Sickle cell trait and the risk of venous thromboembolism among blacks

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People with sickle cell disease have a chronically activated coagulation system and display hemostatic perturbations, but it is unknown whether they experience an increased risk of venous thromboembolism. We conducted a case–control study of venous thromboembolism that included 515 hospitalized black patients and 555 black controls obtained from medical clinics. All subjects were assayed for hemoglobin S and hemoglobin C genotypes. The prevalence of the S allele was 0.070 and 0.032 for case patients and controls, respectively (P < .001). The odds that a patient had sickle cell trait were approximately twice that of a control, indicating that the risk of venous thromboembolism is increased approximately 2-fold among blacks with sickle cell trait compared with those with the wild-type genotype (odds ratio = 1.8 with 95% confidence interval, 1.2-2.9). The odds ratio for pulmonary embolism and sickle cell trait was higher, 3.9 (2.2-6.9).

The prevalence of sickle cell disease was also increased among case patients compared with controls. We conclude that sickle cell trait is a risk factor for venous thromboembolism and that the proportion of venous thromboembolism among blacks attributable to the mutation is approximately 7%. (Blood. 2007;110:908-912)

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Introduction

Genetic risk factors for venous thromboembolism (VTE) in the black population are poorly understood. Common genetic risk factors for VTE in whites rarely are found among blacks.1,2 Although it is well known that individuals with sickle cell disease (SCD) manifest laboratory evidence of a chronically activated coagulation system, it is unknown whether they are at increased risk of VTE. Because a similar but lesser degree of coagulation activation occurs in asymptomatic subjects with sickle cell trait (SCT) and because the amplification phase of coagulation is enhanced on the surface of irreversibly sickled erythrocytes,2 we hypothesized that SCT may be a risk factor for VTE. We therefore evaluated the relationship between VTE and sickle cell disorders in the ongoing Genetic Attributes and Thrombosis Epidemiology case–control study.

Patients, materials, and methods

This study was approved by the Institutional Review Boards of both Emory University, Atlanta, GA, and the Centers for Disease Control and Prevention. Informed consent was obtained in accordance with the Declaration of Helsinki. This study has been described elsewhere.3 Persons, aged 18 to 70, hospitalized at 2 university hospitals in Atlanta, GA, with a recently diagnosed first or recurrent episode of a deep vein thrombosis (DVT) and/or pulmonary embolism (PE) between March 1998 and September 2005 were eligible as cases. During the study period, we approached 1867 patients with a diagnosis of DVT and/or PE for enrollment into the study. A total of 487 refused participation, 36 consented but had no questionnaire data or blood sample, and we could not locate 199 after their discharge from the hospital. Of the 1145 case patients enrolled in the study, 536 identified themselves as black and 515 had DNA results both for hemoglobin S and C and were included in this analysis.

Control subjects were sampled from a list of patients who visited the office of one of 10 physicians at a university-affiliated primary care clinic between January 1997 and September 2005. The list was sampled to obtain controls approximately similar to patients with respect to age, sex, and race. Persons with a history of VTE or currently taking anticoagulant medications were not eligible as controls. We attempted to contact 2956 potential controls and located 2095 of them. Of these, 153 were ineligible attributable to a prior thrombosis or attributable to a mental or physical impairment. Of the remaining 1942 eligible controls, 609 refused to participate and 4 died before we could obtain an interview. A blood sample was not obtained for 20 and hence the final number of controls was 1309. Of these controls, 586 identified themselves as black and 555 had DNA results both for hemoglobin S and C and were included in the analysis.

Diagnoses of DVT were confirmed by contrast venography for 41 cases, by Doppler ultrasonography for 315 cases, by magnetic resonance imaging for 2 cases, by computed tomography (CT) for 37 cases, and by other procedures for 4 cases. The presence of PE was established by angiogram for 7 cases, by ventilation–perfusion lung scan for 52 cases, by CT for 102 cases, by ventilation–perfusion scan and CT for 15 cases, and by other procedures for 2 cases. Only ventilation–perfusion scans judged as high probability of a PE were included in the study. The majority (>95%) of the CT-confirmed cases were done by spiral CT. However, in 1998 and 2000, as many as 6 PE cases may have been diagnosed by conventional CT.

