Platelet PlA2 Allele and Incidence of Coronary Heart Disease
Results From the Atherosclerosis Risk In Communities (ARIC) Study

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Background—The major platelet integrin glycoprotein IIb-IIIa plays a primary role in platelet aggregation and acute thrombus formation at the site of vascular injury. A genetic polymorphism of glycoprotein IIb-IIIa (PlA) has recently been proposed as a potential genetic factor linking to platelet hyperaggregability and increased risk of myocardial infarction. Despite numerous, mostly nonprospective studies, the role of this polymorphism as a clinically relevant, inherited risk factor for coronary heart disease (CHD) is still controversial. The purpose of this study was to determine whether PlA2 is a risk factor for incident CHD and whether it is correlated with increased platelet activation in a case-cohort study nested within a prospective epidemiologic investigation.

Methods and Results—Blood samples were collected and processed from the Atherosclerosis Risk in Communities Study cohort at the baseline examination (1987 to 1989). They were stored at −80°C. PlA1/A2 genotype and plasma β-thromboglobulin levels were determined in 439 incident CHD cases and a reference cohort sample of 544 (of whom 18 were also CHD cases). The prevalence of the PlA2 allele was not different in cases versus noncases. No significant correlation between CHD risk factors and the PlA2 allele was noted either. Platelet activation, as measured by plasma β-thromboglobulin levels, was not enhanced in individuals with the PlA2 allele.

Conclusions—This prospective study indicates that healthy individuals carrying the PlA2 allele do not have an increased risk of CHD. (Circulation. 2000;102:1901-1905.)

Key Words: platelets | glycoproteins | coronary disease

Platelet activation and aggregation play key roles in the process of arterial thrombosis. Successful prevention of arterial thrombotic events with inhibitors of platelet aggregation underscores the critical role that platelet aggregation plays in human arterial thrombotic disorders. Aspirin, which blocks platelet thromboxane A2 synthesis, thereby suppressing platelet aggregation, is efficacious in preventing coronary heart disease (CHD) and thrombotic stroke. Abciximab and other antagonists of fibrinogen binding to glycoprotein (GP)IIb-IIIa have been shown to reduce thrombotic complications after coronary angioplasty or atherectomy.

Platelet hyperaggregability has often been detected in patients with myocardial infarction (MI). Prospective studies further indicate that increased platelet aggregability is an independent risk factor for recurrent MI. The mechanism by which platelet hyperaggregability arises is unclear, but both genetic and environmental factors probably contribute. The genetic polymorphism of GPIIb-IIIa has recently been proposed as a potential factor related to platelet hyperaggregability and increased risk of MI. Binding of fibrinogen and von Willebrand factor to GPIIb-IIIa is the final common pathway by which platelet aggregation occurs. Hence, GPIIb-IIIa occupies a pivotal position in platelet aggregation. A number of polymorphisms have been identified for the GPIIb and GPIIIa genes. A GPIIIa polymorphism at codon 33 (Leu33Pro), also known as PlA1/A2 (or HPA-1a and HPA-1b), was reported by Weiss et al to be associated with acute MI. They reported in a case-control study that the frequency of the GPIIIa Pro33 allele was increased in MI cases. Several other case-control studies have been reported, but the results are inconsistent.

The purpose of this study was to determine whether PlA2 is a risk factor for incident CHD and whether it is related to increased platelet activation in a prospective case-cohort study within the Atherosclerosis Risk in Communities (ARIC) Study cohort.

Methods

Study Population
In 1987 through 1989, the ARIC study recruited a population-based cohort of men and women 45 to 64 years of age from 4 US...
communities. Details of the criteria for inclusion of subjects from the participating communities and relevant epidemiologic data have been presented elsewhere. A total of 15,792 participants completed a home interview and clinic examination. Since enrollment, the subjects in this cohort have been prospectively followed for development of CHD and other vascular events. Participants were contacted annually by telephone to identify all hospitalizations and deaths. Death certificates and discharge lists from local hospitals from ARIC surveillance are also reviewed on a continuous basis to detect events occurring to cohort participants.

Ascertainment of Incident CHD Cases
For the present study, we included all CHD events occurring between ARIC visit 1 (1986 to 1988) and December 31, 1993. The mean follow-up time was 6.2 years and the standard error of the mean was 0.1 years. We defined CHD incidence as (1) a definite or probable MI, (2) a silent MI between examinations detectable by ECG, (3) a definite fatal CHD death, or (4) a coronary revascularization. The ascertainment of CHD cases and criteria for CHD classification have been previously described.

Baseline Measurements
The definitions and methods used for baseline measurements (systolic blood pressure, hypertension, diabetes mellitus, alcohol intake, plasma total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, fibrinogen) were published elsewhere.

Determination of P1 Genotypes
P1A1/A2 genotype was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism, according to a previously published procedure. A 266-bp DNA fragment containing exon 2 was amplified by PCR with primers flanking the exon. The PCR products were digested separately with 2 restriction enzymes, MspI and NciI, (New England Biolabs, Beverly, Mass), and the resulting fragments were visualized and typed after electrophoresis on a 3% agarose gel and staining. A new restriction site is generated as the result of the PlA polymorphism. We did a blind replicate DNA analysis for genotyping the PlA polymorphism. Results from replicate pair analysis (n = 45) showed that the χ² value was 0.939, which indicates very strong agreement between blind duplicates in determining the PlA polymorphism.

Plasma B-Thromboglobulin Assay
Blood samples were collected and processed according to standardized procedures and organizational plans described previously. Plasma level of β-thromboglobulin (β-TG) was determined by an ELISA assay with kits obtained from American Bioproducts. The β-TG assay has a coefficient of variation of 6.8% and a reliability coefficient of 0.83.

