

THE RISK OF RECURRENT DEEP VEIN THROMBOSIS AMONG HETEROZYGOUS CARRIERS OF BOTH FACTOR V LEIDEN AND THE G20210A PROTHROMBIN MUTATION

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ABSTRACT

Background Point mutations in the factor V gene (factor V Leiden) and the prothrombin gene (the substitution of A for G at position 20210) are the most common causes of inherited thrombophilia. Whether or not factor V Leiden increases the risk of recurrent deep vein thrombosis is controversial, and there is no information on the risk of recurrence among carriers of both mutations.

Methods We studied a retrospective cohort of 624 patients who were referred for a first episode of deep vein thrombosis. After excluding 212 patients with other inherited or acquired causes of thrombophilia, we compared 112 patients who were heterozygous carriers of factor V Leiden with 17 patients who were heterozygous for both factor V Leiden and the prothrombin mutation and 283 patients who had neither mutation. The relative risk of recurrent deep vein thrombosis was calculated with use of a proportional-hazards model.

Results Patients who were heterozygous for factor V Leiden alone had a risk of recurrent deep vein thrombosis that was similar to that among patients who had neither mutation (relative risk, 1.1; 95 percent confidence interval, 0.7 to 1.6; $P=0.76$). In contrast, patients who were heterozygous for both factor V Leiden and the prothrombin mutation had a higher risk of recurrent thrombosis than did carriers of factor V Leiden alone (relative risk, 2.6; 95 percent confidence interval, 1.3 to 5.1; $P=0.002$). When the analysis was restricted to patients with spontaneous recurrences (i.e., ones that occurred in the absence of transient risk factors for venous thrombosis), the risk among carriers of both mutations, as compared with carriers of factor V Leiden alone, remained high (relative risk, 3.7; 95 percent confidence interval, 1.7 to 7.7; $P<0.001$), particularly if the first event had also been spontaneous (relative risk, 5.4; 95 percent confidence interval, 2.0 to 14.1; $P<0.001$). In contrast, the risk of recurrence in the presence of transient risk factors was similar among carriers of both mutations and carriers of factor V Leiden alone.

Conclusions The risk of recurrent deep vein thrombosis is similar among carriers of factor V Leiden and patients without this mutation. Carriers of both factor V Leiden and the G20210A prothrombin mutation have an increased risk of recurrent deep vein thrombosis after a first episode and are candidates for lifelong anticoagulation. (N Engl J Med 1999;341:801-6.)

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INHERITED resistance to activated protein C is a thrombophilic condition resulting from a mutant factor V (factor V Leiden).¹ The mutation is relatively common among whites, with a frequency of 5 percent in the general population of European ancestry and 11 to 21 percent among patients with venous thromboembolism.² The estimated risk of deep vein thrombosis is 7 times as high among heterozygous carriers of the mutation as among persons without the mutation, and 80 times as high among homozygotes.³ There is disagreement whether factor V Leiden is associated with an increased risk of recurrent deep vein thrombosis. Several studies reported relative risks of recurrence ranging from 2.4 to 4.1 among patients with deep vein thrombosis who had the mutation as compared with those who did not have the mutation.^{4,5} Other studies, however, failed to find an association between factor V Leiden and recurrent deep vein thrombosis.⁶⁻⁸ This issue is clinically important, because carriers of the mutation should receive lifelong anticoagulant therapy if their risk of recurrent thrombosis is high.

Another thrombophilic mutation has been identified in the 3' untranslated region of the prothrombin gene (the substitution of A for G at position 20210)⁹; the mutant allele is present in 2 percent of the general population¹⁰ and increases the risk of deep vein thrombosis by a factor of 2.7 to 3.8.^{9,11-13} The expected prevalence of both the factor V Leiden mutation and the prothrombin mutation in the general population is about 1 per 1000; the prevalence has been estimated to be 1 to 5 percent among patients with deep vein thrombosis.^{9,11-14} The prevalence of the 20210A allele among selected patients with factor V Leiden and previous venous thromboembolism is 3 to 27 percent.¹¹⁻¹⁶ Because there is preliminary evidence that carriers of the prothrombin 20210A allele who also have other inherited thrombophilic conditions (deficiency of antithrombin III, protein C, or protein S or the presence of factor V Leiden) are more prone to recurrent thrombotic ep-

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isodes than persons with one thrombophilic condition,^{14,17} we evaluated the risk of recurrent deep venous thrombosis among patients who were heterozygous for factor V Leiden in the presence and the absence of the prothrombin mutation as compared with patients with no known causes of inherited thrombophilia.

