Chapter 7

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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_Circulation_. 2008;118:1659-67
ABSTRACT

**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 nondeficient 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 nondeficient 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 nondeficient 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.
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 nondeficient 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 $\geq 140$ mm Hg or $\geq 160$ mm Hg in patients $\geq 60$ years of age, a diastolic blood pressure of $\geq 90$ mm Hg measured on at least 2 occasions, or the use of antihypertensive drugs; diabetes mellitus; 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 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 nondeficient 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 nondeficient subjects diverged until approximately 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 proportional hazards 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 nondeficient subjects was similar. Overall, 50% of deficient subjects had a history of VTE in contrast to only 3% of nondeficient subjects. When probands were excluded from this analysis, 31% of deficient versus 3% of nondeficient relatives had a history of VTE (P<0.001). Moreover, 21% of overall deficient subjects received long-term (ie, ≥12 months) treatment with vitamin K antagonists compared with 2% of nondeficient subjects (median, 10 years; range, 1 to 42 years). Long-term use of antiplatelet agents was similar in deficient (2%) and nondeficient (1%) subjects. Although concomitance of the prothrombin G20210A mutation and factor V Leiden was equally distributed among deficient and nondeficient 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 nondeficient subjects (P=0.06), especially in protein C–deficient subjects, whereas diabetes mellitus tended to be more common in nondeficient 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 nondeficient 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 nondeficient subjects.
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(P=0.006). Compared with the pooled cohort of nondeficient 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).

Figure 1. Recruitment of 3 family cohorts with hereditary protein S, protein C, or antithrombin deficiency.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cohort</th>
<th>Protein S cohort</th>
<th>Protein C cohort</th>
<th>Antithrombin cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>308</td>
<td>109</td>
<td>120</td>
<td>79</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>159 (52%)</td>
<td>57 (52%)</td>
<td>61 (51%)</td>
<td>41 (52%)</td>
</tr>
<tr>
<td>Median age at enrolment, (range), yr</td>
<td>43 (15-89)</td>
<td>43 (15-84)</td>
<td>46 (15-89)</td>
<td>41 (15-84)</td>
</tr>
<tr>
<td>History of VTE, n (%)</td>
<td>153 (50%)</td>
<td>56 (51%)</td>
<td>63 (53%)</td>
<td>34 (43%)</td>
</tr>
<tr>
<td>Long-term VKA, n (%)</td>
<td>65 (21%)</td>
<td>22 (20%)</td>
<td>28 (23%)</td>
<td>15 (19%)</td>
</tr>
<tr>
<td>Concomitance of, n (%)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVIII:C &gt;150 IU/dl</td>
<td>116 (43%)</td>
<td>43 (45%)</td>
<td>51 (49%)</td>
<td>22 (32%)</td>
</tr>
<tr>
<td>PT G20210A</td>
<td>19 (7%)</td>
<td>5 (5)</td>
<td>9 (8)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>44 (15%)</td>
<td>19 (19%)</td>
<td>21 (19%)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>6 (2%)</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>35 (11%)</td>
<td>17 (16%)</td>
<td>9 (8)</td>
<td>9 (11)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>41 (13%)</td>
<td>12 (11%)</td>
<td>20 (17%)</td>
<td>9 (11)</td>
</tr>
<tr>
<td>Smoking history, n (%)</td>
<td>102 (33%)</td>
<td>34 (31%)</td>
<td>44 (37%)</td>
<td>24 (30%)</td>
</tr>
<tr>
<td>Overall ATE, n (%)</td>
<td>31 (10%)</td>
<td>12 (11%)</td>
<td>13 (11%)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>MI</td>
<td>12 (4%)</td>
<td>5 (5)</td>
<td>4 (3)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>11 (4%)</td>
<td>4 (4)</td>
<td>6 (5)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>TIA</td>
<td>8 (3%)</td>
<td>3 (3)</td>
<td>3 (3)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Median age at onset of ATE (range), yr</td>
<td>53 (30-80)</td>
<td>54 (31-80)</td>
<td>43 (30-61)</td>
<td>59 (55-65)</td>
</tr>
</tbody>
</table>

