Clinical implications of deficiencies of protein S, protein C or antithrombin

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Hereditary deficiency of protein C or protein S confers increased risk of arterial thromboembolic events at a young age: results from a large family cohort study

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Summary

Background: whether hereditary protein S, protein C, or antithrombin deficiency is associated with arterial thromboembolism (ATE) and whether history of venous thromboembolism in these subjects predisposes them to subsequent ATE have yet to be determined. Methods and Results: on the basis of pedigree analysis, we enrolled a total of 552 subjects (52% women; mean age, 46-17 years), belonging to 84 different kindreds, in this retrospective family cohort study. Detailed information on previous episodes of venous thromboembolism, ATE, anticoagulant use, and atherosclerosis risk factors was collected. Primary study outcome was objectively verified symptomatic ATE. Of 552 subjects, 308 had protein S (35%), protein C (39%), or antithrombin (26%) deficiency. Overall, annual incidences of ATE were 0.34% (95% confidence interval [CI], 0.23 to 0.49) in deficient *versus* 0.17% (95% CI, 0.09 to 0.28) in non-deficient subjects; the hazard ratio was 2.3 (95% CI, 1.2 to 4.5). Because the risk hazards varied over lifetime, we performed a time-dependent analysis. After adjusting for atherosclerosis risk factors and clustering within families, we found that deficient subjects had a 4.7-fold (95% CI, 1.5 to 14.2; *P*=0.007) higher risk for ATE before 55 years of age *versus* 1.1 (95% CI, 0.5 to 2.6) thereafter compared with non-deficient family members. For separate deficiencies, the risks were 4.6- (95% CI, 1.1 to 18.3), 6.9- (95% CI, 2.1 to 22.2), and 1.1- (95% CI, 0.1 to 10.9) fold higher in protein S-, protein C-, and antithrombin-deficient subjects, respectively, before 55 years of age. History of venous thromboembolism was not related to subsequent ATE (hazard ratio, 1.1; 95% CI, 0.5 to 2.2). Conclusions: compared with non-deficient family members, subjects with protein S or protein C deficiency but not antithrombin deficiency have a higher risk for ATE before 55 years of age that is independent of prior venous thromboembolism.

CHAPTER 7

Introduction

Several coagulation disorders are associated with an increased risk of venous thromboembolism (VTE). These thrombophilic conditions include hereditary deficiencies of protein S, protein C, and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of clotting factors VIII, IX, and XI; and antiphospholipid antibodies (1). Hereditary deficiencies of protein S, protein C, and antithrombin have been recognized as the most potent thrombophilic conditions for VTE (2-6). Recently, we demonstrated that the concomitance of other thrombophilic defects further enhances the risk of VTE associated with these deficiencies (2). Whether hereditary protein S, protein C, or antithrombin deficiency also is involved in the development of arterial thromboembolism (ATE) has still to be elucidated. Evidence of such an association has been derived mainly from case reports (7,8).

In 2003, a link between VTE and atherosclerosis was reported (9). In the ensuing years, several other studies addressed this issue, most of these confirming this association (10). This possible link has thus far been attributed to the sharing of common risk factors by the 2 conditions (9,11). The contribution of thrombophilic defects to the link between VTE and ATE has yet to be defined.

We performed a retrospective follow-up study to assess the risk of ATE in a large series of protein S-, protein C-, or antithrombin-deficient subjects compared with non-deficient family members. Moreover, assuming that VTE and ATE share similar risk factors, we hypothesized that a relationship between VTE and subsequent ATE would be likely, especially in these subjects.

Methods

Subjects

The study contained 3 cohorts of families with hereditary deficiency of protein S, protein C, or antithrombin. Probands were consecutive patients with VTE who had one of these deficiencies. First-degree relatives >15 years of age were identified by pedigree analysis. Because the number of antithrombin-deficient probands was small, second-degree relatives from a deficient parent also were identified. Subjects were enrolled after informed consent was obtained. Detailed data on previous episodes of VTE and ATE, risk factors for atherosclerosis, and anticoagulant treatment were collected by using a standardized questionnaire and reviewing medical records. Blood samples were taken after clinical data had been collected. Probands and relatives were tested for other thrombophilic defects in addition to their index deficiencies, including deficiencies of protein S, protein C, and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of factor VIII; and lupus anticoagulant. Risk factors for atherosclerosis included hypertension, defined as a systolic blood pressure of ≥140 mm Hg or ≥160 mm Hg in patients ≥60 years of age, a diastolic blood pressure of ≥90 mm Hg measured on at least 2 occasions, or the use of antihypertensive drugs; diabetes mel-

litus (12); cigarette smoking; and hyperlipidemia, defined as total cholesterol level >6.5 mmol/L (250 mg/dL), triglycerides >2.5 mmol/L (220 mg/dL), or use of lipid-lowering drugs. The study was approved by the institutional review board of our hospital.

Diagnosis of thromboembolism
ATE was considered established if myocardial infarction, ischemic stroke, transient ischemic attack, or peripheral artery disease was symptomatic and objectively verified. Q-wave and non-Q-wave myocardial infarction was confirmed by typical ECG features, elevated levels of cardiac enzymes, radionuclide imaging techniques, or coronary angiography. Ischemic stroke was documented by computed tomography scanning or magnetic resonance imaging. Transient ischemic attack required neurological symptoms and signs lasting <24 hours. Peripheral artery disease was considered thromboembolic at acute signs and symptoms of ischemia and was documented by arteriography.

VTE was considered established if deep vein 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. Before these techniques were available, VTE was considered established when the patient had received full-dose unfractionated heparin and a vitamin K antagonist for at least 3 months (2).

