:1183-6.
15. Fowkes FGR, Lowe GDO, Housley E, et al. Cross-linked fibrin degradation products, progression of peripheral arterial disease, and risk of coronary heart disease. *Lancet* 1993;342:84-6.
16. Ridker PM, Hennekens CH, Cerskus A, Stampfer MJ. Plasma concentration of cross-linked fibrin degradation product (D-dimer) and the risk of future myocardial infarction among apparently healthy men. *Circulation* 1994;90:2236-40.
17. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Hennekens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. *Lancet* 1993;341:1165-8.
18. Ridker PM, Hennekens CH, Stampfer MJ, Manson JE, Vaughan DE. Prospective study of endogenous tissue plasminogen activator and risk of stroke. *Lancet* 1994;343:940-3.
19. Hamsten A, Wiman B, de Faire U, Blombäck M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N Engl J Med* 1985;313:1557-63.
20. Hamsten A, de Faire U, Walldius G, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 1987;2:3-9.
21. Steering Committee of the Physicians' Health Study Research Group. Final report of the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med* 1989;321:129-35.
22. World Health Organization. Ischemic heart disease registers: report of the Fifth Work Group, including a second revision of the operating protocol, Copenhagen, Denmark, April 26-29, 1971. Copenhagen, Denmark: World Health Organization, Regional Office for Europe, 1971.
23. Hennekens CH, Buring JE. *Epidemiology in medicine*. Boston: Little, Brown, 1987.
24. Anderson FA Jr, Wheeler HB, Goldberg RJ, et al. A population-based perspective of the hospital incidence and case-fatality rates of deep vein thrombosis and pulmonary embolism: the Worcester DVT Study. *Arch Intern Med* 1991;151:933-8.
25. Hirsh J, Genton E, Hull R. *Venous thromboembolism*. New York: Grune & Stratton, 1981.
26. Dalen JE, Alpert JS. Natural history of pulmonary embolism. *Prog Cardiovasc Dis* 1975;17:257-70.
27. Goldhaber SZ, ed. *Pulmonary embolism and deep venous thrombosis*. Philadelphia: W.B. Saunders, 1985.
28. Kniffin WD Jr, Baron JA, Barrett J, Birkmeyer JD, Anderson FA Jr. The epidemiology of diagnosed pulmonary embolism and deep venous thrombosis in the elderly. *Arch Intern Med* 1994;154:861-6.
29. Prandoni P, Lensing AWA, Büller HR, et al. Deep-vein thrombosis and the incidence of subsequent symptomatic cancer. *N Engl J Med* 1992;327:1128-33.

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