

The Pathogenesis of Venous Thromboembolism: Evidence for Multiple Interrelated Causes

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Background: Venous thromboembolism (VTE) is thought to result from interactions between multiple genetic and environmental risk factors.

Objective: To assess the contribution of multiple thrombophilic defects and exogenous risk factors to the absolute risk for VTE.

Design: Retrospective family cohort study.

Setting: Single university hospital.

Participants: 468 relatives of 91 probands with a symptomatic hereditary deficiency of protein S, protein C, or antithrombin.

Measurements: All relatives were tested for 10 thrombophilic deficiencies and defects in addition to the index deficiency and were assessed for exogenous risk factors (surgery, trauma, immobilization, use of oral contraceptives, and pregnancy). The authors compared annual incidences and relative risks for VTE in deficient and nondeficient relatives.

Results: Annual incidences of VTE in relatives with 0, 1, and 2 or more additional thrombophilic deficiencies or defects were 1.16 (95% CI, 0.60 to 2.03), 1.75 (CI, 1.17 to 2.53), and 2.64 (CI, 1.67 to 3.96) per 100 person-years, respectively, compared with 0.06

(CI, 0.002 to 0.33) per 100 person-years in nondeficient relatives without additional deficiencies or defects. Adjusted relative risks were 16.3 (CI, 2.0 to 131.0), 50.3 (6.5 to 389.7), and 102.8 (12.5 to 843.4). Of deficient relatives, 38% with no additional defect, 57% with 1 additional defect, and 81% with 2 or more additional defects had VTE at age 65 years compared with 5% of nondeficient relatives ($P < 0.001$). In deficient relatives with additional deficiencies or defects, exogenous risk factors increased the risk for VTE from 1.20% to 2.51% per year (relative risk, 2.1 [CI, 1.1 to 4.2]).

Limitations: This was a retrospective study without the ability to distinguish interactions between specific thrombophilic deficiencies and defects.

Conclusion: Additional thrombophilic defects and exogenous risk factors increase the risk for VTE in persons with hereditary deficiencies of protein S, protein C, or antithrombin and provide evidence that multiple genetic and environmental risk factors contribute to VTE.

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Venous thromboembolism (VTE) has an incidence of 0.1 to 0.2 per 100 person-years (1, 2). It has been speculated that the development of VTE results from interactions between multiple genetic and environmental risk factors (3). Several inherited or acquired coagulation defects have been identified as VTE risk factors over the past 30 years. Known thrombophilic defects include hereditary deficiencies of protein S, protein C, and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of coagulant factors VIII, IX, and XI; hyperhomocysteinemia; and antiphospholipid antibodies (4). Whether patients with VTE and their relatives should be tested for all of these defects is still debatable. Clinical implications mainly depend on the absolute risk for a first or recurrent episode of VTE in persons with a single defect or in those with a combination of defects. Most common defects are mild risk factors for VTE. In contrast, hereditary deficiencies of protein S, protein C, and antithrombin are strong risk factors but are rare. Although interactions between these deficiencies and 1 or more other thrombophilic defects might increase the risk for VTE, they cannot be studied easily because the prevalence of such combinations is low. Thus far, only a few studies have reported the risk for VTE associated with coinheritance of deficiencies of protein S, protein C, or antithrombin and factor V Leiden or the prothrombin G20210A mutation (5–10). The results

were not consistent, possibly because of small numbers of cases.

We performed a retrospective study to assess the contribution of currently known hereditary thrombophilic defects and exogenous risk factors to the absolute risk for VTE in a large series of protein S-, protein C-, or antithrombin-deficient families.

METHODS

We aimed to assess interactions between known thrombophilic deficiencies and defects, including hereditary deficiencies of protein S, protein C, and antithrombin. Because these deficiencies are strong risk factors for VTE, the effects and clinical impact of interactions with more prevalent and mild thrombophilic defects may be more pronounced than interactions between mild defects. To

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Context

Venous thromboembolism (VTE) is thought to arise from the interaction of environmental and genetic factors in persons predisposed to VTE.

Contribution

The authors studied persons with protein S, protein C, or antithrombin deficiency who were first-degree relatives of persons who had VTE. They assessed each relative for environmental exposures and additional thrombophilic defects. They found that risk for VTE increased with the number of defects and with exposure to environmental risk factors.

Cautions

The findings were not based on the total population of relatives. The authors could not quantify risk for interactions between specific defects and environmental risk factors.

Implications

The risk for VTE increases with the number of thrombophilic defects and environmental risk factors in persons with a hereditary predisposition to VTE. The study provides evidence that VTE arises from interactions between environmental and genetic risk factors and quantifies the risks.

—The Editors

enroll sufficient numbers of affected persons needed for accurate risk estimates, we identified families with these rare deficiencies. The family cohort study design enabled us to assess the clinical impact of interactions from absolute risk estimates.