METHODS

Patients

From November 1994 to December 1998, 624 consecutive unrelated patients who had had a first episode of deep venous thrombosis of the legs or had been given a diagnosis of recurrent venous thromboembolism in the hospital by their attending physicians were referred to two specialized thrombosis centers (in Milan and Rome) for an assessment of the possible causes of thrombophilia. All patients were interviewed about their medical history by physicians before undergoing any laboratory tests, so that the diagnosis of first or recurrent deep venous thrombosis was recorded without knowledge of the results of thrombophilia screening. The presence of known risk factors at the time of any episode of deep venous thrombosis, such as oral-contraceptive use, pregnancy or recent childbirth, surgery, prolonged immobilization (bed rest for at least 10 days, or the need for a leg cast), and trauma, was also recorded.

The diagnosis of a first episode of deep venous thrombosis was considered valid if it had been based on objective methods such as phlebography, ultrasonographic examination, or impedance plethysmography. The diagnosis of contralateral recurrent deep venous thrombosis also had to be objectively established, and recurrences of pulmonary embolism were diagnosed on the basis of perfusion lung scanning or computed tomography. Episodes of ipsilateral deep venous thrombosis or pulmonary embolism that occurred within three months after the first episode of deep venous thrombosis were considered to indicate progression rather than recurrence. Episodes of ipsilateral deep venous thrombosis that occurred more than three months after the first episode of thrombosis were considered to indicate recurrence if the results of the objective tests were worse than those obtained at the time of the initial episode or if a new course of anticoagulant treatment was started.

After giving informed consent, all patients underwent screening for thrombophilia as previously described.¹⁸ DNA samples were analyzed for the presence of the prothrombin gene mutation according to the method of Poort et al.⁹

Of the 624 patients who were referred, 283 had neither mutation and 129 were heterozygous carriers of factor V Leiden, 17 of whom were also heterozygous for the G20210A prothrombin mutation. The remaining 212 patients were excluded from the study because of the presence of one or more of the following: deficiency of antithrombin III, protein C, or protein S; homozygosity for factor V Leiden; an isolated G20210A mutation or a G20210A mutation in combination with thrombophilic traits other than factor V Leiden; cancer or myeloproliferative diseases; autoimmune disorders (including primary antiphospholipid-antibody syndrome); treatment with an oral anticoagulant for more than six months after the first episode of deep venous thrombosis; or a diagnosis of recurrent superficial venous thrombosis without objective signs of deep venous thrombosis.

Statistical Analysis

We used the chi-square test to estimate differences in prevalence among the three groups. We analyzed the interval from the first episode of deep venous thrombosis to a recurrence (uncensored observations) or to referral to the centers (censored observations) in order to estimate the probability of recurrent deep venous thrombosis as a function of time, according to the method of Kaplan and Meier.¹⁹ The probability of recurrent deep venous thrombosis was compared among the groups with use of

the log-rank test; the relative risk of recurrent deep venous thrombosis among patients with one or both mutations as compared with those with neither mutation was estimated with use of a Cox proportional-hazards model, with 95 percent confidence intervals.²⁰

RESULTS

Characteristics of the Patients

The clinical characteristics of the patients are shown in Table 1. Seventeen carriers of the factor V Leiden mutation were also carriers of the G20210A prothrombin mutation; none of the patients were homozygous for the prothrombin mutation. There were no significant differences among the group of patients with neither mutation, the group that was heterozygous for factor V Leiden alone, and the group that was heterozygous for both factor V Leiden and the prothrombin mutation with respect to sex distribution, age at the time of the first episode of deep venous thrombosis, age at referral, interval from the first episode of deep venous thrombosis to recurrence or referral, or rate of spontaneous first thrombotic events. With one exception, the prevalence of known risk factors at the time of the first event did not differ among the groups; pregnancy and recent childbirth were more common among the women who had one or both mutations than among those with neither mutation.

Risk of Recurrent Deep Venous Thrombosis

At the time of referral, the cumulative incidence of recurrent deep venous thrombosis was 30 percent among carriers of factor V Leiden alone and patients with neither mutation. The cumulative incidence was more than twice as high (65 percent) among the 17 carriers of both factor V Leiden and the G20210A prothrombin mutation ($P=0.005$ and $P=0.003$, respectively, by the chi-square test) (Table 2).

