The Relationship Between Polymorphisms in the Endothelial Cell Nitric Oxide Synthase Gene and the Platelet GPIIIa Gene With Myocardial Infarction and Venous Thromboembolism in African Americans* 

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**Study objectives:** To determine whether the polymorphic dinucleotide repeats found in intron 4 of the endothelial cell nitric oxide synthase (ecNOS) gene and the platelet GPIIIa PLA1/A2 polymorphism are associated with myocardial infarction (MI) and venous thromboembolism (VTE) in African Americans. Because these two genes may interact physiologically, the third objective was to determine if there was a relationship between the polymorphisms with respect to MI and VTE.

**Design:** A hospital-based case-control study. After informed consent was obtained, blood used for DNA extraction was drawn from the subjects.

**Setting:** The study was conducted in the Anticoagulant Clinic and the Cardiology Clinic at Grady Memorial Hospital in Atlanta Georgia.

**Patients:** Subjects were recruited from African-American patients with a reported history of MI (n = 110) or VTE (n = 91). Control subjects (n = 185) without a history of cardiovascular or venous disease were recruited from an outpatient clinic.

**Measurements and results:** The 393 ecNOS allele was more common among MI cases (36%; p = 0.01) and VTE cases (35%; p = 0.04) than among control subjects (26%). There was no association between the GPIIIa genotypes and either MI or VTE. However, among the MI subjects, there was a strong association between the ecNOS 393/393 genotype and the PlA2 allele. It was also found that the frequency of the 393 allele was higher in African-American persons (0.26) compared with what has been reported for Australian Caucasians (0.14) and Japanese (0.10).

**Conclusions:** The 393 allele but not the PlA2 allele was significantly associated with both MI and VTE in African Americans. Homozygosity for the 393 allele was significantly associated to the diagnosis of MI prior to the age of 45. The combination of the 393 allele and a PlA2 allele was also highly associated with MI. The frequency of the 393 allele was significantly higher in African Americans than what has been reported for other populations. This study furthers not only extends the association of the 393 allele to VTE but has demonstrated an interaction with the PlA2 allele with respect to MI.  

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**Key words:** African Americans; endothelial cell nitric oxide synthase; glycoprotein IIb/IIIa receptor; myocardial infarction; venous thrombosis

**Abbreviations:** CAD = coronary artery disease; CI = confidence interval; ecNOS = endothelial cell nitric oxide synthase; GP = glycoprotein; MI = myocardial infarction; NO = nitric oxide; OR = odds ratio; PCR = polymerase chain reaction; VTE = venous thromboembolism

Pathophysiologic alterations in nitric oxide (NO) production and/or platelet aggregation are thought to play a role in the pathogenesis of both coronary artery disease (CAD) and the acute coronary syndromes.1–3 There is also evidence to suggest that decreased NO production may also play a role in the development of venous disease.4–8 Under normal hemostatic conditions, NO contributes to the antithrombotic, antiproliferative, and anti-inflammatory properties of the endothelium, in part through the control of vascular tone, leukocyte/platelet adhesion, smooth muscle proliferation, and inhibition of platelet aggregation.8–11 The production of NO in the endothelial cell is regulated by the enzyme endothelial cell nitric oxide synthase (ecNOS).12 Platelet aggregation may also be affected by alterations in the glycoprotein (GP) IIb/IIIa receptor.13 A...
primary determinant of platelet aggregation is the binding of fibrinogen and von Willebrand factor to the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor found on the surface of the platelet. Both fibrinogen and von Willebrand factor are essential to platelet aggregation, and pathologic alterations in these proteins can lead to either increased or decreased platelet aggregation. Platelet aggregation and adherence are not only important in the evolution and progression of an atherosclerotic plaque, but can also initiate the occurrence of a myocardial infarction (MI) through the formation of an occlusive thrombus at the site of a ruptured plaque.