We distinguished between patients with an underlying provocation for their VTE (n = 286) and those without (idiopathic cases, n = 229). Provoked cases included VTE arising in the setting of cancer; in conjunction with the placement of a central line; during or after attendance in the intensive care unit; during or after a pregnancy; or after surgery, an injury, or prolonged immobilization within the previous 4 weeks. These categories were not mutually exclusive. The mean time between the trigger for the event and its diagnosis was less than 1 week for provoked cases. Cases without any of these underlying conditions were considered to be idiopathic. We also distinguished between first VTE events (n = 417) and those occurring in a person with a self-reported history of VTE (n = 98).
Data collection

A whole blood sample was obtained from case patients while in the hospital and from controls at a visit to the Centers for Disease Control and Prevention. Patients were interviewed in the hospital and controls at their visit to the Centers for Disease Control and Prevention. A personal history of hypertension and diabetes was considered positive if subjects reported that a doctor had told them that they had these conditions. Cigarette smoking pertained to self-reported current smoking. A subject was considered obese if his body mass index exceeded 30 kg/m². Family history of VTE was considered positive if the subject reported a history of a blood clot in a first-degree relative. A subject was considered positive on exercise if he reported engaging in a sporting activity or doing physical exercise at least once per month.

Laboratory methods

Blood samples were collected in 0.109 mol/L sodium citrate. DNA was extracted from the whole blood samples according to the manufacturer’s protocol using the Puregene kit from Gentra Systems (Minneapolis, MN) and then stored at −20°C.

Polymerase chain reaction

Polymerase chain reaction (PCR) amplifications were performed with rapid temperature ramping in a DNA Engine Peltier Thermal Cycler (MJ Research, Reno, NV). Proprietary PCR primers were custom-designed for this multiplexed PCR reaction (Tag-It assay; Tm Bioscience, Toronto, ON, Canada). Allele-specific primer extension reactions were completed in a DNA Engine Peltier thermal cycler with proprietary custom-designed primers for the multiplexed reaction.

Luminex technology

The Bioscience Tag-It assay uses precise DNA sequences or probes containing specific proprietary sequences, which function as tags to complement sequences coupled to microspheres. Each microsphere size with a specific fluorescent wavelength or color is assigned to one target allele, allowing reactions with multiplexed targets to be accurately decoded by a Luminex100 (Luminex, Austin, TX). The hybridization reactions were conducted in a 96-well PCR plate and approximately 2500 anti-tag-coupled microspheres were used for each of the 10 single nucleotide polymorphism bases in the multiplex assay. The reporter signals were read on a Bio-Plex system (Bio-Rad, Richmond, CA) powered by Luminex xMap technology and the data were analyzed on Tag-It data analysis version 4.01 software written by Bioscience Corporation.

Validation

A minimum of 5% of the results for each single nucleotide polymorphism was validated by sequencing with BigDye Terminator v1.1 on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Statistical methods

The distribution of genotypes and the prevalence of alleles were compared for case patients and controls using the χ² distribution. We obtained the ratio of the odds of a particular genotype compared with the wild-type genotype for case patients compared with controls, 95% confidence limits for the odds ratio, and 2-tailed P values through the use of unconditional logistic regression. These odds ratios are interpreted as the risk of VTE for persons with a particular genotype divided by the risk among persons with the wild-type genotype. For sparse tables, we used Fisher exact test to obtain P values. The assumption of Hardy-Weinberg equilibrium (HWE) for the hemoglobin (Hb) S and Hb C genotypes was evaluated among controls by the method described by Sham.

Results

The median age of patients was 48 years old (25th percentile 38 years old; 75th percentile 56 years old), whereas that of controls was 49 years old (25th percentile 38 years old; 75th percentile 58 years old). Diabetes was a statistically significant risk factor for VTE (Table 1). Exercise was protective against VTE, even in an analysis restricted to idiopathic cases (P = .004). On the other hand, there was little or no association between obesity and VTE. Family history of VTE in a first-degree relative was a risk factor for VTE (odds ratio [OR] [all cases] = 2.7, 95% CI: 1.8-3.9). The Factor V Leiden and prothrombin mutations were risk factors for VTE, but the findings are only marginally statistically significant because each mutation is rare among blacks.