In general, patients with a first episode of deep venous thrombosis should be advised not to take oral contraceptives and to start antithrombotic prophylaxis during situations that increase the risk of recurrence, such as surgery or pregnancy. Our patients did not always follow these recommendations; therefore, 39 of 131 recurrences (30 percent) occurred among patients with a known risk factor; however, the prevalence of recurrences that were associated with a known risk factor was similar in the three groups: 10 percent among carriers of factor V Leiden alone, 9 percent among patients with neither mutation, and 6 percent among carriers of both factor V Leiden and the prothrombin mutation ($P=0.87$). When only spontaneous recurrences were considered, the cumulative incidence was still higher among patients with both mutations (59 percent) than among those with factor V Leiden alone (21 percent, $P<0.001$) or those with neither mutation (21 percent, $P<0.001$) (Table 2).

Kaplan–Meier analysis showed that the likelihood of thrombosis-free survival after the first event was

TABLE 1. CLINICAL CHARACTERISTICS OF THE PATIENTS WITH DEEP VEIN THROMBOSIS.*

CHARACTERISTIC	PATIENTS WHO WERE HETEROZYGOUS FOR FACTOR V LEIDEN AND G20210A PROTHROMBIN MUTATION (N=17)		PATIENTS WHO WERE HETEROZYGOUS FOR FACTOR V LEIDEN (N=112)		PATIENTS WITH NEITHER MUTATION (N=283)	
	INCIDENCE	RELATIVE RISK (95% CI)†	INCIDENCE	RELATIVE RISK (95% CI)‡	INCIDENCE	RELATIVE RISK (95% CI)‡
Sex						
Male	9		51		120	
Female	8		61		163	
Ratio	1.12		0.84		0.74	
Age at first episode of DVT — yr						
Mean	32		35		36	
Median	29		32		35	
Range	6–67		15–69		6–72	
Age at referral — yr						
Mean	43		43		44	
Median	41		41		44	
Range	21–70		18–75		9–77	
Interval from first episode of DVT to recurrence or referral — yr						
Mean	5		6		6	
Median	3		4		3	
Range	1–19		1–34		1–41	
Spontaneous first episode of DVT — no. of patients (%)	8 (47)		41 (37)		114 (40)	
Risk factors associated with first episode of DVT						
Oral-contraceptive use — no. of female patients/total no. (%)	4/8 (50)		26/61 (43)		55/163 (34)	
Pregnancy or recent childbirth — no. of female patients/total no. (%)	2/8 (25)		22/61 (36)		28/163 (17)	
Surgery — no. of patients/total no. (%)	2/17 (12)		10/112 (9)		42/283 (15)	
Trauma or leg cast — no. of patients/total no. (%)	0/17		7/112 (6)		30/283 (11)	
Bed rest — no. of patients/total no. (%)	1/17 (6)		6/112 (5)		14/283 (5)	

*DVT denotes deep vein thrombosis.

TABLE 2. RELATIVE RISK OF RECURRENT DEEP VEIN THROMBOSIS.*

VARIABLE	PATIENTS WHO WERE HETEROZYGOUS FOR FACTOR V LEIDEN AND G20210A PROTHROMBIN MUTATION (N=17)					PATIENTS WHO WERE HETEROZYGOUS FOR FACTOR V LEIDEN (N=112)			PATIENTS WITH NEITHER MUTATION (N=283)	
	INCIDENCE	RELATIVE RISK (95% CI)†		P VALUE	RELATIVE RISK (95% CI)‡	P VALUE	RELATIVE RISK (95% CI)‡			P VALUE
		no. (%)	no. (%)				no. (%)			
Recurrent DVT	11 (65)	2.6 (1.3–5.1)	0.002	2.7 (1.4–5.0)	<0.001	34 (30)	1.1 (0.7–1.6)	0.76	86 (30)	
Spontaneous recurrent DVT	10 (59)	3.7 (1.7–7.7)	<0.001	3.4 (1.7–6.6)	<0.001	23 (21)	1.0 (0.6–1.6)	0.81	59 (21)	
Spontaneous recurrent DVT after a spontaneous first episode of DVT§	7 (88)	5.4 (2.0–14.1)	<0.001	5.1 (2.2–11.4)	<0.001	10 (24)	1.0 (0.5–2.0)	0.97	33 (29)	
Spontaneous recurrent DVT after a secondary first episode of DVT¶	3 (33)	2.1 (0.6–7.3)	0.22	1.9 (0.6–6.2)	0.26	13 (18)	1.2 (0.6–2.3)	0.65	26 (15)	

*The P values were calculated by the log-rank test. CI denotes confidence interval, and DVT deep vein thrombosis.