Participants

The study comprised 3 cohorts of families with hereditary deficiencies of protein S, protein C, or antithrombin. Proband was consecutive patients with VTE who had 1 of these deficiencies. Primary care physicians referred patients with clinically suspected VTE to 1 of 2 hospitals in our region of the Netherlands; 50% were referred to the thrombosis outpatient clinic of our university hospital. Previous clinical trials did not show differences between patients who were referred to either hospital for this reason. Because it is common practice in the Netherlands to confirm suspected VTE, the proportion of patients who were not referred was probably small. All patients who had VTE confirmed at our outpatient clinic were tested for protein S, protein C, or antithrombin deficiencies, unless they had extensive malignant disease. After a deficiency was established by repeated measurement and after causative acquired conditions were excluded, the patient's first-degree relatives who were older than age 15 years were identified by pedigree analysis and were contacted through the probands. Because the number of antithrombin-

deficient probands was small, second-degree relatives with a deficient parent were also identified. Relatives were assessed for deficiency at the time of identification and then were followed for thromboembolic events from age 15 years to the time of testing. All participants provided informed consent. Physicians at our outpatient clinic collected detailed information about previous episodes of VTE, exposure to exogenous risk factors for VTE, and anticoagulant treatment using a validated questionnaire (11) and by reviewing medical records. The use of oral contraceptives and an obstetric history were documented for women. Blood samples were taken after clinical data had been collected. All relatives were tested for 10 thrombophilic deficiencies and defects in addition to their index deficiencies, including deficiencies of protein S, protein C, antithrombin, and plasminogen; factor V Leiden; prothrombin G20210A; high levels of factors VIII, IX, and XI; hyperhomocysteinemia; and lupus anticoagulant.