Laboratory studies
Protein S and protein C antigen levels were measured by ELISA (reagents obtained from DAKO, Glostrup, Denmark); activity of protein C (Berichrom Protein C, Dade Behring, Liederbach, Germany) and antithrombin (Coatest, Chromogenix, Mölndal, Sweden) was measured by chromogenic substrate assays. Normal ranges (mean ± SD) were determined in 393 healthy blood donors who had no (family) history of thromboembolism, were not pregnant, and had not used oral contraceptives for at least 3 months. Protein S deficiency type I was defined by lowered total protein S antigen levels (<68 IU/dL). Protein C deficiency types I and II were defined by reduced levels of protein C antigen (<63 IU/dL) and/or activity (<64 IU/dL); antithrombin deficiency was defined by decreased levels of antithrombin activity (<74 IU/dL). Deficiencies were considered inherited if they were confirmed by measurement of a second sample that was collected 3 months later and were found in at least 2 family members, whereas acquired conditions were excluded. If a discrepancy was found 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 reactions (13,14). Factor VIII:C was measured by 1-stage clotting assay and was considered increased at levels >150 IU/dL. Lupus anticoagulant was defined by abnormal values of dilute Russell viper venom time, activated partial thromboplastin time, and tissue

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                              Patients tested
                                (n = 1600)
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                                    ▼
                            Established deficiencies
                                 (n = 95)
                                    │──────────────────▶ Deceased or unknown
                                    │                     relatives (n = 4)
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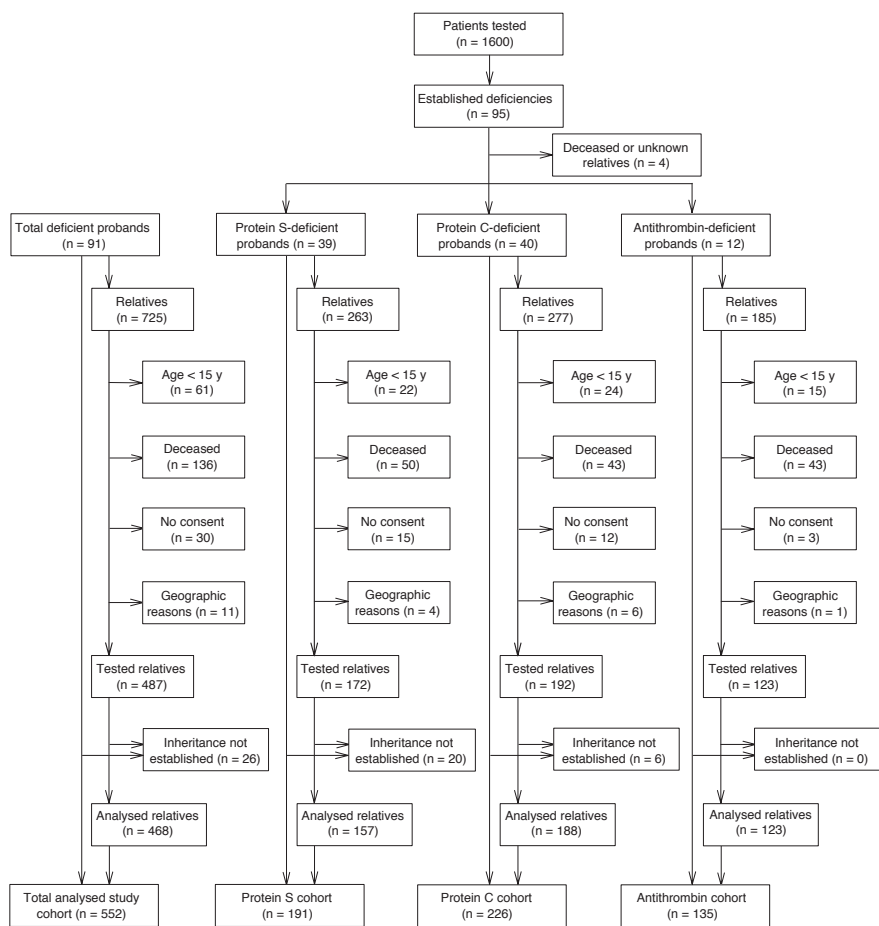| Total deficient probands (n = 91)	Protein S-deficient probands (n = 39)	Protein C-deficient probands (n = 40)	Antithrombin-deficient probands (n = 12)
Relatives (n = 725)	Relatives (n = 263)	Relatives (n = 277)	Relatives (n = 185)
Age < 15 y (n = 61)	Age < 15 y (n = 22)	Age < 15 y (n = 24)	Age < 15 y (n = 15)
Deceased (n = 136)	Deceased (n = 50)	Deceased (n = 43)	Deceased (n = 43)
No consent (n = 30)	No consent (n = 15)	No consent (n = 12)	No consent (n = 3)
Geographic reasons (n = 11)	Geographic reasons (n = 4)	Geographic reasons (n = 6)	Geographic reasons (n = 1)
Tested relatives (n = 487)	Tested relatives (n = 172)	Tested relatives (n = 192)	Tested relatives (n = 123)
Inheritance not established (n = 26)	Inheritance not established (n = 20)	Inheritance not established (n = 6)	Inheritance not established (n = 0)
Analysed relatives (n = 468)	Analysed relatives (n = 157)	Analysed relatives (n = 188)	Analysed relatives (n = 123)
Total analysed study cohort (n = 552)	Protein S cohort (n = 191)	Protein C cohort (n = 226)	Antithrombin cohort (n = 135)

Figure 1. Recruitment of 3 family cohorts with hereditary protein S, protein C, or anti-thrombin deficiency.

Table 1. Characteristics of the study population.