Genetic changes in the genes encoding either for ecNOS or the GPIIb/IIIa receptor could contribute to the development of CAD. Recent studies have reported an association of DNA polymorphisms in both the ecNOS and GPIIIa genes with both CAD and MI. In a Australian case-control study of CAD, Wang et al reported that, among white subjects with a history of smoking, homozygosity for the ecNOS4a allele was more common among cases than control subjects. Current or former smokers homozygous for the ecNOS4a allele also more frequently reported a history of MI than did smokers with other ecNOS genotypes. These investigators did not find such associations among nonsmokers. In a Japanese case-control study that was focused on MI, Ichihara et al reported an association between the ecNOS4a allele and MI in both smoking and non-smoking Japanese subjects. This association was stronger among subjects who lacked other risk factors for MI. Although controversial, the PA2/PA2 polymorphism of the GPIIIa gene has been associated with an increased risk of MI among whites in the United States and an increased risk of CAD among low-risk German patients. Because gene prevalence, and perhaps gene-disease associations, vary according to ethnicity, we assessed whether the ecNOS and the PA2/PA2 polymorphisms were associated with MI in African Americans. Also, because in vitro studies have shown that smoking can impair NO synthase in saphenous vein strips, we investigated whether there was a relationship between the ecNOS allele and venous thromboembolism (VTE) among our African-American subjects. Because of the physiologic relevance to platelet aggregation, we also examined the interactions between these two genes and the risk of MI and VTE.

**Materials and Methods**

At Grady Memorial Hospital, a large, urban public hospital in Atlanta, GA, all African-American patients with a history of VTE who were attending an anticoagulant clinic and patients with a history of MI attending a cardiology clinic were eligible for inclusion as cases in the study. In the anticoagulant clinic, 123 individuals with a possible VTE were approached and 122 (99%) agreed to participate. Four patients were excluded as cases because their medical records did not support a VTE diagnosis. An additional 27 patients were excluded because they had a self-reported history of heart disease, stroke, or other types of arterial thrombosis. Of the remaining 91 cases, 55 had deep-vein thrombosis, 34 had deep-vein thrombosis complicated by a pulmonary embolism, and two had an inferior vena cava thrombus. Eighty-three of the cases (91%) were confirmed by either a venogram, Doppler ultrasound, a pulmonary angiogram, or a ventilation-perfusion scan. Among the eight VTE cases for whom radiologic confirmation of the diagnosis could not be located, a tentative VTE diagnosis was established by clinical history and clinical signs and symptoms. The placement of an inferior vena cava filter in three of the eight cases and the administration of thrombolytic therapy prior to a venogram in another case strongly supported the clinical diagnosis.

In the cardiology clinic, 125 African-American patients were approached and all agreed to participate. Fifteen cases were excluded because their medical records did not support a diagnosis of MI. The present analysis is restricted to 110 African-American cases. Ninety-five of the cases (86%) were confirmed by ECG and/or cardiac enzymes. The remaining 15 patients had a history of MI with the clinical diagnosis supported by cardiac scan thallium studies showing evidence of a fixed myocardial defect suggestive of myocardial scarring or a cardiac catheterization that was compatible with an old MI. Ninety-five of the 110 cases (86%) had evidence of CAD at cardiac catheterization. Eighty-two of these 95 cases (85%) had significant CAD (≥ 50% occlusion of at least one major coronary artery).

We selected control subjects from among African-American outpatients attending a clinical laboratory for routine blood tests who had approximately the same distribution of age and sex as the cases. Persons with a history of heart attack, stroke, or blood clots were ineligible as control subjects. Of 207 eligible control subjects asked to participate, 185 (89%) agreed.

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 and lifestyle habits; a personal history of VTE, MI, and other medical problems; and a family history of blood clots, stroke, or heart attack. In addition to the subjects described above, cord blood was obtained from infants for an ongoing study at another Atlanta hospital, Crawford W. Long Hospital, and was used for the purpose of estimating gene frequencies by race.

**DNA Analysis**

After informed consent was obtained, blood samples were collected in 0.109M sodium citrate. DNA was extracted from 3
mL of whole blood using the Genta DNA extraction kit (Minneapolis, MN) per the manufacturer’s instructions and stored at −20 C. Polymerase chain reaction (PCR) was used to amplify DNA fragments in the ecNOS and GPIIIa genes. Following amplification of exon 2 of the GPIIIa gene, the PCR product was digested with the enzymes Msp I and Nco I in order to differentiate between the PlA1 and PlA2 alleles. The GPIIIa genotypes were denoted as PlA1/PlA1, PlA1/PlA2, and PlA2/PlA2. After electrophoresis of the digested product on a 1% agarose gel, results were analyzed by determining the restriction enzyme pattern displayed in the ethidium bromide–stained agarose gel.

