Relation of Three Genetic Traits to Venous Thrombosis in an African-American Population

Anne Dilley,1 Harland Austin,1,2 W. Craig Hooper,1 Cathy Lally,1,2 Maria J. A. Ribeiro,3 Nanette Kass Wenger,3,4 Victor Silva,3,4 Peggy Rawlins,4 and Bruce Evatt1

A mutation in the Factor V gene (Factor V Leiden), a variant in the 5,10-methylenetetrahydrofolate reductase gene (MTHFR), and an insertion/deletion polymorphism of the angiotensin I-converting enzyme gene (ACE) may be related to abnormal blood clotting. The authors examined the associations between these genetic traits and venous thrombosis among African Americans. This study comprised 93 patients with venous thrombosis and 185 control subjects attending clinics at an urban, public hospital in Atlanta, Georgia, in 1995-1996. Subjects' DNA was extracted from blood and assayed for these genetic traits. Odds ratios were obtained from logistic regression and used as a measure of association between each genetic trait and venous thrombosis. Factor V Leiden was unrelated to venous thrombosis, but the mutation was too rare among our African-American subjects to evaluate adequately its relation to venous thrombosis. The homozygous and heterozygous genotypes for the V allele of the MTHFR gene were unrelated to venous thrombosis (odds ratio = 0.9, 95% confidence interval 0.5–1.8). Subjects with the deletion/deletion ACE polymorphism experienced a moderate increase in venous thrombosis risk compared with persons with the other genotypes (odds ratio = 1.5, 95% confidence interval 0.9–2.6). However, women with this ACE genotype experienced no increased risk (odds ratio = 0.9, 95% confidence interval 0.5–1.9), whereas men with this genotype had nearly three times the risk (odds ratio = 2.8, 95% confidence interval 1.2–6.2; p value for interaction = 0.06). These data indicate that the prevalence of Factor V Leiden and the V allele of the MTHFR gene is low among African Americans. The D allele of the ACE gene is equally prevalent among African Americans and whites and may be related to venous thrombosis among African-American men. Am J Epidemiol 1998;147:30-5.

Complications of deep vein thrombosis, which include pulmonary embolism and chronic venous insufficiency, are associated with significant mortality and morbidity (1). Past studies have reported that, while approximately 30 percent of deep vein thromboses are due to deficiencies in the anticoagulant proteins (protein S, protein C, and antithrombin III), the causes for the remaining cases are largely unknown (2).

Recent studies have identified three genetic traits that may directly or indirectly affect blood coagulability. Based on earlier work by Marlar et al. (3), in which it was determined that activated protein C (APC) controlled clot formation by the proteolytic inactivation of the coagulation factors Va and VIIIa, the study by Dahlback et al. (4) described an impaired APC response, termed APC resistance (APC-R), in a patient with a history of unexplained thrombosis. In 1994, Bertina et al. (5) identified the molecular basis of APC-R as a defect in Factor V (Factor V Leiden) involving the mutation of Arg506 to Gln506. APC-R has been implicated as a cause of venous thrombosis in several epidemiologic studies (6–8).

Hyperhomocysteinemia is a consequence of either inherited or acquired alterations in the transsulfuration or remethylation pathway. A recently described polymorphism in the 5,10-methylenetetrahydrofolate reductase gene (MTHFR) that encodes for a key enzyme involved in remethylation is associated with reduced MTHFR activity and elevated levels of plasma homocysteine (9). The polymorphism is due to a C to T substitution at nucleotide 677 that converts alanine to a valine (9). Elevated levels of plasma homocysteine have been associated with an increased risk of venous thrombosis (10–12).
In humans, an insertion/deletion polymorphism of the angiotensin I-converting enzyme gene (ACE) has been identified in intron 16. Of the three genotypes I/I, I/D, and D/D, the D/D genotype has been associated with the highest plasma ACE activity, the I/D genotype with intermediate ACE activity, and the I/I genotype with the lowest ACE activity (13). ACE converts angiotensin I to the potent vasoconstrictor, angiotensin II, and inactivates bradykinin, a vasodilator (14). Thus, one mechanism by which elevated ACE activity may cause chronic vascular disease is by inducing chronic vasoconstriction (15, 16). High ACE levels are also thought to interfere with the fibrinolytic system by increasing levels of plasminogen activator inhibitor 1 (17). Although the D/D genotype has been related to cardiovascular disease in several epidemiologic studies (18, 19), the relation of the ACE polymorphism and venous thrombosis has not been evaluated.

Almost all of the epidemiologic evidence evaluating the association between these genes and venous and arterial thrombosis pertains to white populations. The purpose of the present study was to determine if these genetic characteristics are associated with venous thrombosis among African Americans.