Variable	Total cohort			Protein S cohort			Protein C cohort			Antithrombin cohort		
	Deficient	Non-deficient	P	Deficient	Non-deficient	P	Deficient	Non-deficient	P	Deficient	Non-deficient	P
Subjects, n	308	244		109	82		120	106		56	56	
Women, n (%)	159 (52)	128 (52)	0.93	57 (52)	44 (54)	0.88	61 (51)	54 (51)	1.00	41 (52)	30 (54)	0.86
Median age at enrolment, y (range)	43 (15-89)	47 (15-92)	0.18	43 (15-84)	47 (15-84)	0.43	46 (15-89)	48 (15-86)	0.54	41 (16-92)	46 (16-92)	0.45
History of VTE, n (%)	153 (50)	7 (3)	<0.001	56 (51)	5 (6)	<0.001	63 (53)	2 (2)	<0.001	34 (43)	0 (0)	<0.001
Long-term VKA, n (%)	65 (21)	4 (2)	<0.001	22 (20)	1 (1)	<0.001	28 (23)	2 (2)	<0.001	15 (19)	1 (2)	0.002
Concomitance of, n (%)*												
FVIII:C >150 IU/dl	116 (43)	68 (34)	0.04	43 (45)	22 (29)	0.04	51 (49)	31 (39)	0.18	22 (32)	15 (32)	1.00
PT G20210A	19 (7)	19 (9)	0.50	5 (5)	6 (8)	0.54	9 (8)	7 (8)	1.00	5 (7)	6 (13)	0.35
Factor V Leiden	44 (15)	37 (17)	0.71	19 (19)	11 (14)	0.43	21 (19)	24 (25)	0.31	4 (6)	2 (4)	0.35
Diabetes, n (%)	6 (2)	12 (5)	0.06	3 (3)	5 (6)	0.29	2 (2)	5 (5)	0.26	1 (1)	2 (4)	0.57
Hyperlipidemia, n (%)	35 (11)	20 (8)	0.25	17 (16)	10 (12)	0.54	9 (8)	7 (7)	1.00	9 (11)	3 (5)	0.36
Hypertension, n (%)	41 (13)	29 (12)	0.70	12 (11)	14 (17)	0.29	20 (17)	9 (8)	0.08	9 (11)	6 (11)	1.00
Smoking history, n (%)	102 (33)	62 (26)	0.06	34 (31)	26 (32)	1.00	44 (37)	24 (23)	0.03	24 (30)	12 (21)	0.32
Overall ATE, n (%)	31 (10)	13 (5)†	0.06	12 (11)	4 (5)	0.19	13 (11)	5 (5)	0.14	6 (8)	4 (7)†	1.00
MI	12 (4)	4 (1.6)	0.13	5 (5)	1 (1)	0.24	4 (3)	2 (2)	0.69	3 (4)	1 (2)	0.64
Ischemic stroke	11 (4)	4 (1.6)	0.20	4 (4)	3 (4)	1.00	6 (5)	1 (1)	0.12	1 (1)	0 (0)	1.00
TIA	8 (3)	4 (1.6)	0.56	3 (3)	0 (0)	0.26	3 (3)	2 (2)	1.00	2 (3)	2 (4)	1.00
Median age at onset of ATE, y (range)	53 (30-80)	64 (41-78)	0.006	54 (31-80)	65 (41-72)	0.47	42 (30-61)	58 (52-70)	0.02	59 (55-65)	69 (63-78)	0.03

VTE denotes venous thromboembolism; Long-term VKA, use of vitamin K antagonists for ≥12 months; PT G20210A, the prothrombin G20210A mutation mutation; ATE arterial thromboembolism; MI, myocardial infarction; TIA, cerebral transient ischemic attack.

* Of total study cohort, 15%, 10% and 8% of subjects were not tested for FVIII:C, the prothrombin G20210A mutation and Factor V Leiden, respectively. Of 416 tested subjects, 2 were positive for lupus anticoagulant.

† One subject in this group had peripheral artery disease.

thromboplastin inhibition, which was normalized by the addition of phospholipids to the subject's plasma (15). In probands and symptomatic relatives, blood samples were collected at least 3 months after a thrombotic event. If they were on long-term treatment with acenocoumarol, a short-acting vitamin K antagonist, samples were taken after treatment had been interrupted for at least 2 weeks; meanwhile, nadroparin was given subcutaneously.

Statistical analysis

We calculated overall and age-specific absolute risks of ATE in subjects with protein S, protein C, or antithrombin deficiency separately and in the pooled cohorts of deficient and non-deficient subjects. Because all probands were selected on the basis of VTE and the presence of protein S, protein C, or antithrombin deficiency, they were included in the current analysis for ATE. Annual incidences were calculated by dividing the number of symptomatic subjects by the total number of observation years. Observation time was defined as the period from 15 years of age until the first episode of ATE or the end of the study, considering that thrombosis is rare at younger ages. The 95% confidence intervals (CIs) around the annual incidences were assessed with the Poisson distribution assumption. Kaplan-Meier methods were used for survival plots. On the basis of the observation that Kaplan-Meier curves in the pooled cohorts of deficient and non-deficient subjects diverged until ≈55 years of age and then showed less divergence, the assumption for proportional hazards for the final model was not met over the entire observation period. Therefore, we chose a time-dependent Cox proportionalhazards model for the analyses of index deficiencies with a cutoff point set at 55 years of age (16). A multivariable model with additional adjustment for clustering of ATE within families that used the robust sandwich method in SAS version 9.1 (SAS Institute Inc, Cary, NC) was applied to all variables that yielded a value of $P<0.15$ from the univariable model (16). Results were expressed as hazard ratios with 95% CIs and P values.

Continuous variables were expressed as median values and ranges; categorical data, as numbers and frequencies. Differences between groups were evaluated by the Student *t*-test or Mann-Whitney *U*-test, depending on the normality of data for continuous data, and by the Fisher exact test for categorical data. Statistical significance was considered at a 2-tailed $P<0.05$. Statistical analyses were performed with SAS software, version 9.1. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Subjects

Sixteen hundred consecutive patients with VTE were screened over 12 years to identify 91 probands with 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 subjects in our study. Figure 1 details the reasons for exclusion of 257 relatives. Sixty-one relatives (8%) were <15 years of age; 136 (19%) died before enrollment; 30 (4%) refused or could not provide consent because of mental illness; and 11 (1.5%) were not enrolled for geographic reasons. Nineteen additional relatives and their 7 probands were excluded because inheritance of the index deficiency could not be established. The remaining 468 relatives and 84 probands were analyzed: 191 subjects in the protein S cohort, 226 in the protein C cohort, and 135 in the antithrombin cohort (Figure 1).

Characteristics of the study subjects are summarized in the Table. Deficiencies were demonstrated in 48% of relatives and were equally distributed among men and women. Age of enrollment in deficient and non-deficient subjects was similar. Overall, 50% of deficient subjects had a history of VTE in contrast to only 3% of non-deficient subjects. When probands were excluded from this analysis, 31% of deficient *versus* 3% of non-deficient relatives had a history of VTE (*P*<0.001). Moreover, 21% of overall deficient subjects received long-term (i.e., ≥12 months) treatment with vitamin K antagonists compared with 2% of non-deficient subjects (median, 10 years; range, 1 to 42 years). Long-term use of antiplatelet agents was similar in deficient (2%) and non-deficient (1%) subjects. Although concomitance of the prothrombin G20210A mutation and factor V Leiden was equally distributed among deficient and non-deficient subjects, factor VIII levels >150 IU/dL were more prevalent in deficient subjects. Of risk factors for atherosclerosis, smoking history tended to be more frequent in deficient than non-deficient subjects (*P*=0.06), especially in protein C-deficient subjects, whereas diabetes mellitus tended to be more common in non-deficient subjects (*P*=0.06).