As described by Wang et al., the area of tandem repeats in intron 4 of ecNOS was PCR-amplified. Heterozygosity and homozygosity were determined by the size of the amplified fragment. Size was determined either by sizing fragments in a 1% agarose gel after electrophoresis or by using the GeneScan software after gel electrophoresis on a DNA sequencer (ABI Prism 377) or capillary electrophoresis on an automated genetic analyzer (model 310; ABI). The GeneScan software sizes and quantifies the amplified DNA fragment subsequent to automated fluorescent scanning detection. This approach allows the labeling of different DNA fragments with up to three different colors of fluorescent dyes, and then using another color dye to label a internal standard for multiplex loading and electrophoresis of four colors in DNA lane/ run. The internal size standard allows the software to automatically determine DNA fragment size. Samples that were sized using an agarose gel were reanalyzed using GeneScan. No discrepancies were noted between the two methods. Results were independently verified by two laboratory workers.

**Statistical Methods**

Odds ratios (ORs) were used as a measure of association between the genes and VTE or MI risk and are interpreted as the relative risk of disease for the “exposed” compared with the “unexposed.” For ORs in the paper, we constructed exact confidence intervals (CIs) and exact mid-p values. We used logistic regression for trend tests. For example, for the P1A2 allele, we constructed a model with scores of 0 for those without the P1A2 allele, 1 for those with one P1 A2 allele, and 2 for those with two alleles. The GPIII genotype was expressed by the number of the P1A2 allele, we constructed a model with scores of 0 for those without the P1A2 allele, 1 for those with one P1 A2 allele, and 2 for those with two alleles. The genetic distribution of the P1A2 allele was compared with control subjects (p = 0.01). MI cases were also more likely to report a family history of blood clots, stroke, and/or heart attack (66%) than were the control subjects (56%; p < 0.001). MI cases were also more likely to have ever smoked cigarettes (83%) compared with control subjects (56%; p < 0.001). VTE cases and control subjects were similar with respect to cigarette smoking and family history of vascular disease.

**ecNOS Genotype**

After amplification of the polymorphic intron 4 region, Wang et al. and Ichihara et al. identified two alleles that were designated as ecNOS4a and ecNOS4b. The size of the most common allele, ecNOS4a, was 420 base pairs, while the rare allele ecNOS4b was 393 base pairs. In addition to these two alleles, we identified three alleles of 339, 366, and 447 base pairs in this African-American population. For the sake of simplicity, these five alleles will be referred to by base pair size. The genotypes are denoted as 420/420, 420/393, 393/393, 447/393, 447/420, and 366/393. The ecNOS genotypes are displayed in Table 1 and the distributions are in Hardy-Weinberg equilibrium for the two case groups and for the control subjects.

**The Relationship between ecNOS and MI**

The carrier frequency of the 393 allele was 36% for MI cases and 26% for control subjects (p = 0.01). The OR for subjects who had one 393 allele was 1.8 (p = 0.03) compared with those who did not have a 393 allele, and the OR for subjects homozygous for 393 was 2.2 (p = 0.07) compared to subjects who did not have a 393 allele (Table 2). The result of the logistic trend test indicates that each additional 393 allele is associated with approximately a 60% increase in MI risk (p = 0.01). Seven of 30 cases (23%) with a diagnosis of MI before the age of 45 years were homozygous for the 393 allele, whereas only 7 of 76 patients (9%) who received the diagnosis after