The distribution of genotypes at codon 6 (E>V) characterizing the Hb S mutation was statistically significantly different for case patients and controls (P < .001; Table 2). The odds of SCT among patients (number of patients with the Hb AS genotype divided by number of patients with the wild-type genotype, Hb AA) is almost 2-fold that of the corresponding odds among controls (P = .006). The odds ratio adjusted for age and sex and for potential risk factors for VTE (history of diabetes, hypertension, physical inactivity,

Table 1. Distribution of odds ratio, P values, and 95% confidence intervals according to putative risk factors† for case patients and controls

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Case patients</th>
<th>Controls</th>
<th>OR§ overall</th>
<th>P value‡</th>
<th>OR§ idiopathic</th>
<th>95% CI§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>219</td>
<td>296</td>
<td>224</td>
<td>331</td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>131</td>
<td>384</td>
<td>85</td>
<td>470</td>
<td>2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>256</td>
<td>259</td>
<td>258</td>
<td>297</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Obesity</td>
<td>232</td>
<td>283</td>
<td>217</td>
<td>338</td>
<td>1.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Exercise</td>
<td>211</td>
<td>302</td>
<td>255</td>
<td>300</td>
<td>0.83</td>
<td>0.13</td>
</tr>
<tr>
<td>Family history of venous thromboembolism</td>
<td>90</td>
<td>321</td>
<td>49</td>
<td>465</td>
<td>2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>6</td>
<td>509</td>
<td>1</td>
<td>554</td>
<td>6.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Prothrombin mutation</td>
<td>4</td>
<td>511</td>
<td>0</td>
<td>555</td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

CI indicates confidence interval.  
†Risk factors are self-reported except for the factor V Leiden and prothrombin mutation.  
‡Odds ratio (OR) and P values are adjusted for age and sex.  
§Odds ratio pertains to 229 idiopathic cases and all controls with adjustment for age and sex.  
¶Matching factor.  
||Unadjusted (crude) results with exact mid P value or lower confidence limit.)
cigarette smoking, body mass index, and the factor V Leiden mutation) was hardly changed (OR = 1.8 with 95% CI: 1.1-2.8). The odds ratio for SCT and VTE was slightly higher among subjects with a family history (OR = 2.3), but this odds ratio is not statistically different from the corresponding odds ratio pertaining to subjects without a family history of VTE. Eight patients and no controls had the Hb SS genotype (SCD-SS, exact test, P = .002). The prevalence of the S allele is 0.070 and 0.032 for case patients and controls, respectively (P < .001). The Hb S genotypes are in HWE among controls.

A total of 286 of the cases were provoked and 229 were idiopathic. The odds ratio for SCT among the idiopathic cases was 1.8 (1.1-3.1), whereas among the provoked cases, the odds ratio was 1.9 (1.1-3.1). Ninety-eight of the cases were recurrent events, whereas 417 were first events. The odds ratios for the SCT for recurrent and first events were 1.9 (0.95-4.0) and 1.8 (1.1-2.9), respectively. Because all of these odds ratios are approximately 2 and are not statistically significantly different from each other, we prefer the overall odds ratio for all cases displayed in Table 2. Of the 8 patients with the Hb SS genotype, 7 were provoked and one was idiopathic, whereas 3 were recurrent and 5 were first events.

The prevalence of SCT differed according to type of VTE (Table 3). The prevalence was similar for controls and for patients with DVT only. On the other hand, the prevalence of SCT among cases of PE was markedly elevated compared with controls. Patients with both DVT and PE had an intermediate prevalence of SCT. The mean age of patients with and without SCT was 43 years old and 48 years old, respectively (P = .01).

At codon 6 (E>K) reflecting the Hb C mutation, 501 patients and 458 controls had the wild-type genotype, 14 cases and 7 controls were heterozygous for the Hb C mutation, and no subject was homozygous for the C mutation. Although the odds of heterozygosity for the Hb C mutation among case patients were approximately twice that of controls, the difference is not statistically significant (OR = 2.2; 95% CI: 0.9-5.5; P = .09). The odds ratio for heterozygosity for the Hb C mutation was slightly higher for PE-only cases (OR = 2.8) than for DVT-only cases (OR = 2.1), but these odds ratios are not statistically significantly different from each other. The Hb C genotypes were in HWE among controls.

Five patients and no controls were compound heterozygotes for the Hb S and Hb C mutations (SCD-SC), a difference that is statistically significant (Fisher exact P = .026). Three of these patients had DVT only, whereas 2 had PE only.