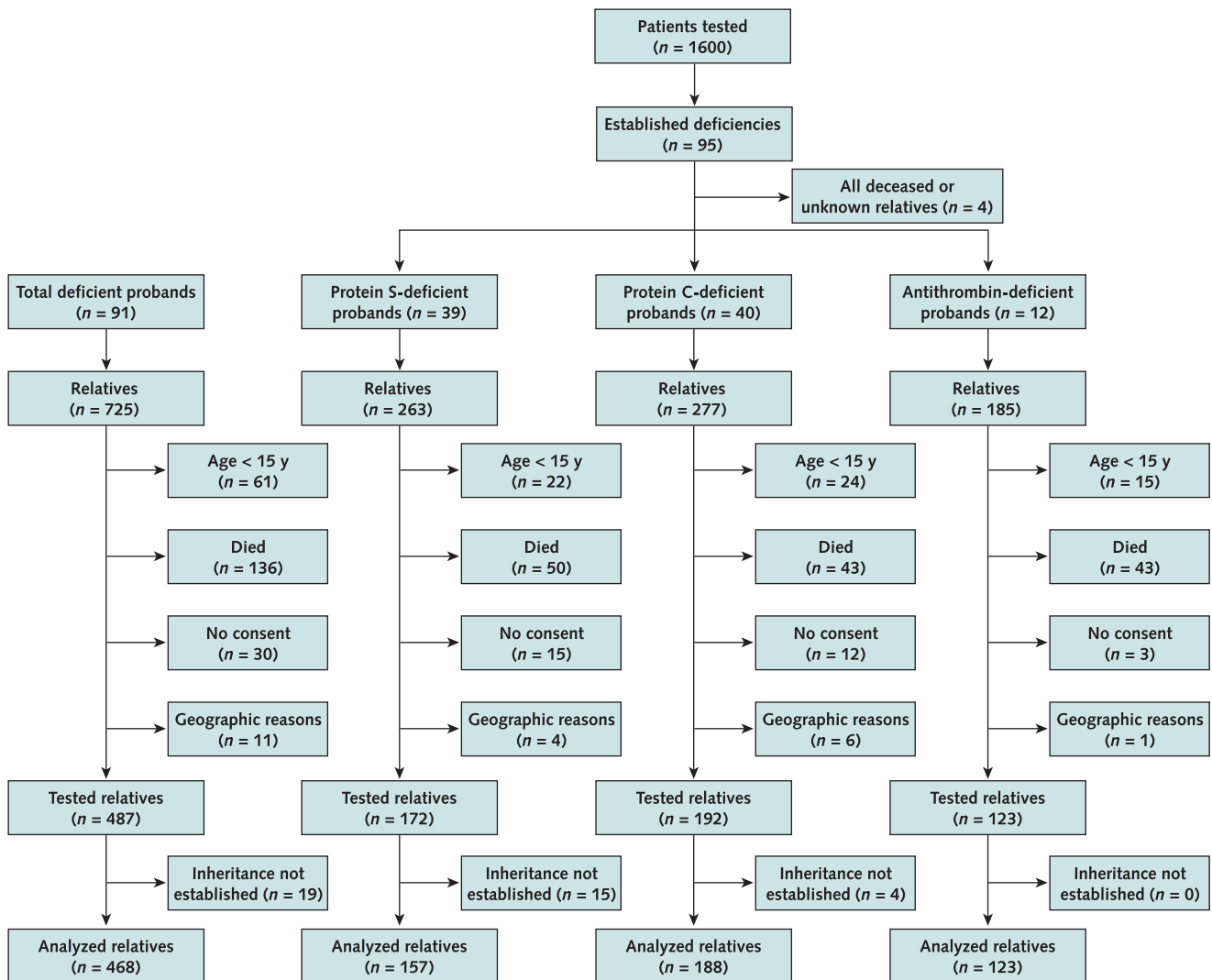
Diagnosis of VTE

Venous thromboembolism was considered established if deep venous thrombosis was confirmed by compression ultrasonography or venography, and pulmonary embolism was confirmed by ventilation–perfusion lung scanning, spiral computed tomography scanning, or pulmonary angiography. When these techniques were not yet available, VTE was considered established when the patient had received full-dose unfractionated heparin and a vitamin K antagonist for at least 3 months. Venous thromboembolism was considered secondary if it had occurred at or within 3 months after exposure to exogenous risk factors, including major surgery, trauma, immobilization for more than 7 days, oral contraceptives, hormone replacement therapy, pregnancy, and malignant disease. In the absence of these risk factors, VTE was considered primary.

Laboratory Studies

Protein S and protein C antigen levels were measured by enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark); activity of protein C (Berichrom Protein C, Dade Behring, Marburg, Germany), antithrombin (Coatest, Chromogenix AB, Mölndal, Sweden), and plasminogen (S2251, Chromogenix AB) was measured by chromogenic substrate assays. Levels of protein S, protein C, and antithrombin were expressed as a percentage of the levels measured in pooled plasma set at 100%. Normal ranges were determined in 393 healthy blood donors who had no family history of VTE, were not pregnant, and had not used oral contraceptives for at least 3 months. Protein S deficiency type I was defined by decreased free and total protein S levels, that is, below normal ranges, and protein S deficiency type III was defined by decreased free protein S levels and normal total protein S levels. After we had demonstrated that type III protein S deficiency was not a risk factor for thrombosis, families with this deficiency were excluded from the analysis (12). Protein C deficiency type I and type II were defined by decreased levels or ac-

Figure 1. Recruitment of 3 family cohorts with hereditary deficiencies of protein S, protein C, or antithrombin.



tivity of protein C antigen, and antithrombin deficiency was defined by decreased levels of antithrombin activity. Deficiencies were considered inherited if they were confirmed by measuring a second sample that was collected 3 months later and were found in at least 2 family members. Relatives with acquired conditions were excluded. If there was a discrepancy between the results of the 2 tests, a third sample was tested. A deficiency was considered acquired, through use of oral contraceptives or pregnancy, unless it was confirmed at least 3 months after withdrawal of oral contraceptives or delivery, respectively. Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reaction (13, 14). Factors VIII:C, IX:C, and XI:C were measured by 1-stage clotting assays (Amelung GmbH, Lemgo, Germany) and were increased at levels above 150%. Lupus anticoagulant was defined by abnormal values of dilute Russell viper venom

time, activated partial thromboplastin time, and tissue thromboplastin inhibition, which normalized by adding phospholipids to the participant's plasma (15). Fasting and postmethionine-loading levels of homocysteine were measured by high-performance liquid chromatography (16). Hyperhomocysteinemia was defined as a fasting homocysteine level above $18.5 \mu\text{mol/L}$, a postloading level above $58.8 \mu\text{mol/L}$, or both, as described in a Dutch population (17). Blood samples were collected from probands and relatives at least 3 months after VTE. If probands or relatives were receiving long-term treatment with vitamin K antagonists, samples were taken after treatment had been interrupted for at least 2 weeks; in the meantime, nadroparin was given subcutaneously. Additional tests were performed on plasma stored at -80°C from relatives who had been enrolled at a time when 1 of more of these deficiencies or defects had not yet been recognized as risk factors for VTE.

Table 1. Characteristics of 468 Relatives of Families with Inherited Protein S, Protein C, or Antithrombin Deficiency*