**Table 1—Distribution of Control Subjects, MI Cases, and VTE Cases According to the ecNOS and GPIII Genotypes**

<table>
<thead>
<tr>
<th>ecNOS genotype</th>
<th>Control Subjects (n = 185)</th>
<th>MI Cases (n = 110)</th>
<th>VTE Cases (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>420/420</td>
<td>84 (45)</td>
<td>37 (34)</td>
<td>30 (33)</td>
</tr>
<tr>
<td>420/393</td>
<td>18 (10)</td>
<td>6 (5)</td>
<td>8 (9)</td>
</tr>
<tr>
<td>420/447</td>
<td>63 (34)</td>
<td>45 (41)</td>
<td>37 (41)</td>
</tr>
<tr>
<td>366/393</td>
<td>5 (3)</td>
<td>7 (6)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>393/393</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>393/393</td>
<td>15 (8)</td>
<td>14 (13)</td>
<td>12 (13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GPIII genotype</th>
<th>Control Subjects (n = 185)</th>
<th>MI Cases (n = 110)</th>
<th>VTE Cases (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PlA1/PlA1</td>
<td>144 (78)</td>
<td>91 (83)</td>
<td>73 (80)</td>
</tr>
<tr>
<td>PlA1/PlA2</td>
<td>39 (21)</td>
<td>18 (16)</td>
<td>17 (19)</td>
</tr>
<tr>
<td>PlA2/PlA2</td>
<td>2 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

**RESULTS**

The mean age of control subjects, MI cases, and VTE cases was 56 (range, 21 to 93 years), 55 (range, 29 to 84 years), and 54 years (range, 18 to 89 years), respectively. Forty-nine percent of the control subjects were male, as were 65% of the MI cases and 42% of the VTE cases. MI patients were more likely to report a family history of blood clots, stroke, and/or heart attack (66%) than were the control subjects (43%; p < 0.001). MI cases were also more likely to have ever smoked cigarettes (83%) compared with control subjects (56%; p < 0.001). VTE
the age of 45 years had the 393/393 genotype (p = 0.05). No sex difference in the relationship between ecNOS and MI was noted. There was no evidence of statistical interaction between ecNOS, MI, and smoking in this study.

The Relationship between ecNOS and VTE

The carrier frequency of the 393 allele was 35% and 26% for VTE cases and control subjects, respectively (p = 0.04). The OR for VTE for subjects who had one 393 allele was 1.5 (p = 0.12) compared with those who did not have a 393 allele, and the OR for subjects homozygous for the 393 allele was 2.1 (p = 0.10) compared with subjects who had no 393 allele (Table 3). The result of the logistic trend test indicates that each additional 393 allele is associated with approximately a 50% increase in VTE risk (p = 0.04). The ORs for the recessive allele model (393/393 vs 420/393 and 420/420) and the dominant allele model (393/393 and 420/393 vs 420/420) were about equal, and the finding for the dominant allele model was nearly statistically significant (p = 0.06). It should be noted, however, that eight of our cases did not have radiographic confirmation of VTE; nevertheless, a diagnosis of possible VTE was strongly supported by clinical evidence.

The Relationship Between GPIIIa and MI and VTE

The distribution of the GPIIIa genotypes was similar for MI cases, VTE cases, and the control subjects (Table 1), indicating a lack of association between GPIIIa genotypes and either disease. The prevalence of the PlA2 allele among control subjects, MI cases, and VTE cases was 12%, 9%, and 10%, respectively. The prevalence of this allele was not statistically significantly different between MI cases and control subjects (p = 0.20), nor for VTE cases and control subjects (p = 0.20). The overall prevalence of the PlA2 allele among all 386 of our African-American subjects is 10.6% (95% CI, 8.5%, 13.0%).

### Table 2—Distribution of MI Cases and Control Subjects and the ORs According to the ecNOS Polymorphisms

<table>
<thead>
<tr>
<th>ecNOS</th>
<th>MI Cases</th>
<th>Control Subjects</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>420/420†</td>
<td>44</td>
<td>102</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>420/393†</td>
<td>52</td>
<td>68</td>
<td>1.8</td>
<td>1.1–2.9</td>
</tr>
<tr>
<td>393/393</td>
<td>14</td>
<td>15</td>
<td>2.2</td>
<td>0.9–4.9</td>
</tr>
</tbody>
</table>

*393/393 vs (420/393 and 420/420): OR = 1.6; 95% CI, 0.8 to 3.6. (393/393 and 420/393) vs 420/420: OR = 1.8; 95% CI, 1.1 to 3.0. 393/393 vs 420/393 vs 420/420: OR = 1.6; p trend = 0.01.
†Includes 18 control subjects and 7 MI cases with the 420/447 and 420/339 genotypes.