Assuming that the odds ratio for SCT in this study (approximately 2) estimates the relative risk of VTE for carriers versus noncarriers and that the prevalence of SCT among blacks is approximately 7%, then the population attributable risk (PAR) for VTE and SCT is estimated as

\[
PAR = 0.07/(0.07 \times 2) + 0.93 \approx 0.07.
\]

Discussion

The major finding of this study was that persons with SCT experienced approximately a 2-fold increased risk of VTE compared with persons with the wild-type genotype, Hb AA. This increased risk was observed both for idiopathic and provoked VTE as well as for first and recurrent VTE. In addition, PE risk (without DVT) was significantly increased (approximately 4-fold) among those with SCT, whereas the risk of a DVT (without PE) was not meaningfully increased. The increase in risk of DVT with PE among persons with SCT was intermediate. Heterozygosity for Hb C was more prevalent among case patients than controls, but the sample size was too small to conclude with confidence that Hb C was a risk factor for VTE. A secondary finding of interest was that persons with sickle cell disease (SCD-SS and SCD-SC) also experienced an increased risk of VTE.

Although it has been long recognized that patients with SCD manifest an activated coagulation system, a persuasive link between SCD and VTE has not been established. Because SCD is associated with considerable morbidity and frequent hospitalizations, it is difficult to distinguish if an increased risk of VTE is an indirect result of complications of SCD, such as immobility, or if the increased risk is a direct result of enhanced basal activation of the coagulation system attributable to the sickling process. Our observation that asymptomatic persons with Hb AS also experienced an increased risk of VTE suggests that the increased VTE risk associated with SCD is attributable, at least in part, to the effect of sickling erythrocytes on coagulation rather than to secondary health conditions.

That persons with SCT experience an increased risk of VTE is biologically plausible. SCD is associated with increased circulating tissue factor procoagulant factor, increased markers of thrombin generation, decreased levels of natural anticoagulant proteins, and evidence of platelet and fibrinolytic system activation. The hemostatic perturbations among persons with SCT are not as pronounced but, nonetheless, are apparent. For example, laboratory markers of coagulation activation such as TAT complexes, d-dimers, and prothrombin fragment F1.2 are elevated in persons with Hb AS compared with those with Hb AA, and were the highest among those with Hb SS. The amplification phase of coagulation or the assembly of the prothrombinase complex is enhanced on the

<table>
<thead>
<tr>
<th>Type of VTE</th>
<th>Hb AS*</th>
<th>Hb AA</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>35 (6.3)</td>
<td>520</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>DVT only</td>
<td>23 (7.0)</td>
<td>307</td>
<td>1.1</td>
<td>0.65-1.9</td>
</tr>
<tr>
<td>PE only</td>
<td>24 (21.9)</td>
<td>91</td>
<td>3.9</td>
<td>2.2-6.9</td>
</tr>
<tr>
<td>DVT/PE</td>
<td>9 (14.5)</td>
<td>53</td>
<td>2.5</td>
<td>1.2-5.5</td>
</tr>
</tbody>
</table>

— indicates not applicable.

*Number of patients (% with sickle cell trait) shown. Eight cases with Hb SS are excluded.

Table 3. Distribution of subjects and the odds ratio with 95% confidence limits according to type of venous thromboembolism and sickle cell trait
surface of irreversibly sickled erythrocytes. Membranes of sickle cell erythrocytes show loss of normal phospholipid asymmetry, and the resulting abnormal phosphatidylserine exposure is thought to contribute to the hemostatic perturbations seen in persons with SCD. Subclinical sickling of red cells probably occurs in most persons with SCT and may explain such complications as splenic infarction at high altitudes, essential hematuria, loss of renal concentrating ability, and sudden death after extreme exertion. Humphries et al described a case report of a 23-year-old man with multiple episodes of recurrent venous thrombosis in which the SCT was the only identified potential risk factor. Our study now provides epidemiologic evidence that another clinical manifestation of SCT is an increased risk of VTE that also may result from subclinical sickling of red cells.

We do not have a biologic explanation for the observation that the association between SCT and PE was stronger than that for DVT. However, there is a precedent that risk factors for DVT and PE, although they overlap considerably, can differ meaningfully. For example, it is generally accepted that the association between factor V Leiden and DVT is stronger for DVT than it is for PE, and recent data demonstrate that the prothrombin G20210A is conversely more likely to be associated with PE than DVT. More to the point, in a report of the National Hospital Discharge Survey, 0.44% of black patients with SCD had a discharge diagnosis of PE compared with approximately 0.12% of blacks without SCD. On the other hand, the prevalence of a discharge diagnosis of DVT among blacks with SCT was 0.44%, approximately the same (0.40%) as hand, the prevalence of a discharge diagnosis of DVT among hospitalized black men, PE was found in 2.2% of the patients with SCD.13 Subclinical sickling of red cells probably occurs in most persons with SCT and may explain such complications as splenic infarction at high altitudes, essential hematuria, loss of renal concentrating ability, and sudden death after extreme exertion. Humphries et al described a case report of a 23-year-old man with multiple episodes of recurrent venous thrombosis in which the SCT was the only identified potential risk factor. Our study now provides epidemiologic evidence that another clinical manifestation of SCT is an increased risk of VTE that also may result from subclinical sickling of red cells.