MATERIALS AND METHODS

All patients with a venous thrombosis attending an anticoagulant clinic at a large, urban public hospital in Atlanta, Georgia, in 1995–1996 were eligible for inclusion as cases in the study. We approached 137 of these patients, and 136 (99 percent) agreed to participate. Two patients were excluded as cases because their medical records did not support a venous thrombosis diagnosis. Twenty-seven patients were excluded because they had a history of heart disease, stroke, or other arterial thromboses. The present analysis is restricted to 93 African-American cases (14 white cases were excluded). Of these 93 cases, 51 had a deep vein thrombosis only, 34 had a deep vein thrombosis with a pulmonary embolism, and two had an inferior vena cava thrombus. Eighty-one of the cases were confirmed by a venogram, ultrasound examination, an angiogram, or a ventilation-perfusion scan. The two cases with an inferior vena cava thrombus were confirmed by computerized tomography. For 10 cases, we could not find radiologic confirmation of the diagnosis in the medical record and therefore relied on the clinical diagnosis.

We selected controls from among outpatients attending a clinical laboratory for routine blood tests and frequency matched them to cases on age (within 10 years), sex, and race. We excluded as controls persons with a history of heart attack, stroke, or blood clots. Of 225 eligible control subjects asked to participate, 200 (89 percent) agreed. The controls in the present analysis are restricted to 185 African Americans (15 white controls were excluded).

Participation in the study entailed granting permission to review medical records, an in-person interview, and the collection of 20 ml of blood. The questionnaire elicited information on basic demographics, lifestyle habits, a personal history of thrombosis and other medical problems, and a family history of blood clots, stroke, or heart attack.

Laboratory methods

The presence of Factor V Leiden, zygosity for the insertion/deletion polymorphism of the ACE gene, and zygosity for the alanine/valine polymorphism of the MTHFR gene were determined from DNA extracted from a blood sample. Blood samples were collected in 0.109 M sodium citrate. DNA was extracted from 3 ml of whole blood using a Gentra DNA Extraction kit (Minneapolis, Minnesota) per the manufacturer's instructions and stored at −20°C. Polymerase chain reaction was used to amplify DNA fragments in the Factor V (20), MTHFR (9), and ACE (18) genes. Restriction enzyme analysis for Factor V Leiden and MTHFR was carried out using MnlI and HindIII, respectively. The digested products were then run on an ethidium bromide-stained 3 percent metaphor gel, and the results were determined from the restriction enzyme digestion pattern. A subset of samples was selected at random and confirmed by direct nucleotide sequencing. The insertion/deletion polymorphisms were determined by sizing the amplified product in an ethidium bromide-stained 1.5 percent agarose gel. In order to ensure stringency for the D/D genotype, dimethyl sulfoxide was included in the polymerase chain reaction mixture. Furthermore, all D/D results were confirmed with the use of a third primer set that discriminated between I/D and D/D. Polymerase chain reaction results for the genes were confirmed by both direct nucleotide sequencing and by GENESCAN software analysis following electrophoresis on a model 377 automated ABI DNA sequencer (Applied Biosystems, Foster City, California) on a subset of randomly selected samples. Quality control for the DNA analyses was maintained by the use of both positive and negative controls in each set of analyzed samples, and results were confirmed independently by two different laboratory workers.

Statistical methods

We created separate logistic regression models to assess the relation between venous thrombosis and Factor V Leiden, the ACE polymorphism, and the MTHFR polymorphism. Since all subjects were either
heterozygous for the Factor V Leiden mutation or homozygous for its absence, the Factor V defect was classified simply as present versus absent. The MTHFR genotypes are denoted as alanine/alanine (A/A), alanine/valine (A/V), and valine/valine (V/V). The ACE genotypes are denoted as insertion/insertion (I/I), insertion/deletion (I/D), and deletion/deletion (D/D). We analyzed the MTHFR polymorphism by considering the expression of the V allele either as dominant (V/V and A/V vs. A/A) or as recessive (V/V vs. A/V and A/A). Additionally, we fit a logistic model with scores of 0 for the A/A genotype, 1 for the A/V genotype, and 2 for the V/V genotype. This model assumes that the presence of an additional V allele is associated with a multiplicative increase in venous thrombosis risk. We performed an analogous analysis for the D allele for the ACE gene. The odds ratio was used as a measure of association between the genes and venous thrombosis (21).

We included a continuous variable for age and an indicator for sex in our logistic models. Alcohol consumption, cigarette smoking, and family history of thrombosis were evaluated as potential confounders. Alcohol habit pertained to average lifetime use. Since these factors did not confound any of the gene-disease associations, we obtained the odds ratios relating the genetic traits and venous thrombosis from a logistic model that included terms only for the matching factors (age and sex). All reported \( p \) values are two-tailed, and 95 percent confidence intervals are used.