Risk of arterial thromboembolic events
ATE occurred in 11% (protein S), 11% (protein C), and 8% (antithrombin) of deficient subjects compared with 5%, 5%, and 7% of non-deficient subjects, respectively (the Table). Median age at onset of the first episode of ATE was 11 years lower in pooled cohorts of deficient subjects *versus* non-deficient subjects (*P*=0.006). Compared with the pooled cohort of non-deficient subjects, this difference was most prominent in subjects with protein C deficiency (22 years; *P*<0.001), followed by protein S-deficient subjects (10 years; *P*=0.14) and antithrombin-deficient subjects (5 years; *P*=0.29).

The probability of ATE-free survival was 87% and 98% at 55 years of age and 71% and 74% at 73 years of age in deficient and non-deficient subjects, respectively (Figure 2). Overall, the annual incidences of ATE in the pooled cohorts were 0.34% (95% CI, 0.23 to 0.49) in deficient subjects *versus* 0.17% (95% CI, 0.09 to 0.28) in non-deficient subjects (hazard ratio, 2.3; 95% CI, 1.2 to 4.5; *P*=0.01). However, on the basis of the observation that the Kaplan-Meier curves in the pooled cohorts diverged until ≈55 years of age and then showed less divergence (Figure 2), we opted for time-dependent analysis of the index deficiencies (age <55 *versus* ≥55 years).

Figure 3 depicts the annual incidences of ATE in deficient and non-deficient subjects <55 and ≥55 years of age compared with age- and sex-weighted annual incidences in the general population (17,18). Annual incidences in deficient subjects after 55 years of age were several-fold higher than before 55 years of age, but the former were similar to the age- and sex-weighted annual incidence in the general population (17,18). In contrast, annual incidences before 55 years of age were significantly higher in subjects with protein S, protein C, or any deficiency compared with the general population (17,18). Figure 4 shows the univariable association of various variables with the risk for ATE. Compared with non-deficient subjects, the risk for ATE was 5.6-fold (95% CI, 1.7 to 19.1; *P*=0.006) higher in individuals with any deficiency (i.e., protein S, protein C, or antithrombin) before 55 years of age. It was 1.3-fold (95% CI, 0.6 to 3.0; *P*=0.51) higher thereafter. The high risk for ATE conferred by any deficiency before 55 years of
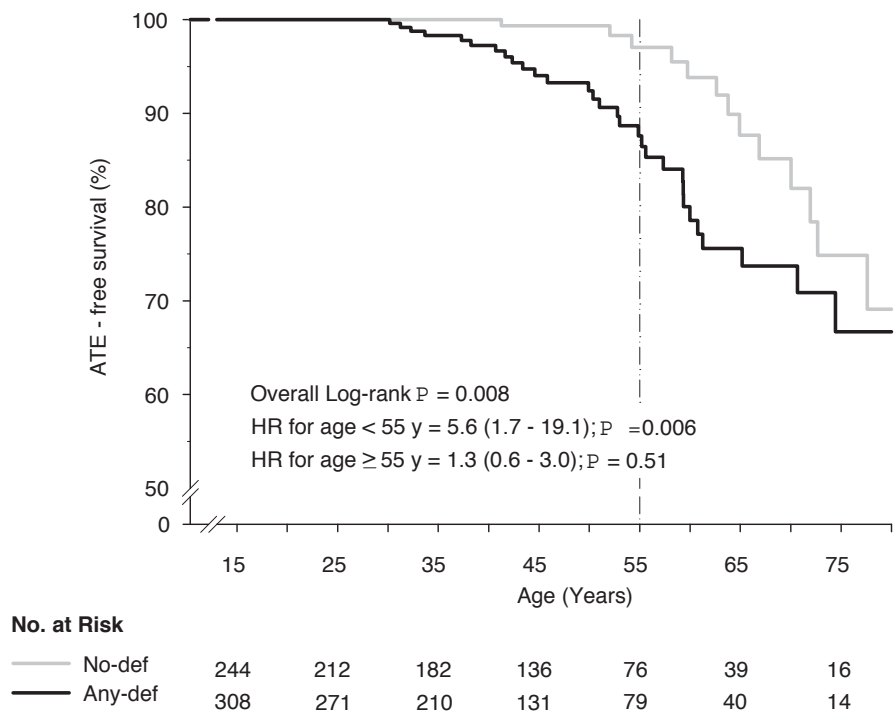

Figure 2. Event-free survival comparing subjects with and without any deficiency. HR indicates, hazard ratio; No-def, no deficiency; and Any-def, any deficiency (i.e., protein S, protein C, or antithrombin deficiency).

age was confined to protein S- or protein C-deficient subjects. Although factor VIII:C levels >150 IU/dL (*P*=0.16) and the prothrombin G20210A mutation (*P*=0.14) tended to be positively related to ATE, factor V Leiden did not show a similar trend. However, subjects with factor V Leiden had significantly lower prevalence of hyper-tension and smoking compared with non-carriers P≤0.02). Of 416 tested subjects, only 2 were positive for lupus anticoagulant. History of VTE was not related to subsequent ATE (hazard ratio, 1.0; 95% CI, 0.5 to 1.9; *P*=0.96). After the duration of anticoagu-

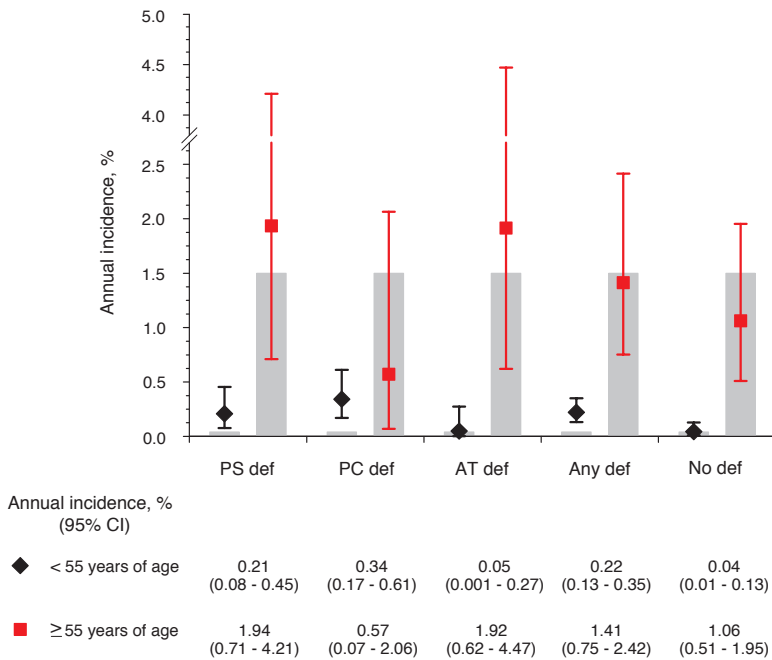| Annual incidence, % (95% CI)	PS def	PC def	AT def	Any def	No def
◆ < 55 years of age	0.21 (0.08 - 0.45)	0.34 (0.17 - 0.61)	0.05 (0.001 - 0.27)	0.22 (0.13 - 0.35)	0.04 (0.01 - 0.13)
■ ≥55 years of age	1.94 (0.71 - 4.21)	0.57 (0.07 - 2.06)	1.92 (0.62 - 4.47)	1.41 (0.75 - 2.42)	1.06 (0.51 - 1.95)