In a retrospective study of approximately 65,000 consecutively hospitalized black men, PE was found in 2.2% of the patients with SCT compared with 1.5% of those with normal hemoglobin. As indicated by these investigators, this finding must be interpreted with caution because the magnitude of the difference is small and surrogate markers were used for the diagnosis of PE. Our study supports the hypothesis that SCT is related to increased VTE risk, especially PE. In addition, the observation that events occur at an earlier age among cases with SCT compared with those without the trait provides some support for a causal interpretation of the finding, insofar as disease attributable to a genetic defect often occurs earlier in life than does idiopathic disease. On the other hand, in a study of 355 hospitalized men with SCT, HbS concentration was not related to PE. However, because all subjects in this study had SCT, this study is not directly relevant to the issue of whether SCT is a risk factor for PE.

Our study had limitations. The participation rate of subjects, especially case patients, was somewhat low. However, the observation that the prevalence of SCT among our controls, 6.2%, is comparable with that observed (6.7%) in a large cross-sectional study of 30,400 healthy blacks in the Washington, DC, metropolitan area suggests that the decision to participate in the study was not related to the sickle cell genotype. The observation that the SCT/VTE association was seen both for provoked and idiopathic cases contradicts the belief that the association is an artifact arising from the use of sick, hospitalized cases. Another limitation of the study is that PE was not ruled out for patients diagnosed only with a DVT, nor was a DVT ruled out for patients diagnosed only with PE. However, any resulting misclassification would tend to attenuate differences in the associations between PE and SCT and DVT and SCT and, hence, is an unlikely explanation of the findings in Table 3.

On the other hand, the study had some major strengths. It was sufficiently large to rule out chance as an explanation of its findings. The assays for SCT were performed in a blinded fashion and hence systematic bias arising from the laboratory was unlikely. The observations that the genotype frequencies for Hb S and Hb C were in HWE among controls and that the prevalence of the S allele in our controls was nearly identical to that observed in a large population-based survey provides credence to our laboratory methods. Thus, we believe that chance and bias are unlikely explanations of our findings and therefore favor a causal interpretation of the study.

A major implication of this study is the recognition of a potentially important inherited form of thrombophilia in the black population. The most common inherited risk factors for VTE in Americans of European descent, factor V Leiden and the prothrombin G20210A mutation, are rare in blacks. However, despite the absence of these 2 genetic risk factors, blacks have approximately the same or a slightly higher rate of VTE than do whites and are more likely to be diagnosed with idiopathic PE. Our study findings provide a partial explanation for this paradox. That is, among whites, the prevalence of the prothrombin G20210A mutation is approximately 3% with a relative risk between 2 and 3. Using a relative risk of 2.0, the proportion of VTE among whites attributable to the prothrombin mutation is approximately 3%. As estimated previously, the proportion of VTE among blacks attributable to Hb AS is approximately 7%. Thus, if our finding is valid, SCT is a more important cause of VTE among blacks than is the prothrombin mutation among whites. We note that 34 (37%) of the 91 subjects determined to have SCT were unaware that they had the trait. This observation implies that improvements in educational efforts regarding sickle cell status among blacks are needed.

In summary, we have found that blacks with SCT experience approximately a 2-fold increased risk of VTE. The association is much stronger for PE than it is for DVT without PE. Physicians treating blacks with SCT should be aware that such patients are at increased risk for a VTE, especially PE.

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Authorship

Contribution: H.A., C.L., N.F.D., C.W., and W.C.H. designed the study and collected the data; J.M.B. and W.C.H. were responsible for all the laboratory work; data management and analysis were done by H.A. and C.L.; all of the authors contributed to the writing, although H.A. and N.S.K. were the principal authors; N.S.K. provided the encouragement to evaluate sickle cell trait in our study population. Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

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