Variable	All Families†		P Value	Protein S–Deficient Families‡		P Value	Protein C–Deficient Families‡		P Value	Antithrombin-Deficient Families‡		P Value
	Deficient	Nondeficient		Deficient	Nondeficient		Deficient	Nondeficient		Deficient	Nondeficient	
Relatives, n	224	244		75	82		82	106		67	56	
Women, n (%)	110 (49)	128 (52)	0.52	36 (48)	44 (54)	0.52	40 (49)	54 (51)	0.88	34 (51)	30 (54)	0.86
Median age at enrollment, (range), y	41 (15–89)	47 (15–92)	0.050	41 (15–84)	47 (15–84)	0.20	45 (15–89)	48 (15–86)	0.33	39 (15–84)	46 (16–92)	0.21
VTE, n	69	7	<0.001	22	5	<0.001	25	2	<0.001	22	0	<0.001
Median age at onset, (range), y	30 (5–64)	28 (18–48)	0.52	31 (16–64)	28 (18–48)	0.64	31 (16–63)	31 (20–42)	0.43	29 (5–60)		
Deep venous thrombosis, n (%)	55 (80)	4 (57)		16 (73)	3 (60)		22 (88)	1 (50)		17 (77)		
Pulmonary embolism, n (%)	7 (10)	1 (14)		3 (14)	0 (0)		3 (32)	1 (50)		1 (5)		
Other locations, n (%)‡	7 (10)	2 (29)		3 (14)	2 (40)		0	0		4 (18)		
Primary VTE, n (%)	33 (48)	1 (14)	0.41	13 (59)	1 (20)	0.115	11 (44)	0	0.27	9 (41)		
Secondary to major surgery, trauma, or immobilization, n (%)§	15 (22)	3 (43)		6 (27)	1 (20)		6 (24)	2 (100)		3 (14)		
Oral contraceptive use, n (%)	9 (13)	1 (14)		2 (9)	1 (20)		6 (24)	0		1 (5)		
Pregnancy or puerperium, n (%)	12 (17)	2 (29)		1 (5)	2 (40)		2 (8)	0		9 (41)		
Smoking, n (%)	32 (46)	2 (29)	0.45	12 (55)	2 (40)	0.65	11 (44)	0	0.50	9 (41)		
Median exposures to exogenous risk factors, (range), n	1 (0–5)	1 (0–5)	0.062	1 (0–5)	1 (0–5)	0.134	1 (0–5)	1 (0–5)	0.194	1 (0–5)	1 (0–5)	0.070
Observation period, person-years	4174	6425		1464	2196		1579	2738		1131	1491	
Annual incidence (95% CI), n/100 person-years	1.65 (1.29–2.09)	0.11 (0.04–2.22)		1.50 (0.94–2.28)	0.23 (0.07–0.53)		1.58 (1.02–2.34)	0.07 (0.01–0.26)		1.94 (1.22–2.94)	0.00 (0.0–0.25)	
Adjusted relative risk (95% CI)	16.8 (7.2–39.3)	1		16.2 (6.1–43.4)			16.2 (6.4–41.2)			18.3 (6.7–50.1)		

* VTE = venous thromboembolism.

† Familial inheritance established. Individual relatives within families may be deficient or nondeficient in protein S, protein C, or antithrombin.

‡ Includes caval, mesenteric, axillary, and cerebral veins.

§ No cases of venous thromboembolism secondary to estrogen replacement therapy or malignant disease.

|| Adjusted for clustering in families.

Statistical Analysis

We compared the absolute risk for VTE in deficient and nondeficient relatives in each of the 3 cohorts and in the pooled cohorts. Probands were excluded from the analysis to avoid selection bias. Annual incidences were calculated by dividing the number of symptomatic relatives by the total number of observation-years. Ninety-five percent CIs were calculated around the incidence rates by using the Poisson distribution assumption. Observation time was defined as the period from age 15 years until the first thrombotic episode or until study entry, because VTE is rare in younger persons.

Crude relative risks were based on annual incidences, and 95% confidence limits were calculated by using the binomial probability model (conditional small-sample approach) (18). To account for clustering within families, outcome rates were analyzed by using random-effects logistic regression with Gaussian distribution in Stata, version 9.1 (Stata Corp., College Station, Texas), which resulted in adjusted relative risks and 95% confidence limits.

The effects of additional thrombophilic defects were assessed in separate and pooled cohorts. Because combinations of defects were numerous, relatives were categorized

according to the number of concomitant defects, provided that the risk for VTE was comparable for individual defects.

Continuous variables were expressed as median values and ranges, and categorical data were expressed as counts and percentages. Differences between groups were evaluated by using the Student *t*-test or the Mann–Whitney U test, depending on the normality of data, for continuous data and by using the Fisher exact test for categorical data. A 2-tailed *P* value less than 0.05 indicated statistical significance.

Analyses were performed by using SAS software, version 6.12 (SAS Institute, Inc., Cary, North Carolina), and Stata, version 9.1.

Role of the Funding Source

The institutional review board of our hospital approved the study. We did not receive external funding to conduct the study.

RESULTS

Sixteen hundred consecutive patients with VTE were screened over 12 years to identify 91 probands, for a 5.7%

prevalence of protein S, protein C, or antithrombin deficiency. For living relatives, response rates between 90% and 97% per cohort allowed us to identify 725 relatives who were the participants in our study. **Figure 1** details the reasons for excluding 257 relatives. Sixty-one (8%) relatives were younger than age 15 years, 136 (19%) died before enrollment (VTE was a possible cause of death in 8 [6%]), 30 (4%) would not or could not provide consent because of mental illness, and 11 (15%) could not be studied because of geographic distance. Nineteen additional relatives also were excluded because inheritance of the index deficiency could not be established (15 relatives of 5 probands in the protein S cohort and 4 relatives of 2 probands in the protein C cohort). The remaining 468 relatives were analyzed, comprising 263 first-degree relatives of 39 protein S type I–deficient probands (protein S cohort), 277 first-degree relatives of 40 protein C–deficient probands (protein C cohort), and 185 relatives (92 first- and 93-second degree) of 12 antithrombin-deficient probands (antithrombin cohort) (**Figure 1**). Seventy-one of these 468 relatives were excluded from analyses of additional deficiencies or defects because tests could not be performed in all cases (for example, because of shortage of plasma).