Figure 3. Annual incidences of ATE in subjects with hereditary protein S, protein C, or antithrombin deficiency <55 and ≥55 years of age. Solid diamonds (black) and squares (red) indicate annual incidences of ATE <55 and ≥55 years of age, respectively. The corresponding 95% CIs are represented by vertical error bars. The vertical gray bars represent the age- and sex-weighted ATE in the general population (ARIC study for myocardial infarction17 and Framingham study for cerebrovascular and peripheral artery disease (18). PS def indicates protein S deficiency; PC def, protein C deficiency; AT def, antithrombin deficiency; Any def, any (i.e., protein S, protein C, or anti-thrombin) deficiency; No def, no deficiency.

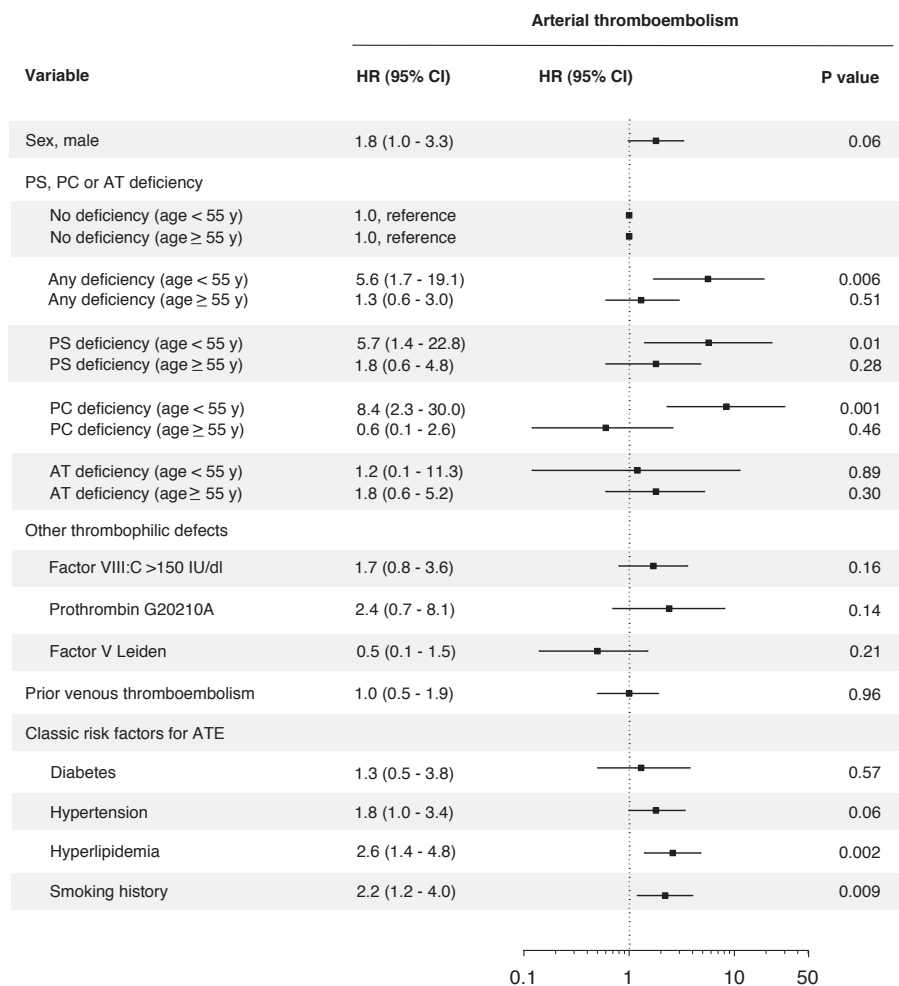| Variable	Arterial thromboembolism		
	HR (95% CI)	HR (95% CI)	P value
Sex, male	1.8 (1.0 - 3.3)		0.06
PS, PC or AT deficiency			
No deficiency (age < 55 y)	1.0, reference		
No deficiency (age ≥ 55 y)	1.0, reference		
Any deficiency (age < 55 y)	5.6 (1.7 - 19.1)		0.006
Any deficiency (age ≥ 55 y)	1.3 (0.6 - 3.0)		0.51
PS deficiency (age < 55 y)	5.7 (1.4 - 22.8)		0.01
PS deficiency (age ≥ 55 y)	1.8 (0.6 - 4.8)		0.28
PC deficiency (age < 55 y)	8.4 (2.3 - 30.0)		0.001
PC deficiency (age ≥ 55 y)	0.6 (0.1 - 2.6)		0.46
AT deficiency (age < 55 y)	1.2 (0.1 - 11.3)		0.89
AT deficiency (age ≥ 55 y)	1.8 (0.6 - 5.2)		0.30
Other thrombophilic defects			
Factor VIII:C >150 IU/dl	1.7 (0.8 - 3.6)		0.16
Prothrombin G20210A	2.4 (0.7 - 8.1)		0.14
Factor V Leiden	0.5 (0.1 - 1.5)		0.21
Prior venous thromboembolism	1.0 (0.5 - 1.9)		0.96
Classic risk factors for ATE			
Diabetes	1.3 (0.5 - 3.8)		0.57
Hypertension	1.8 (1.0 - 3.4)		0.06
Hyperlipidemia	2.6 (1.4 - 4.8)		0.002
Smoking history	2.2 (1.2 - 4.0)		0.009

Figure 4. Univariable proportionalhazards analysis of association with the time to the first arterial thromboembolic event. Solid squares indicate the hazard ratio of ATE with the corresponding 95% CIs represented by the horizontal error bars. Index deficiencies were analyzed as time-dependent variables, i.e., age <55 and ≥55 years. HR indicates hazard ratio; PS, protein S; PC, protein C; and AT, antithrombin.

lant treatment was subtracted from the observation period and 4 ATEs that occurred while these subjects were on anticoagulant therapy were excluded, the risk estimates remained unchanged (hazard ratio, 1.1; 95% CI, 0.5 to 2.2; P=0.86). Of the classic risk factors for atherosclerosis, hyperlipidemia and history of smoking were associated with ATE (P<0.01). Furthermore, hypertension and male sex tended to be associated with ATE (P=0.06).