### RESULTS

The distribution of cases and controls according to basic demographics and history of other illnesses is displayed in table 1. The mean ages for cases and controls are 55 and 56 years, respectively (ranges, 18–89 years for cases and 21–93 years for controls). Fifty-four of the cases and 94 of the controls are women. Cases and controls were similar with respect to a history of high blood pressure or diabetes, a family history of any vascular disease, cigarette habit, and alcohol consumption. Eight cases had a history of cancer. Cancer was more common among controls, some of whom were obtained from the general medicine clinic that includes cancer patients.

In table 2, the odds ratios relating venous thrombosis and each of the three genetic traits are displayed. The Factor V mutation is not associated with venous thrombosis among these study subjects. However, the odds ratio pertaining to Factor V Leiden is imprecise because of the low prevalence of the mutation among study subjects. The prevalence of the V allele among our study subjects also was too low to evaluate adequately the recessive allele model (V/V vs. A/V and A/A). However, the dominant allele model (V/V and A/V vs. A/A) was more informative. There was little or no association between venous thrombosis and the combined homozygous and heterozygous genotypes of the V allele compared with the A/A genotype (see table 2). The overall prevalence of the V allele was 9.1 percent and 10.0 percent among cases and controls, respectively (\( p > 0.20 \)). This observation further supports the belief that there is little or no association between the MTHFR polymorphism and venous thrombosis.

The odds ratio for the ACE D/D genotype compared with the other two ACE genotypes combined is moderately elevated but not statistically significantly so (\( p = 0.13 \)). Among women, the odds ratio for the D/D genotype compared with the other two combined is near its null value of unity (odds ratio = 0.9, 95 percent confidence interval 0.5–1.9), whereas among men the D/D genotype is associated with about a tripling of venous thrombosis risk (odds ratio = 2.8, 95 percent confidence interval 1.2–6.2; \( p = 0.01 \)). This difference between the sex-specific odds ratios for the D/D genotype approaches statistical sig-

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**TABLE 1.** Distribution of cases and controls according to basic demographic characteristics and a history of selected diseases, Atlanta, Georgia, 1995–1996

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n = 185)</th>
<th>Cases (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>91</td>
<td>49</td>
</tr>
<tr>
<td>Female</td>
<td>94</td>
<td>51</td>
</tr>
<tr>
<td>History of cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>155</td>
<td>84</td>
</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>138</td>
<td>75</td>
</tr>
<tr>
<td>Yes</td>
<td>47</td>
<td>25</td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>34</td>
</tr>
<tr>
<td>Yes</td>
<td>122</td>
<td>66</td>
</tr>
<tr>
<td>Family history of vascular disease*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>106</td>
<td>57</td>
</tr>
<tr>
<td>Yes</td>
<td>79</td>
<td>43</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>82</td>
<td>44</td>
</tr>
<tr>
<td>Ever smoked</td>
<td>103</td>
<td>56</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondrinker</td>
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<td>26</td>
</tr>
<tr>
<td>&lt;1 per day</td>
<td>56</td>
<td>30</td>
</tr>
<tr>
<td>1–2 per day</td>
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<td>12</td>
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<tr>
<td>≥3 per day</td>
<td>58</td>
<td>31</td>
</tr>
<tr>
<td>Age (mean years)</td>
<td>Cases (mean years)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>55</td>
</tr>
</tbody>
</table>

* A history of blood clots, stroke, or heart attack.
nificance \((p = 0.06)\). The odds ratios for the \textit{D/D} \ and \textit{I/I} genotypes combined versus the \textit{I/I} genotype are below unity for both men and women.

**DISCUSSION**

This case-control study allowed us to examine the association of three genetic traits with venous thrombosis in African Americans, as well as to compare the prevalences of these traits with those observed in whites. We did not find an association between \textit{Factor V Leiden} and venous thrombosis in this African-American study population. However, lack of statistical power precluded us from an adequate assessment of this gene (the minimal detectable odds ratio to obtain 80 percent power for a two-tailed test at a level of 5 percent for \textit{Factor V Leiden} was about 6.3). We anticipated such low statistical power based upon our interim report that the overall prevalence of \textit{Factor V Leiden} is significantly lower in African Americans than in whites (22).