Table 1 summarizes the characteristics of the relatives. Deficiencies were demonstrated in 48% of relatives and were equally distributed among men and women. Median follow-up in deficient and nondeficient relatives was 16 years (range, 0 to 61 years) and 26 years (range, 0 to 77 years), respectively. Venous thromboembolism occurred in 29%, 31%, and 33% of relatives with protein S deficiency, protein C deficiency, and antithrombin deficiency, respectively, compared with 6%, 2%, and 0% of nondeficient relatives, respectively. The median age at onset of the first episode of VTE was 30 years. Fifty-two percent of events in relatives with a deficiency had identifiable exogenous

risk factors. Overall, the numbers of relatives with exogenous risk factors and the median number of exogenous risk factors were comparable in deficient and nondeficient relatives.

Annual incidences of VTE were 1.50 per 100 person-years, 1.58 per 100 person-years, and 1.94 per 100 person-years in protein S–deficient relatives, protein C–deficient relatives, and antithrombin-deficient relatives, respectively, compared with 0.23, 0.07, and 0.0 per 100 person-years in nondeficient relatives, respectively (**Table 1**). Relative risks adjusted for clustering in families with deficient relatives were 16.2 (95% CI, 6.1 to 43.4) for protein S deficiency, 16.2 (CI, 6.4 to 41.2) for protein C deficiency, and 18.4 (CI, 6.7 to 50.1) for antithrombin deficiency compared with nondeficient relatives.

One or more additional deficiencies or defects were demonstrated in 61% of the 397 relatives who were completely tested. One additional defect was demonstrated in 164 relatives (41%), and 2 or more additional defects were demonstrated in 79 relatives (20%). Four relatives from 4 unrelated families had a second deficiency of protein S, protein C, or antithrombin, but because inheritance of second deficiencies was not established, these deficiencies were considered to be acquired and were not counted as additional thrombophilic defects in risk analyses. Plasminogen deficiency was demonstrated in only 5 relatives. Lupus anticoagulant was not found in any participants. The remaining defects were equally distributed among deficient and nondeficient relatives in each cohort (**Table 2**).

Annual incidences of VTE in deficient relatives with 2 or more concomitant deficiencies or defects compared with deficient relatives without concomitant deficiencies or defects were 2.14 (CI, 1.03 to 3.94) versus 0.63 (CI, 0.08 to 2.29) per 100 person-years for protein S deficiency, 2.56 (CI, 1.03 to 5.28) versus 0.98 (CI, 0.20 to 2.87) per 100

Table 2. Prevalence of Additional Thrombophilic Deficiencies and Defects in 468 Relatives of Families with Inherited Protein S, Protein C, or Antithrombin Deficiency

Additional Thrombophilic Deficiencies or Defects	All Families		P Value	Protein S–Deficient Families		P Value	Protein C–Deficient Families		P Value	Antithrombin-Deficient Families		P Value
	Deficient (n = 224)	Nondeficient (n = 244)		Deficient (n = 75)	Nondeficient (n = 82)		Deficient (n = 82)	Nondeficient (n = 106)		Deficient (n = 67)	Nondeficient (n = 56)	
Second deficiency, %*												
Protein S deficiency	0.5 (210)	0 (233)	0.47				1 (80)	0 (103)	0.40	0 (55)	0 (48)	
Protein C deficiency	0.5 (189)	0 (235)	0.45	0 (65)	0 (82)					1 (42)	0 (47)	0.74
Antithrombin deficiency	0.9 (222)	1 (243)	1.0	0 (74)	0 (82)		1 (81)	1 (105)	0.40			
Plasminogen deficiency, %*	1 (208)	1 (229)	1.0	1 (74)	0 (81)	0.48	1 (79)	2 (101)	1.0	0 (55)	1 (47)	0.46
Factor V Leiden, %*†	14 (203)	17 (220)	0.42	17 (66)	14 (77)	0.82	17 (77)	25 (96)	0.26	7 (60)	4 (47)	0.69
Prothrombin G20210A, %*†	7 (196)	9 (216)	0.46	3 (65)	8 (76)	0.29	10 (74)	8 (93)	0.78	7 (57)	13 (47)	0.34
Factor VIII level >150%, %*	39 (189)	34 (202)	0.34	38 (63)	29 (75)	0.28	44 (70)	39 (80)	0.51	32 (56)	32 (47)	1.0
Factor IX level >150%, %*	9 (149)	15 (209)	0.075	11 (54)	19 (72)	0.23	7 (55)	13 (90)	0.29	8 (40)	13 (47)	0.49
Factor XI level >150%, %*	11 (196)	11 (211)	1.0	11 (65)	16 (73)	0.46	12 (74)	8 (90)	0.43	9 (57)	8 (48)	1.00
Hyperhomocysteinemia, %*	17 (186)	17 (191)	1.0	19 (63)	19 (69)	1.0	19 (68)	10 (77)	0.160	13 (55)	27 (45)	0.123
Fasting homocysteine level >18.5 μmol/L, %	13	13	1.0	13	12	1.0	16	9	0.22	9	20	0.151
Postloading homocysteine level >58.5 μmol/L, %	10	9	1.0	15	16	1.0	5	3	1.0	9	6	1.0
Lupus anticoagulant, %*	0 (166)	0 (177)		0 (52)	0 (62)		0 (63)	0 (71)		0 (51)	0 (47)	

* The numbers in parentheses are the numbers of relatives who were tested.