Multivariable analysis with additional adjustment for clustering within families was applied to any deficiency, the prothrombin G20210A mutation, hypertension, hyperlipidemia, smoking history, and sex. Of these variables, any deficiency before 55 years of age, hypertension, hyperlipidemia, and smoking were independently associated with ATE (P≤0.03) (Figure 5). The adjusted hazard ratio for ATE conveyed by any deficiency before 55 years of age was 4.7 (95% CI, 1.5 to 14.2; P=0.007) *versus* 1.1 (95% CI, 0.5 to 2.6; P=0.84) thereafter. Adjusted hazard ratios conferred by hypertension, hyperlipidemia, and smoking were each ≈2-fold elevated. Adjusted hazard ratios conferred by separate deficiencies were 4.6 (95% CI, 1.1 to 18.3; P=0.03), 6.9 (95% CI, 2.1 to 22.2; P=0.001), and 1.1 (95% CI, 0.1 to 10.9; P=0.94) in subjects with protein S, protein

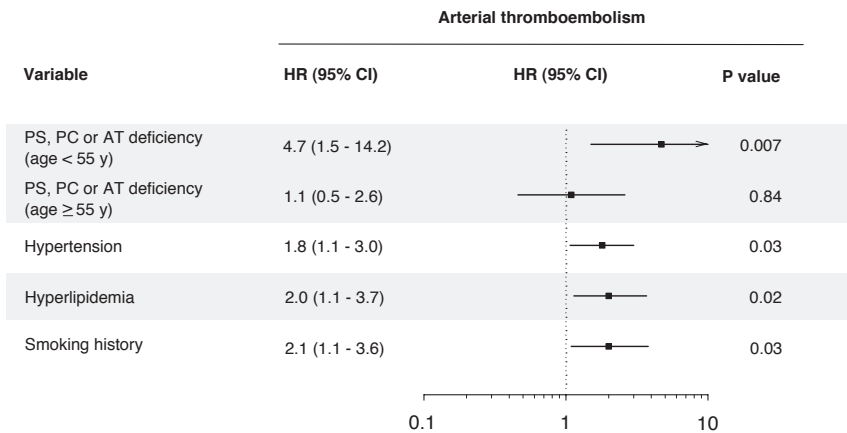|	Arterial thromboembolism		
Variable	HR (95% CI)	HR (95% CI)	P value
PS, PC or AT deficiency (age < 55 y)	4.7 (1.5 - 14.2)		0.007
PS, PC or AT deficiency (age ≥ 55 y)	1.1 (0.5 - 2.6)		0.84
Hypertension	1.8 (1.1 - 3.0)		0.03
Hyperlipidemia	2.0 (1.1 - 3.7)		0.02
Smoking history	2.1 (1.1 - 3.6)		0.03

Figure 5. Multivariable proportionalhazards analysis. Solid squares indicate the hazard ratio of ATE with the corresponding 95% CIs represented by the horizontal error bars. Any deficiency was analyzed as a time-dependent variable (i.e., age <55 and ≥55 years). The hazard ratios (HR) are adjusted for clustering of ATE within families. PS indicates protein S; PC, protein C; and AT, antithrombin.

C, and antithrombin deficiencies, respectively, before 55 years of age (data not shown). No significant interaction was found between smoking and any deficiency or between other atherosclerosis risk factors or any of the concomitant thrombophilic defects (i.e., elevated factor VIII:C, the prothrombin G20210A mutation, and factor V Leiden) and any deficiency (P≥0.37).

Discussion

Overall, the lifetime risk of ATE was ≈2-fold higher in subjects with any deficiency (i.e., protein S, protein C, or antithrombin) compared with non-deficient subjects. However, the high risk of ATE conferred by any deficiency was evident only until ≈55 years of age, a 5-fold risk increase. Moreover, subjects with any deficiency were on average 11 years younger at the onset of ATE compared with non-deficient subjects. Interestingly, only protein S and protein C deficiencies were related to ATE before 55 years of age. Antithrombin deficiency was not related to a significantly increased risk either before 55 years of age or thereafter. Subjects with both a history of VTE and any deficiency had a risk of subsequent ATE similar to that of subjects with any deficiency alone.

Only a few previous studies and several case reports have reported on this issue (7,8). A comparison with our study is hampered by differences in design. In a family cohort study, arterial events were observed in 8% of 144 protein S- or protein C-deficient subjects and in 1% of 94 antithrombindeficient subjects (4). Annual incidences in that study were stratified into types of ATE, sex, and age according to the Framingham study (18). However, because of the limited number of events, annual incidences were only estimated in a few strata, which hindered an appropriate comparison with our study. Furthermore, data on non-deficient relatives and conventional risk factors for atherosclerosis were not provided. In a large study in carriers of familial thrombophilia (the European Prospective Cohort on Thrombophilia [EPCOT] study) (19), overall annual incidences of myocardial infarction and/or ischemic stroke after 20 years of age were 0.15%, 0.18%, and 0.15% in subjects with protein S (n=111), protein C, (n=150), and antithrombin deficiency (n=92), respectively. When we confined our analysis to >20 years of age, annual incidences of myocardial infarction and/or ischemic stroke were 0.32% (protein S), 0.32% (protein C), and 0.21% (antithrombin) in deficient subjects and 0.19% in non-deficient subjects. It is likely that the risks of ATE in the EPCOT study (19) were underestimated because annual incidences in their control group also were remarkably low, only 0.03% in men (mean age, 58 years) and 0.01% in women (mean age, 58 years). Moreover, information on cardiovascular risk factors and on whether the recorded events were objectively verified was not available. In a case-control study, ATE was recorded more frequently in 88 cases with protein S, protein C, or antithrombin deficiency (19% ATE) compared with control subjects with VTE without these deficiencies (1% ATE) (20). Although the control group in this study might not be appropriate because not all deficient subjects had history of VTE, no difference in ATE prevalence was found among protein S-, protein C-, or antithrombin-defi-

cient subjects. Another case-control study reported significantly lower plasma levels of activated protein C in young patients with myocardial infarction (n=231) compared with healthy controls (n=231) (21). In a Japanese study, subjects with established inherited protein C deficiency and either myocardial infarction (n=10) or ischemic stroke (n=11) were on average 11 and 7 years younger at onset of myocardial infarction and ischemic stroke, respectively, than control subjects with myocardial infarction (n=42) or ischemic stroke (n=48) with normal protein C levels (22). Finally, the prospective epidemiological Atherosclerosis Risk in Communities (ARIC) study reported that plasma protein C appeared protective against ischemic stroke but not myocardial infarction (23).