We found no association between smoking and alcohol use and venous thrombosis in this study. However, we believe that our use of hospital controls precludes a valid assessment of the relation of such lifestyle factors and venous thrombosis. This is so because many controls were attending the hospital for treatment of tobacco- and alcohol-related diseases. Furthermore, we suspect that our study subjects’ self-reported data may be inaccurate, biasing the odds ratios for such factors toward the null. For these reasons, we have not emphasized these findings in the paper nor have we estimated the odds ratios relating these factors and venous thrombosis. Nonetheless, despite this limitation of our study, we believe it is valid for the purpose of evaluating the role of these three genes in the etiology of venous thrombosis in African Americans.

Several epidemiologic studies have found a direct association between elevated plasma homocysteine levels and venous thrombosis (10–12). However, to our knowledge, the relation between the \textit{MTHFR} polymorphism and venous thrombosis has not been investigated. Frost et al. (9) reported that persons homozygous for the \textit{V} allele had higher plasma homocysteine levels than did persons with the \textit{A/V} and \textit{A/A} genotypes who had similar plasma homocysteine levels. We could not adequately evaluate the risk of venous thrombosis associated with the \textit{V/V} genotype because the prevalence of the \textit{V} allele was too low among our study subjects (minimal detectable odds ratio of about 5.5). However, the dominant allele model \((V/V \text{ vs. } A/V \text{ vs. } A/A)\) was more informative, and these genotypes were unrelated to venous thrombosis (minimal detectable odds ratio of about 2.3). Thus, this analysis, as well as the observation that the prevalence of the \textit{V} allele was nearly identical among cases and controls, provides moderate support against the belief that the heterozygous genotype for the \textit{V} allele is associated with increased venous thrombosis risk. In a recent study from Ohio, the prevalence of the \textit{V} allele was significantly lower among African Americans (10 percent) than it was among whites (30 percent) (23). The prevalence of the \textit{V} allele among our controls was also 10 percent, providing additional evidence that the \textit{V}
allele is much less common among African Americans than it is among whites in the United States.

The D/D ACE genotype has been linked to an increased risk of cardiovascular disease in numerous epidemiologic studies (18, 24, 25), although one large prospective study did not find the positive association (26). We are unaware of any epidemiologic studies of the ACE polymorphism and venous thrombosis. The dominant allele model (D/D and I/D vs. I/I) in our study was unrelated to venous thrombosis. We found a moderate, though not statistically significant, elevated odds ratio of 1.5 for venous thrombosis for persons with the D/D genotype compared with persons with the I/D or I/I genotypes for men and women combined. However, our sex-specific analysis indicates that, among African-American men, the risk of venous thrombosis is about tripled for those with the D/D genotype. The D/D genotype is not associated with increased risk among African-American women. The interaction with sex was almost statistically significant. We have no biologic explanation for this finding and would be inclined to attribute it to chance were it not for the fact that two other studies of the ACE polymorphism also reported sex differences. In a cross-sectional study, Schunkert et al. (27) found that the odds of left ventricular hypertrophy for men with the D/D genotype were increased by about 2.6-fold, while the corresponding odds for women were 1.2. The difference in odds ratios between men and women in that study is nearly statistically significant (p = 0.07, our calculation). In a study of 182 white persons with coronary artery disease compared with 338 control subjects, Beohar et al. (19) reported an odds ratio for the D/D genotype of 2.0 for men and about 0.9 for women. This sex difference also appears to be statistically significant (p = 0.02, our calculation). Thus, considered in the context of these two studies, our findings that the D/D ACE polymorphism may increase venous thrombosis risk in African-American men, but not women, are more plausible.

The prevalence of the D allele among controls in our study is 55 percent, and the distribution of the three genotypes is consistent with the Hardy-Weinberg equilibrium. This prevalence is nearly identical to that (56 percent) reported for 2,340 US physicians, most of whom are white (26). Among African Americans living in Michigan and Florida, the prevalence of the D allele was reported as 58 percent and 60 percent, respectively (28, 29), though 85 percent of these subjects (n = 165) were hypertensive. The prevalence of the D allele was 49 percent among the 39 normotensive subjects in these two studies. Among 80 Nigerian bank workers, the prevalence of the D allele was 59 percent (30). Thus, in contrast to the D allele, the V allele for the MTHFR gene, the prevalence of the ACE D allele is similar in whites and African Americans.

In summary, the present study was too small to investigate adequately the relation between venous thrombosis and the Factor V Leiden defect or the V/V genotype among African Americans. It does, however, support the growing body of evidence that indicates that the prevalences of the Factor V Leiden mutation and the V allele for the MTHFR gene are considerably lower among African Americans compared with whites, in contrast to the prevalence of the D allele for the ACE gene that is comparable among whites and African Americans. The study also suggests that the D/D genotype for the ACE gene is related to venous thrombosis among African-American men. This latter finding requires confirmation in larger epidemiologic studies.

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REFERENCES