### Table 3—Distribution of VTE Cases and Control Subjects and the ORs According to the ecNOS Polymorphisms

<table>
<thead>
<tr>
<th>ecNOS</th>
<th>VTE Cases</th>
<th>Control Subjects</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>420/420†</td>
<td>39</td>
<td>102</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>420/393</td>
<td>40</td>
<td>68</td>
<td>1.5</td>
<td>0.9–2.6</td>
</tr>
<tr>
<td>393/393</td>
<td>12</td>
<td>15</td>
<td>2.1</td>
<td>0.9–4.9</td>
</tr>
</tbody>
</table>

*393/393 vs (420/393 and 420/420): OR = 1.7; 95% CI, 0.8 to 3.9. (393/393 and 420/393) vs 420/420: OR = 1.6; 95% CI, 1.0 to 2.7. 393/393 vs 420/393 vs 420/420: OR = 1.5; P trend = 0.04.
†Includes 18 control subjects and 7 MI cases with the 420/447 and 420/339 genotypes.
††Includes 5 control subjects and 7 MI cases with the 447/393 genotype.

### The Interaction Between ecNOS and GPIIIa Genotypes

The association between the ecNOS genotypes and MI risk according to the presence of the PlA2 allele is displayed in Table 4. Among subjects homozygous for the PlA1 allele, there was an erratic, nonsignificant association between the addition of the ecNOS 393 allele and MI risk (p > 0.20). Among these subjects without a PlA2 allele, the OR for MI comparing at least one 393 allele to the absence of the 393 allele (dominant model) is 1.5; this finding was not statistically significant (p = 0.12). On the other hand, among subjects who had at least one PlA2 allele, the OR for MI comparing at least one 393 allele to the absence of the 393 allele to the absence of the 393 allele was markedly increased (4.3) and highly statistically significant (p = 0.01). A statistical test of equivalence of these two ORs (1.5 vs 4.3) was not statistically significant (p = 0.16). A comparable pattern in the corresponding ORs for VTE was observed, but the difference

### Table 4—Distribution of MI Cases and Control Subjects and the ORs According to the ecNOS Genotype Stratified by the GPIIIa Polymorphism

<table>
<thead>
<tr>
<th>ecNOS Genotype</th>
<th>MI Cases</th>
<th>Control Subjects</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPIII A1/A1*</td>
<td>420/420</td>
<td>39</td>
<td>77</td>
<td>1.0</td>
</tr>
<tr>
<td>420/393</td>
<td>42</td>
<td>52</td>
<td>1.6</td>
<td>0.9–2.8</td>
</tr>
<tr>
<td>393/393</td>
<td>10</td>
<td>15</td>
<td>1.3</td>
<td>0.5–3.2</td>
</tr>
<tr>
<td>GPIII A1/A2 or A2/A2</td>
<td>420/420</td>
<td>5</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>420/393</td>
<td>10</td>
<td>16</td>
<td>3.1</td>
<td>0.9–11.6</td>
</tr>
<tr>
<td>393/393</td>
<td>4</td>
<td>0</td>
<td>infinite</td>
<td>3.2–infinity</td>
</tr>
</tbody>
</table>

*For GPIII A1/A1: OR, 1.5 (95% CI, 0.9 to 2.6) for (393/393 and 420/393) vs 420/420.
†For GPIII A1/A2 or A2/A2: OR, 4.3 (95% CI, 1.3 to 15.5) for (393/393 and 420/393) vs 420/420.
was less striking and the two ORs (1.6 and 1.9) did not differ in a statistically significantly manner (p > 0.20).

Discussion

Both clinical and laboratory research has established a link between a dysfunctional ecNOS enzyme, platelets, and cardiovascular disease. Investigations have now begun to correlate DNA polymorphisms or mutations in the ecNOS gene with loss of ecNOS activity or with cardiovascular disease. In addition, polymorphisms in the gene(s) encoding platelet receptor proteins responsible for platelet aggregation such as GPIIIa have been another area for investigation for possible association with both venous and arterial disease.