On the other hand, in other case-control studies, prevalences of these deficiencies were similar between cases with ischemic stroke (7,24) or myocardial infarction (7,25,26) and matched controls, even young patients (25,26). Furthermore, in the ARIC study, low levels of plasma protein C and antithrombin were not related to coronary heart disease (27). However, the lack of association between protein S, protein C, or antithrombin deficiency and risk for ATE in these studies could be explained by the low prevalence of these hereditary deficiencies in the general population. This notion is given further credence by the finding that more common, but weaker, thrombophilic defects (i.e., factor V Leiden, the prothrombin G20210A mutation) were more frequently related to myocardial infarction (25,26,28) and/or overall coronary disease (i.e., myocardial infarction or coronary stenosis) (28). In addition, it could be speculated that protein S, protein C, or antithrombin deficiency in these studies might be acquired rather than hereditary, considering that acquired deficiencies are more prevalent. The latter is consistent with the exceptionally high prevalence of these deficiencies (up to 4%) in control subjects (24).

Surprisingly, the increased risk for ATE was confined to subjects with protein S and protein C deficiencies. Subjects with antithrombin deficiency had a risk for ATE comparable to that for non-deficient subjects. That this difference is attributable by chance, for instance, as a result of the smaller cohort of subjects with antithrombin deficiency, could be argued because high risk for ATE conferred by protein S or protein C but not antithrombin deficiency also was reported earlier (4,8). It could be speculated that the higher risk for ATE in subjects with protein C deficiency could be ascribed to the potent cytoprotective effects of the protein C pathway (29). Why protein S deficiency, rather than deficiency of antithrombin, was associated with ATE may be the synthesis of protein S by endothelial cells (30), whereas antithrombin is synthesized by hepatocytes. Endothelial injury as a trigger of thrombosis may be enhanced by a preexisting defect in protein S synthesis at the site of injury. Furthermore, some cytoprotective effects also have been attributed to protein S (30,31).

Since its first description (9), the amount of data indicating a link between VTE and subsequent ATE has been increasing (10). In our study, subjects with prior VTE had a risk for ATE similar to that of subjects without prior VTE. This lack of association could not be ascribed to use of vitamin K antagonists. On the basis of our esti-

mated hazard risk, even in a larger sample, a link between VTE and subsequent ATE seems unlikely in these subjects. The potential link between VTE and subsequent ATE is believed to be due to the sharing of common pathophysiological mechanisms (9). Because protein S and protein C deficiencies but not antithrombin deficiency were significantly related to ATE in our study, it could be speculated that endothelial dysfunction rather than coagulation disorders is the main actor in the link between VTE and subsequent ATE. Moreover, atherosclerosis risk factors are somewhat stronger risk factors for VTE than are the prothrombin G20210A mutation and factor V Leiden for ATE (11,28). However, it remains unclear why subjects with both prior VTE and protein S or protein C deficiency had a risk for ATE similar to that of subjects with these deficiencies alone.

Concomitant thrombophilic events apparently aggregated in our families. The prevalence of factor V Leiden was 3 to 5 times higher in families with protein S or C deficiency than that reported in the general population (14% to 25% *versus* 5%) (5). The prothrombin G20210A mutation was more prevalent in families with any of the 3 deficiencies than in the general population (5% to 13% *versus* 2%), as were increased factor VIII:C levels (29% to 49% *versus* 11%) (5). Aggregation of these concomitant thrombophilic defects was independent of the index deficiencies, except that factor VIII:C levels >150 IU/dL were more frequently observed in subjects with protein S or C deficiency. The latter might be secondary to these deficiencies per se because, in theory, low levels of activated protein C or protein S may result in less FVIII inactivation. In contrast to VTE (2), the concomitance of factor VIII:C levels >150 IU/dL, the prothrombin G20210A mutation, or factor V Leiden was not related to higher risk for ATE compared with subjects with the index deficiencies alone.

One may consider screening for protein S or protein C deficiency in young subjects with ATE if a family history is suspected or positive for thrombophilia because diagnosis of these deficiencies has clinical implications for the prevention of VTE. Further studies are needed to demonstrate clinical utility of thrombophilia screening for primary or secondary prevention of ATE.

The main limitation of this study is its retrospective design. Consequently, because patients were not routinely screened for atherosclerosis risk factors and the information on these factors was self-reported and/or derived from medical records, it is possible that the true incidence of these risk factors has been underestimated in asymptomatic subjects. This would have resulted in a slightly lower adjusted hazard ratio for ATE conferred by protein S, protein C, or antithrombin deficiency. Referral bias may have been introduced by the university hospital setting but was probably reduced by testing all consecutive patients with VTE for deficiencies. Because probands were consecutive patients with VTE and the response rate of eligible relatives was high, selection bias was probably limited. Although we cannot exclude the possibility that more deficient than non-deficient subjects died of VTE or ATE, this potential source of bias would have resulted in an underestimated risk for ATE in deficient subjects. Moreover, hereditary deficiencies were not associated with a reduced life expectancy in previous

studies (32,33). Despite these limitations, we believe that this is the first study to document the age-dependent elevated risk for ATE conveyed by hereditary protein S or protein C deficiency but not antithrombin deficiency in these thrombophilic families.

Conclusions
This study delineates an increased risk for ATE conferred by protein S or protein C deficiency before age 55 years compared with non-deficient family members. In contrast, antithrombin deficiency was not associated with a significantly elevated risk for ATE either before age 55 years or thereafter. Prior VTE was not related to subsequent ATE in these subjects.