† All carriers were heterozygous. Relatives with multiple additional defects were counted accordingly.

Table 3. Risk for Venous Thromboembolism Associated with Multiple Thrombophilic Defects in Relatives with and without Protein S, Protein C, or Antithrombin Deficiency*

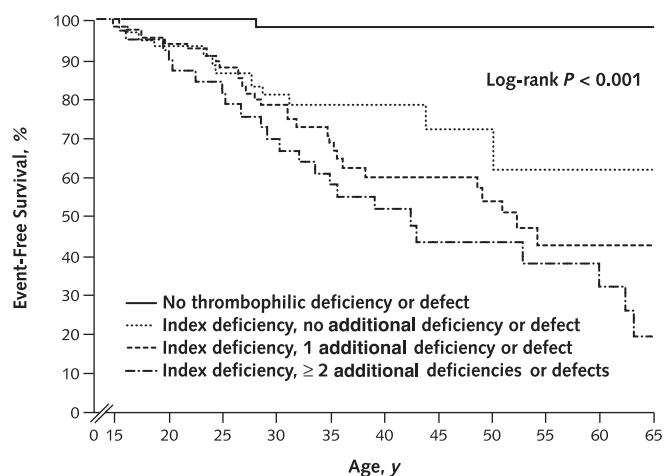
Variable	Relatives without Protein S, Protein C, or Antithrombin Deficiency			Relatives with Protein S, Protein C, or Antithrombin Deficiency		
	No Additional Deficiencies or Defects	1 Additional Deficiency or Defect	≥2 Additional Deficiencies or Defects	No Additional Deficiencies or Defects	1 Additional Deficiency or Defect	≥2 Additional Deficiencies or Defects
Relatives, <i>n</i>	80	89	36	74	75	43
Relatives with event, <i>n</i>	1	2	4	12	28	23
Observation period, <i>y</i>	1681	2464	1297	1033	1597	872
Annual incidence (95% CI), <i>n</i> /100 person-years	0.06 (0.002–0.33)	0.08 (0.01–0.29)	0.31 (0.08–0.79)	1.16 (0.60–2.03)	1.75 (1.17–2.53)	2.64 (1.67–3.96)
Adjusted relative risk (CI)†	1	1.9 (0.2–21.3)	10.4 (1.1–99.1)	16.3 (2.0–131.0)	50.3 (6.5–389.7)	102.8 (12.5–843.4)

* Relatives who were not completely tested for concomitant thrombophilic defects (39 nondeficient and 32 deficient) are excluded.
 † Adjusted for clustering in families.

person-years for protein C deficiency, and 4.55 (CI, 1.67 to 9.90) versus 1.70 (CI, 0.68 to 3.51) per 100 person-years for antithrombin deficiency. Annual incidences of VTE in pooled cohorts increased from 0.06 to 0.31 per 100 patient-years in nondeficient relatives with an increasing number of additional deficiencies or defects and from 1.16 to 2.64 per 100 person-years in deficient relatives (Table 3). Compared with nondeficient relatives without another thrombophilic deficiency or defect, adjusted relative risks were 10.4 (CI, 1.1 to 99.1) for nondeficient relatives with 2 or more other deficiencies or defects and 102.8 (CI, 12.5 to 843.4) for deficient relatives with 2 or more additional deficiencies or defects.

With respect to the time of onset of VTE, the annual incidence before age 50 years (0.7 [CI, 0.53 to 0.88]) was not significantly different from that between ages 50 and 65 years (1.14 [CI, 0.59 to 2.00]) (*P* = 0.122). Event-free survival in protein S–, protein C–, and antithrombin-deficient relatives was comparable (data not shown). At age 65 years, 66% of deficient relatives had VTE compared with 5% of nondeficient relatives. Additional deficiencies or defects decreased the probability of event-free survival (Figure 2). Among deficient relatives, VTE had occurred in 38% with no other deficiency or defect, 57% with 1 additional deficiency or defect, and 81% with 2 or more additional deficiencies or defects at age 65 years (*P* < 0.001).

Figure 2. Event-free survival in relatives with a deficiency of protein S, protein C, or antithrombin (index deficiency) and one or more additional thrombophilic deficiencies or defects.



Exposed relatives, <i>n</i>	80	56	50	35	13	6
No thrombophilic deficiency or defect	80	56	50	35	13	6
Index deficiency, no additional deficiency or defect	74	47	30	17	7	2
Index deficiency, 1 additional deficiency or defect	75	63	46	25	17	6
Index deficiency, ≥ 2 additional deficiencies or defects	43	35	24	15	9	7

Table 4. Risk for Venous Thromboembolism Associated with a Single Additional Thrombophilic Defect in Protein C-, Protein S-, or Antithrombin-Deficient Relatives