This preliminary study not only confirmed those of Wang et al and Ichihara et al regarding a positive association between CAD and MI and a tandem repeat polymorphisms in the 5’ region of the ecNOS gene, but has also extended these observations to VTE and another racial group. In addition to the polymorphisms previously reported, we identified three rare repeat polymorphisms in intron 4 that consisted of 447, 366, and 339 base pairs. While Wang et al found that only the homozygous 393/393 genotype was associated with CAD in smokers, our data indicated that the heterozygous genotypes 420/393 and 447/393 were also associated with an increased risk of MI in both smokers and nonsmokers, a finding consistent with that of Ichihara et al. Because most of our subjects had a history of smoking, we had insufficient statistical power to evaluate interactions between smoking and the ecNOS genotypes in this study. This study also found a significant association between MI at a younger age and homozygosity for the 393 genotype. As indicated by the data, 23% of patients with a diagnosis of MI prior to the age of 45 years were homozygous for 393, whereas only 9% were homozygous if the diagnosis of MI was made after 45 years. In view of the recent finding that impaired endothelium-dependent dilatation is a risk factor for young adults with a family history of CAD, it may be suggested that this genotype could be useful as a genetic screening tool for premature CAD.

The observation that the 393 allele was associated with VTE, although new, is not surprising because it has been reported that smoking can impair NO synthase in saphenous vein strips. Furthermore, in a rabbit model, NO in the microvasculature has a greater antithrombotic effect in venules than in arterioles. The association between ecNOS and VTE in African Americans was particularly interesting because three common genetic risk factors for VTE found in whites—factor V Leiden, methyltetrahydrofolate reductase (MTHFR) 677 C->T, and prothrombin 20210G->A variant—are rare among African Americans.

The ecNOS 393 allele frequency among our African-American control subjects (0.26) was significantly higher than the 0.14 reported for control subjects by Wang and colleagues (p < 0.001) and the 0.10 for control subjects reported by Ichihara and colleagues. Likewise, the frequency of the 393/393 genotype among our MI cases (0.13) and our VTE cases (0.15) was higher than that in the Australian study (0.04) and the Japanese study (0.02). We obtained DNA from cord blood for the purpose of comparing certain gene frequencies at birth between African Americans and US whites. The prevalence for the 393 allele was 0.31 for African-American infants (n = 328) and 0.18 for white infants (n = 112; p < 0.01). There was no significant difference in the 393 allele frequency between the African-American infants and the African-American control subjects in our study.

In contrast to what was initially reported for US whites, there was no association with the PlA polymorphisms and MI in our study of African Americans. In that initial communication, it was reported that African Americans were excluded because of the lower population frequency of the PlA2/PlA2 genotype. However, in our study, the gene frequency for African Americans was similar to that reported for whites. This discrepancy may reflect regional differences in gene frequencies among African Americans. Weiss and colleagues reported the PlA2 polymorphisms to be determinant of MI at an early age (ie, prior to age 60 years). We found no difference in the prevalence of the PlA2 allele among young MI patients vs older MI patients. In any event, with the exception of two reports, other studies have also failed to demonstrate a relationship between PlA with cardiovascular disease. Similar to the findings of Ridker et al, we likewise found no association between PlA2 and VTE. Despite the lack of consistent confirmatory reports, the potential significance of the PlA2 allele was underscored by the observation that the effect of the ecNOS 393 allele on MI risk, but not on VTE risk, was stronger among subjects who had at least one PlA2 allele compared with subjects homozygous for the PlA1 allele. A similar interaction was recently reported by Carter et al, who found that young subjects with the PlA2 allele and high cholesterol levels had a higher risk of MI. Although our sample size was small, these findings suggest that the PlA2 polymorphisms may...
interact with other gene polymorphisms in the pathogenesis of arterial disease.

Clinical and epidemiologic studies have established that cardiovascular diseases and VTE are not a consequence of a single gene or any one environmental factor, but rather represent a complex pathology that is caused by multiple interactions between genetic and environmental factors. These interactions occur over a continuum, with disease initiation as one end point and clinical presentation as the other end point. A key question that confronts investigators today is not only defining what gene-environment interactions are important in cardiovascular disease, but at which point along the continuum they act. Our focus in this report was on two physiologically relevant genes that are known to influence platelets, a major component of the clot. It could be argued that clot formation is the pivotal event along the continuum that leads to clinical presentation of disease. Consequently, the analysis of molecular events that contribute to clot formation may identify markers that may have predictive value. Our data suggest that the ecNOS polymorphism alone, and together with the PlA polymorphism, may be useful in identifying individuals at high risk for cardiovascular disease. Their predictive value may be improved when environmental factors that are known to negatively affect NO synthase, such as smoking, hypercholesterolemia, and low-density lipoprotein, are included in the analysis.

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