REFERENCES

1. Caprini JA, Glase CJ, Anderson CB, Hathaway K. Laboratory markers in the diagnosis of venous thromboembolism. Circulation. 2004;109(suppl):I-4-I-8.
2. Brouwer JL, Veeger NJ, Kluin-Nelemans HC, van der Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. Ann Intern Med. 2006;145:807-15.
3. Sanson BJ, Simioni P, Tormene D, Moia M, Friederich PW, Huisman MV, Prandoni P, Bura A, Rejto L, Wells P, Mannucci PM, Girolami A, Büller HR, Prins MH. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood. 1999;94:3702-6.
4. De Stefano V, Leone G, Mastrangelo S, Tripodi A, Rodeghiero F, Castaman G, Barbui T, Finazzi G, Bizzi B, Mannucci PM. Clinical manifestations and management of inherited thrombophilia: retrospective analysis and follow-up after diagnosis of 238 patients with congenital deficiency of antithrombin III, protein C, protein S. Thromb Haemost. 1994;72:352-8.
5. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet. 1999;353:1167-73.
6. Simioni P, Sanson BJ, Prandoni P, Tormene D, Friederich PW, Girolami B, Gavasso S, Huisman MV, Büller HR, Wouter ten Cate J, Girolami A, Prins MH. Incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost. 1999;81:198-202.
7. Boekholdt SM, Kramer MH. Arterial thrombosis and the role of thrombophilia. Semin Thromb Hemost. 2007;33:588-96.
8. Girolami A, Simioni P, Scarano L, Girolami B. Venous and arterial thrombophilia. Haematologica. 1997;82:96-100.
9. Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, Prins MH, Girolami A. An association between atherosclerosis and venous thrombosis. N Engl J Med. 2003;348:1435-41.
10. Sorensen HT, Horvath-Puho E, Pedersen L, Baron JA, Prandoni P. Venous thromboembolism and subsequent hospitalisation due to acute arterial cardiovascular events: a 20-year cohort study. Lancet. 2007;370:1773-9.
11. Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. Circulation. 2008;117:93-102.
12. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2003;26(suppl 1):S5-S20.
13. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369:64-7.
14. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood.

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1996;88:3698-703.

15. Exner T, Triplett DA, Taberner D, Machin SJ. Guidelines for testing and revised criteria for lupus anticoagulants: SSC Subcommittee for the Standardization of Lupus Anticoagulants. Thromb Haemost. 1991;65:320-2.

16. Allison PD. Survival Analysis Using the SAS System : A Practical Guide. Cary, NC: SAS Institute Inc; 1995.

17. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y. Heart disease and stroke statistics: 2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2008;117:e25-e146.

18. Thom TJ, Kannel WB, Silbershatz H, D'Agostino RB. Incidence, prevalence, and mortality of cardiovascular diseases in the United States. In: Alexander RW, Schlant RC, Fuster V, eds. Hurst's The Heart. 9th ed. New York, NY: McGraw-Hill; 1998:3-17.

19. Vossen CY, Rosendaal FR. Risk of arterial thrombosis in carriers of familial thrombophilia. J Thromb Haemost. 2006;4:916-8.

20. Simioni P, Zanardi S, Saracino A, Girolami A. Occurrence of arterial thrombosis in a cohort of patients with hereditary deficiency of clotting inhibitors. J Med. 1992;23:61-74.

21. Zorio E, Navarro S, Medina P, Estelles A, Osa A, Rueda J, Cubillo P, Aznar J, Espana F. Circulating activated protein C is reduced in young survivors of myocardial infarction and inversely correlates with the severity of coronary lesions. J Thromb Haemost. 2006;4:1530-6.

22. Sakata T, Kario K, Katayama Y, Matsuyama T, Kato H, Miyata T. Studies on congenital protein C deficiency in Japanese: prevalence, genetic analysis, and relevance to the onset of arterial occlusive diseases. Semin Thromb Hemost. 2000;26:11-6.

23. Folsom AR, Rosamond WD, Shahar E, Cooper LS, Aleksic N, Nieto FJ, Rasmussen ML, Wu KK. Prospective study of markers of hemostatic function with risk of ischemic stroke: the Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation. 1999;100:736-42.

24. Hankey GJ, Eikelboom JW, van Bockxmeer FM, Lofthouse E, Staples N, Baker RI. Inherited thrombophilia in ischemic stroke and its pathogenic subtypes. Stroke. 2001;32:1793-9.

25. Rallidis LS, Belesi CI, Manioudaki HS, Chatziioakimidis VK, Fakitsa VC, Sinos LE, Laoutaris NP, Apostolou TS. Myocardial infarction under the age of 36: prevalence of thrombophilic disorders. Thromb Haemost. 2003;90:272-8.

26. Segev A, Ellis MH, Segev F, Friedman Z, Reshef T, Sparkes JD, Tetro J, Pauzner H, David D. High prevalence of thrombophilia among young patients with myocardial infarction and few conventional risk factors. Int J Cardiol. 2005;98:421-4.

27. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atheroscle-

CHAPTER 7

rosis Risk in Communities (ARIC) Study. Circulation. 1997;96:1102-8.

28. Ye Z, Liu EH, Higgins JP, Keavney BD, Lowe GD, Collins R, Danesh J. Seven haemostatic gene polymorphisms in coronary disease: metaanalysis of 66,155 cases and 91,307 controls. Lancet. 2006;367:651-8.

29. Mosnier LO, Zlokovic BV, Griffin JH. The cytoprotective protein C pathway. Blood. 2007;109:3161-72.

30. Rezende SM, Simmonds RE, Lane DA. Coagulation, inflammation, and apoptosis: different roles for protein S and the protein S-C4b binding protein complex. Blood. 2004;103:1192-1201.

31. Liu D, Guo H, Griffin JH, Fernandez JA, Zlokovic BV. Protein S confers neuronal protection during ischemic/hypoxic injury in mice. Circulation. 2003;107:1791-6.

32. Rosendaal FR, Heijboer H, Briët E, Büller HR, Brandjes DP, de Bruin K, Hommes DW, Vandenbroucke JP. Mortality in hereditary antithrombin-III deficiency: 1830 to 1989. Lancet. 1991;337:260-2.

33. Allaart CF, Rosendaal FR, Noteboom WM, Vandenbroucke JP, Briët E. Survival in families with hereditary protein C deficiency, 1820 to 1993. BMJ. 1995;311:910-3.

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