Variable	Total Relatives, n	Relatives with Event, n	Observation Period, y	Annual Incidence (95% CI), n/100 person-years	Crude Relative Risk (95% CI)
No deficiency and no other defect	80	1	1681	0.06 (0.002–0.33)	1.0 (reference)
Deficiency and 1 additional defect					
Factor V Leiden	11	6	114	5.26 (1.93–11.46)	88.5 (13.1–2048)
Prothrombin G20210A	2	1	56	1.79 (0.05–9.95)	30.0 (0.8–1171)
Factor VIII level >150%	38	14	955	1.47 (0.80–2.46)	24.6 (4.4–525)
Factor IX level >150%	3	1	80	1.25 (0.03–6.97)	21.0 (0.5–819)
Factor XI level >150%	9	3	161	1.86 (0.38–5.45)	31.3 (3.3–825)
Hyperhomocysteinemia	12	1	247	0.41 (0.01–2.26)	6.8 (0.2–265)

The risk for VTE in relatives who had an index deficiency combined with additional factor V Leiden or prothrombin G20210A, not accounting for other thrombophilic deficiencies or defects, was estimated to enable a comparison with previous studies. Annual incidences were 3.23 (CI, 1.76 to 5.41) per 100 person-years in deficient relatives who were carriers of factor V Leiden versus 1.55 (CI, 1.16 to 2.04) per 100 person-years in deficient relatives who were not carriers of factor V Leiden (relative risk, 2.1 [CI, 1.2 to 3.8]). The annual incidence of VTE was 3.95 (CI, 1.70 to 7.78) per 100 person-years for deficient relatives who were carriers of prothrombin G20210A versus 1.66 (CI, 1.25 to 2.15) per 100 person-years for deficient relatives who were not carriers of prothrombin G20210A (relative risk, 2.4 [CI, 1.1 to 5.0]).

Table 4 summarizes risk estimates in deficient relatives with a single additional defect, as established by negative results on tests for other defects. Annual incidences ranged from 0.41 (CI, 0.01 to 2.26) per 100 person-years for hyperhomocysteinemia to 5.26 (CI, 1.93 to 11.46) per 100 person-years for factor V Leiden. Compared with nondeficient relatives without any of the tested defects, relative risks ranged from 6.8 (CI, 0.2 to 265) for hyperhomocysteinemia to 88.5 (CI, 13.1 to 2048) for factor V Leiden.

Exposure to exogenous risk factors increased the annual incidence from 0.0 (CI, 0.0 to 1.43) per 100 person-years to 0.07 (CI, 0.0 to 0.39) per 100 person-years in nondeficient relatives without another deficiency or defect, and from 0.12 (CI, 0.0 to 0.68) per 100 person-years to 0.17 (CI, 0.06 to 0.40) per 100 person-years in nondeficient relatives with at least 1 concomitant deficiency or defect (Table 5). The annual incidence increased from 0.51 (CI, 0.01 to 2.84) per 100 person-years to 1.32 (CI, 0.66 to 2.35) per 100 person-years in deficient relatives without additional defects and from 1.20 (CI, 0.57 to 2.30) per 100 person-years to 2.51 (CI, 1.80 to 3.41) per 100 person-years in deficient relatives with concomitant defects.

DISCUSSION

This study shows that the risk for VTE associated with hereditary deficiencies of protein S, protein C, or antithrombin depends on the presence of other thrombophilic

defects and on exogenous risk factors. The highest incidence (2.64 per 100 person-years) was found in deficient relatives who had 2 or more additional deficiencies or defects. This was 2 times the incidence in deficient relatives without additional deficiencies or defects and 44 times the incidence in relatives without any of the tested thrombophilic deficiencies or defects.

Annual incidences in deficient relatives, regardless of additional defects, were 1.50 (protein S deficiency), 1.58 (protein C deficiency), and 1.94 (antithrombin deficiency) per 100 person-years. These incidences are in agreement with those reported from previous studies. The annual incidences in retrospective studies ranged from 1.0 to 3.1 per 100 person-years for protein S deficiency, from 1.0 to 3.0 per 100 person-years for protein C deficiency, and from 1.0 to 2.9 per 100 person-years for antithrombin deficiency (19–21). In prospective studies, these values were 1.3, 1.6, and 4.0 per 100 person-years, respectively (22, 23).

Our analysis of additional defects included measures of factor V Leiden; prothrombin G20210A; increased levels of factors VIII, IX, and XI; and hyperhomocysteinemia. One or more of these defects were demonstrated in 61% of 397 relatives. All defects were equally distributed among deficient and nondeficient relatives. The prevalence of factor V Leiden was 3 to 5 times higher than that reported in the healthy population (5%) (24) in families with protein S or C deficiency, whereas the prevalence approached 5% in families with antithrombin deficiency. Prothrombin G20210A was more prevalent in families with all 3 deficiencies than in the healthy population (1% to 2%) (25), as were increased factor VIII levels and hyperhomocysteinemia (by definition, 10% in the healthy population). Many defects apparently aggregated in these families, independent of inheritance, and thereby enhanced concomitance.

Only a few previous studies have reported on additional thrombophilic defects in persons with protein S, protein C, or antithrombin deficiency, and all addressed a single other factor (5–9).