†The comparison group is the group of patients who were heterozygous for factor V Leiden.

‡The comparison group is the group of patients with neither mutation.

§The percentages are calculated on the basis of the total number of spontaneous first episodes of deep vein thrombosis: 8 among the patients who were heterozygous for both mutations, 41 among those who were heterozygous for factor V Leiden, and 114 among those with neither mutation.

¶The percentages are calculated on the basis of the total number of secondary first episodes of deep vein thrombosis: 9 among the patients who were heterozygous for both mutations, 71 among those who were heterozygous for factor V Leiden, and 169 among those with neither mutation.

similar among carriers of factor V Leiden and patients with neither mutation (relative risk of recurrence among the carriers, 1.1; $P=0.76$) (Table 2). Stratification of the risk of recurrence among patients who were heterozygous for factor V Leiden alone as compared with those with neither mutation according to whether the first event was spontaneous or due to known risk factors or to whether the recurrence was spontaneous or due to known risk factors did not change the results substantially (Table 2). Analysis of the time-to-recurrence curves according to sex showed that the presence of factor V Leiden did not significantly increase the risk of recurrent deep venous thrombosis among men (relative risk, 1.1; 95 percent confidence interval, 0.6 to 1.9; $P=0.77$) or women (relative risk, 1.0; 95 percent confidence interval, 0.5 to 1.7; $P=0.89$).

The overall risk of recurrent deep venous thrombosis among patients who were heterozygous for both factor V Leiden and the G20210A prothrombin mutation was 2.6 times as high as that among patients who were heterozygous for factor V Leiden alone and 2.7 times as high as that among patients with neither mutation (Table 2). The relative risk of spontaneous recurrent deep venous thrombosis was 3.7 among patients with both mutations, as compared with patients who were heterozygous for factor V Leiden alone (Table 2). Further stratification of the risk of recurrent thrombosis among patients who were heterozygous for both mutations according to whether the first episode of thrombosis was spontaneous or due to known risk factors produced statistical instability because of the small number of patients, as reflected by the wide 95 percent confidence intervals. Yet the spontaneous occurrence of an initial episode of thrombosis further increased the relative risk, to 5.4, as compared with the patients who were heterozygous for factor V Leiden alone (Table 2). The risk of recurrence of a spontaneous episode of deep venous thrombosis was not increased by the fact that the initial episode occurred in the presence of known risk factors (Table 2).

DISCUSSION

After a first episode of deep venous thrombosis, the risk of recurrence is higher among patients with a persistent risk factor (such as cancer or inherited thrombophilia) than among those with a transient risk factor (such as oral-contraceptive use, pregnancy or recent childbirth, surgery, or prolonged immobilization).²¹ There is currently no general consensus on the optimal duration of treatment with oral anticoagulants after a first episode of deep venous thrombosis in patients with inherited thrombophilia. Usually, long-term treatment is not recommended if the first episode occurred in association with known transient risk factors, whereas long-term treatment is considered appropriate when deep venous thrombosis

developed spontaneously.^{18,22,23} The decision should take into account the type and severity of the genetic defect, the number of genetic defects, the site of the first thrombotic event, and whether there is a family history of thromboembolic episodes. The risk of recurrent thrombosis has to be weighed against the risk of major bleeding that is associated with long-term oral anticoagulation, which is estimated to range from 1.1 percent to 3.8 percent per patient-year.^{24,25}

The high prevalence of factor V Leiden among patients with deep venous thrombosis prompted studies to determine the risk of recurrence associated with this mutation after a first episode of deep venous thrombosis. An increase in the risk ranging from 2.4 to 4.1 has been reported by some authors^{4,5} but not by others.⁶⁻⁸ Some of these studies suffered from limitations related to the relatively small number of patients who had the mutation⁴⁻⁶ or to the short observation period.^{4,6,7} In addition, most of them were carried out before the identification of the G20210A prothrombin mutation as a cause of inherited thrombophilia. Therefore, some discrepancies in the results may be due to the simultaneous presence of this genetic defect in some carriers of factor V Leiden. This view is supported by the observation that in small series of patients with venous thrombosis and inherited thrombophilia, patients who had the G20210A prothrombin mutation had a higher risk of recurrence than those with a single thrombophilic trait.^{14,17}