VTE denotes venous thromboembolism; Long-term VKA, use of vitamin K antagonists for ≥12 months; PT G20210A, the prothrombin G20210A 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.
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 nondeficient 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 nondeficient 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 approximately 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 2. Event-free survival comparing subjects with and without any deficiency.](image)

HR indicates hazard ratio; No-def, no deficiency; and Any-def, any deficiency (ie, protein S, protein C, or antithrombin deficiency).
Figure 3 depicts the annual incidences of ATE in deficient and nondeficient subjects <55 and ≥55 years of age compared with age- and sex-weighted annual incidences in the general population.\textsuperscript{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.\textsuperscript{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.\textsuperscript{17,18}

![Figure 3](image-url)

**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 (dark grey) 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 infarction\textsuperscript{17} and Framingham study for cerebrovascular and peripheral artery disease\textsuperscript{18}). PS def indicates protein S deficiency; PC def, protein C deficiency; AT def, antithrombin deficiency; Any def, any (ie, protein S, protein C, or antithrombin) deficiency; No def, no deficiency.
Figure 4 shows the univariable association of various variables with the risk for ATE. Compared with nondeficient 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 (ie, protein S, protein C, or antithrombin). Figure 4. Univariable proportional hazards 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, ie, age <55 and ≥55 years. HR indicates hazard ratio; PS, protein S; PC, protein C; and AT, antithrombin.
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 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 hypertension and smoking compared with noncarriers (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 anticoagulant 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 about 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 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 (ie, elevated factor VIII:C, the prothrombin G20210A mutation, and factor V Leiden) and any deficiency (P≥0.37).
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<table>
<thead>
<tr>
<th>Variable</th>
<th>Arterial thromboembolism</th>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>PS, PC or AT deficiency (age &lt; 55 y)</td>
<td>4.7 (1.5 - 14.2)</td>
</tr>
<tr>
<td>PS, PC or AT deficiency (age ≥ 55 y)</td>
<td>1.1 (0.5 - 2.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.8 (1.1 - 3.0)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>2.0 (1.1 - 3.7)</td>
</tr>
<tr>
<td>Smoking history</td>
<td>2.1 (1.1 - 3.6)</td>
</tr>
</tbody>
</table>

Figure 5. Multivariable proportional hazards 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 (ie, 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.
DISCUSSION

Overall, the lifetime risk of ATE was about 2-fold higher in subjects with any deficiency (ie, protein S, protein C, or antithrombin) compared with nondeficient subjects. However, the high risk of ATE conferred by any deficiency was evident only until approximately 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 nondeficient 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.\textsuperscript{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 antithrombin-deficient subjects.\textsuperscript{4} Annual incidences in that study were stratified into types of ATE, sex, and age according to the Framingham study.\textsuperscript{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 nondeficient 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),\textsuperscript{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 nondeficient subjects. It is likely that the risks of ATE in the EPCOT study\textsuperscript{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).\textsuperscript{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-deficient 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).\textsuperscript{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.\textsuperscript{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.\textsuperscript{23}

On the other hand, in other case-control studies, prevalences of these deficiencies were similar between cases with ischemic stroke\textsuperscript{7,24} or myocardial infarction\textsuperscript{7,25,26} and matched controls, even young patients.\textsuperscript{25,26} Furthermore, in the ARIC study, low levels of plasma protein C and antithrombin were not related to coronary heart disease.\textsuperscript{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 (ie, factor V Leiden, the prothrombin G20210A mutation) were more frequently related to myocardial infarction\textsuperscript{25,26,28} and/or overall coronary disease (ie, myocardial infarction or coronary stenosis).\textsuperscript{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.\textsuperscript{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 nondeficient 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.\textsuperscript{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. Why protein S deficiency, rather than deficiency of antithrombin, was associated with ATE may be the synthesis of protein S by endothelial cells, 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.

Since its first description, the amount of data indicating a link between VTE and subsequent ATE has been increasing. 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 estimated 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. 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. 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%). 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%). 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, 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 nondeficient 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.\textsuperscript{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 nondeficient 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