Factor V Leiden was associated with a relative risk for VTE of 1.2 to 3.8 in protein S-deficient relatives, 1.3 to

Table 5. Contribution of Exogenous Risk Factors and Additional Thrombophilic Deficiencies or Defects to the Risk for Venous Thromboembolism in Relatives with or without a Deficiency of Protein S, Protein C, or Antithrombin*

Index Deficiency	Additional Deficiencies or Defects	Exposure to Exogenous Risk Factor	Relatives with Event, <i>n</i>	Observation Period, <i>y</i>	Annual Incidence (95% CI), <i>n</i> /100 person-years	Crude Relative Risk (95% CI)
–	–	–	0	258	0.0 (0.00 to 1.43)	
–	–	+	1	1423	0.07 (0.00 to 0.39)	
–	+	–	1†	823	0.12 (0.00 to 0.68)	1.0 (reference)
–	+	+	5‡	2939	0.17 (0.06 to 0.40)	1.4 (0.2 to 12.0)
+	–	–	1	197	0.51 (0.01 to 2.84)	1.0 (reference)
+	–	+	11	836	1.32 (0.66 to 2.35)	2.6 (0.3 to 20.1)
+	+	–	10§	837	1.20 (0.57 to 2.20)	1.0 (reference)
+	+	+	41	1632	2.51 (1.80 to 3.41)	2.1 (1.1 to 4.2)

* The plus sign indicates relatives with protein S, protein C, or antithrombin deficiency, and the minus sign indicates relatives without protein S, protein C, or antithrombin deficiency.

† 1 event among 25 relatives with 1 additional defect and 0 events among 8 relatives with >1 additional defect.

‡ 1 event among 64 relatives with 1 additional defect and 4 events among 28 relatives with >1 additional defect.

§ 5 events among 23 relatives with 1 additional defect and 5 events among 13 relatives with >1 additional defect.

|| 23 events among 51 relatives with 1 additional defect and 18 events among 30 relatives with >1 additional defect.

2.0 in protein C–deficient relatives, and 1.9 to 5.1 in antithrombin-deficient relatives (5–8). When deficient carriers of prothrombin G20210A were compared with deficient noncarriers, relative risks were 1.7 to 2.3 for protein S deficiency, 0.8 for protein C deficiency, and 1.2 for antithrombin deficiency (8, 9). In contrast to previous studies, we estimated the risk for VTE associated with multiple other thrombophilic defects. However, relative risks were similar when we analyzed for the presence of factor V Leiden or prothrombin G20210A in our sample in the same way as in previous studies, that is, regardless of other defects to enable a proper comparison. Risk estimates for individual additional thrombophilic defects in deficient relatives who had negative results on tests for other defects showed a wide range of annual incidences, from 0.41 per 100 person-years in deficient relatives with hyperhomocysteinemia to 5.26 per 100 person-years in deficient relatives who were factor V Leiden carriers. This finding suggests variations in interactions between different additional defects and deficiencies of protein S, protein C, and antithrombin. Of note, CIs were wide as a result of small numbers.

Exogenous risk factors also contributed to the risk for VTE. In deficient relatives, the annual incidence of VTE was 0.51 per 100 person-years in the absence of additional defects and exogenous risk factors. It increased to 1.32 per 100 person-years with exogenous risk factors, 1.20 per 100 person-years with additional defects, and to 2.51 per 100 person-years with both additional defects and exogenous risk factors. Of note, anticoagulant thromboprophylaxis routinely applied at surgery may have influenced the risk estimates. In our high-risk sample, there was no convincing evidence of an increase in the annual incidence of VTE with age.

This retrospective study has several limitations. Many events were not confirmed by objective techniques, because such techniques were not available at the time of event

onset. Although the risk for VTE might therefore have been overestimated, one should expect a similar effect in deficient and nondeficient relatives. Tests of all other thrombophilic deficiencies or defects were not completed in all relatives, mainly because the amount of plasma collected at enrollment was not sufficient. We categorized relatives according to the number of additional deficiencies or defects, although the risk for VTE associated with each of these might not be mutually comparable. In fact, an analysis of dual combinations of the index deficiency and a single additional defect suggested differences in risk. However, the subgroups of relatives with dual combinations were too small as a result of numerous combinations to expect more precise risk estimates. Referral bias may have been introduced by the university hospital setting but was probably reduced by testing all consecutive patients with VTE for deficiencies. Selection bias was minimized by the high response rate of eligible relatives. Although we cannot exclude the possibility that more deficient than nondeficient relatives died of VTE, this potential source of bias would have resulted in an underestimated risk for VTE. Moreover, hereditary deficiencies were not associated with a reduced life expectancy in previous studies (26, 27). The selection of probands with a symptomatic deficiency alters penetrance and consequently may have influenced risk estimates; however, such a selection represents clinical practice. Neither asymptomatic participants nor their relatives will be tested for hereditary deficiencies, alone or in combination with other thrombophilic defects, because thus far positive test results do not have clinical implications. Despite these limitations, we believe that this is the first study to document the incremental risk for VTE conveyed by multiple thrombophilic defects in 90% of relatives of VTE probands tested for all known hereditary thrombophilic defects.

In conclusion, we provide evidence that VTE is a result of interactions among multiple genetic and environ-

mental risk factors. The presence of additional thrombophilic defects as well as exposure to exogenous risk factors were important determinants of the high risk for VTE in persons with hereditary deficiencies of protein S, protein C, or antithrombin.

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