To evaluate the risk of recurrent deep venous thrombosis among patients with factor V Leiden alone or in association with the G20210A prothrombin mutation, we carried out a retrospective cohort study. None of the patients had other risk factors for thrombosis, such as deficiencies of naturally occurring coagulation inhibitors, cancer, or myeloproliferative or autoimmune diseases. Although hyperhomocysteinemia has been reported as an important synergistic risk factor for deep venous thrombosis in patients with factor V Leiden,²⁶ we chose not to include it among the exclusion criteria, because the retrospective nature of our study and the possible changes in the homocysteine levels as a result of changes in dietary habits during the observation period might have produced a misleading classification of the patients.

Patients who had only factor V Leiden had a risk of recurrent deep venous thrombosis that was nearly identical to that among patients with neither mutation, irrespective of the outcome analyzed (any type of recurrence, spontaneous recurrence, or spontaneous recurrence after either a spontaneous first episode or an episode due to known risk factors). The presence of both factor V Leiden and the G20210A prothrombin mutation increased the risk of recurrent deep venous thrombosis by a factor of 2.6 and the risk of a spontaneous recurrence by a factor of 3.7, as compared with the risk among patients who

were heterozygous for factor V Leiden alone. The relative risk of recurrent deep venous thrombosis among carriers of both mutations, as compared with carriers of factor V Leiden alone, was not significantly increased when the first episode occurred in the presence of a known risk factor.

We do not know whether the increased risk of recurrence among heterozygous carriers of factor V Leiden and the G20210A prothrombin mutation is due to an interaction between the two mutations or simply to the presence of the mutant prothrombin gene. The latter possibility is not supported by the results of two recent prospective studies, which failed to find an increased risk of recurrent deep venous thrombosis in association with the G20210A prothrombin mutation.^{8,27}

Although we studied a selected group of patients, our results can be applied to the majority of patients with factor V Leiden (with or without the G20210A prothrombin mutation) and a previous episode of deep venous thrombosis. In our initial retrospective cohort of 624 consecutive patients, the prevalence of heterozygosity for factor V Leiden was 21 percent, in close agreement with previous data obtained in consecutive patients with deep venous thrombosis.² In our cohort, a large fraction of first events was associated with oral-contraceptive use or pregnancy or recent childbirth, yet when the analysis was restricted to male patients or to patients who had had a spontaneous first episode of deep venous thrombosis, there was a low risk of recurrence associated with the presence of factor V Leiden alone, as compared with the absence of both mutations.

The retrospective nature of our study and the use of recurrence of deep venous thrombosis as the primary outcome may have led to bias. The diagnosis of a first episode of deep venous thrombosis or recurrence was made by the attending hospital physicians before the patients were referred to the thrombosis centers, and each episode was validated by the staff members of the centers before laboratory screening was conducted, so that the interviewers were unaware of the genotype of the patients. Moreover, the period after referral to the centers was not considered, because patients with a diagnosis of inherited thrombophilia were likely to be observed carefully for the onset of recurrent deep venous thrombosis, whereas patients with no identified cause of thrombosis in many cases had no further contact with the centers. Thus, it is unlikely that the rate of recurrence was overestimated in patients with mutations causing thrombophilia. A number of recurrences may have been missed because of the retrospective evaluation of medical records and because episodes were counted as recurrent only in the presence of objective documentation. However, there is no reason to think that the pattern of underestimation should have differed among the three groups.

From a practical point of view, our findings suggest that heterozygous carriers of factor V Leiden with no additional thrombophilic traits should not receive lifelong anticoagulation after a first episode of deep venous thrombosis and that they should be treated like patients with no known mutations. This suggestion is also supported by a recent study of the optimal duration of anticoagulant treatment after a spontaneous first episode of deep venous thrombosis.²⁵ That study failed to find an association between factor V Leiden and recurrent thromboembolism. Our results also support the use of thorough screening for thrombophilia in patients with deep venous thrombosis, because a finding of heterozygosity for both factor V Leiden and the G20210A prothrombin mutation should prompt lifelong treatment with oral anticoagulants. Such a recommendation appears to apply in particular to patients in whom the first episode of deep venous thrombosis occurred in the absence of transient risk factors. Prospective cohort studies are needed to confirm our findings.

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