Cohort Sample
We used a case-cohort design for the present study in which information on GPIIIa Leu33Pro polymorphism and plasma level of β-TG with 25.9% in noncases (Table 1). These differences were not statistically significant (P < 0.79). There was no difference in genotype distribution between whites and blacks.

As previously reported, there was a higher proportion of risk factors (systolic blood pressure, total cholesterol, total triglyceride, cigarette smoking, hypertension and diabetes mellitus, P < 0.001 for each) in our cases than in noncases. The cases were older than noncases (56.2 versus 53.8 years, P < 0.0001), and there were more men in cases than noncases (70.8% versus 42.2%, P < 0.0001). The proportion of blacks was not significantly different between cases and noncases (26.6% versus 25.1%, P = 0.7). There was no statistically significant difference in the genotype frequency between cases versus noncases within and between the two races (Table 1). PlA1 and PlA2 allele frequencies were also not significantly different between cases and noncases (P = 0.91) within and between the two races after adjusting for the confounding effects of age, race, and sex; 69.5% of our incident cases had acute coronary syndrome, whereas the
remaining 30.5% had silent MI or severe coronary stenosis requiring coronary revascularization. When the latter was excluded from analysis, the difference in PI^A1 alleles between cases and noncases remained statistically insignificant.

To exclude possible false-negative results, we determined the minimal detectable difference in A1A1 prevalence between cases and noncases by a 2-sided $\chi^2$ test. The minimal detectable difference was 18%, given an $\alpha$ error of 0.05 and a $\beta$ error of 0.2, based on the case (n=439) and noncase (n=526) size and the observed A1A1 frequency in noncases (74.1%). Thus, we should be able to detect a difference of 18% in A1A1 prevalence between cases and noncases. Our finding of an insignificant difference in A1 and A2 alleles between cases and noncases is not due to false-negative results.

Table 2 shows the characteristics of the cohort reference subjects with respect to the presence or absence of the PI^A2 allele. There was no significant difference in any of the variables considered. The weighted, proportional hazards regression analysis showed that PI^A2 genotype was not significantly associated with the time to development of CHD after controlling for potentially confounding effects of other risk factors such as age, sex, ethnic background, hypertension, diabetes mellitus, total cholesterol, total triglycerides, HDL-C, fibrinogen, and smoking (RR=1.37; 95% CI=0.89 to 2.11).

**Discussion**

We found no increase in risk of incident CHD in individuals heterozygous or homozygous for the PI^A2 allele in our community-based, prospective, case-cohort study. Previous reports have provided contradictory information. A case-control study by Weiss et al.\(^7\) suggested an association between acute MI and the PI^A2 allele. This association was stronger in patients <60 years of age when compared with those >60 years of age. Similar findings were reported in another large nonprospective study involving 405 white patients who underwent angiography for investigation of chest pain or suspected coronary artery disease.\(^11\) In another study, acute MI with multiple-vessel atherosclerotic disease was associated significantly with the PI^A2 allele, but an association between the extent of coronary artery disease and presence of the PI^A2 allele was not observed.\(^20\) The prevalence of the PI^A2 allele was 50% in men having a MI before the age of 47 years.\(^21\) A statistical interaction between the PI^A2 allele and cholesterol levels, as they combine to affect MI prevalence, has also been noted.\(^12\) In a retrospective study of 178 Spanish men <50 years of age, Batalla et al.\(^22\) found a positive association of the PI^A2 allele with high LDL-cholesterol concentrations, but the frequency of the PI^A2 allele in subjects with CHD was not different from that of age- and sex-matched control subjects. All of these studies were nonpro-

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**TABLE 1. Age-, Race-, and Sex-Adjusted Prevalence of PI^A1/A2 in Cases and Noncases**

<table>
<thead>
<tr>
<th>GPIla Genotype</th>
<th>Cases (n=439), %</th>
<th>Noncases (n=526), %</th>
<th>Cases (n=334), %</th>
<th>Noncases (n=397), %</th>
<th>Cases (n=105), %</th>
<th>Noncases (n=129), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1A1</td>
<td>73.1</td>
<td>74.1</td>
<td>72.0</td>
<td>71.4</td>
<td>73.9</td>
<td>82.1</td>
</tr>
<tr>
<td>A1A2 + A2A2</td>
<td>26.9</td>
<td>25.9</td>
<td>28.0</td>
<td>28.6</td>
<td>26.1</td>
<td>17.9</td>
</tr>
</tbody>
</table>

**TABLE 2. Baseline Characteristics of ARIC Cohort Random Reference Subjects With Respect to Presence or Absence of PI^A2 Allele**

<table>
<thead>
<tr>
<th>Variable</th>
<th>A1A1 (n=399)</th>
<th>A1A2/A2A2 (n=145)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at visit 1, y</td>
<td>53.8</td>
<td>54.2</td>
<td>0.62</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>119</td>
<td>118</td>
<td>0.56</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>216</td>
<td>210</td>
<td>0.20</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>126</td>
<td>114</td>
<td>0.13</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>54.1</td>
<td>54.4</td>
<td>0.84</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>301</td>
<td>297</td>
<td>0.55</td>
</tr>
<tr>
<td>Smoking, cigarette-y</td>
<td>254</td>
<td>252</td>
<td>0.96</td>
</tr>
<tr>
<td>Ethanol intake, g/wk</td>
<td>35.7</td>
<td>34.9</td>
<td>0.93</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>28.5</td>
<td>30.0</td>
<td>0.83</td>
</tr>
<tr>
<td>Men, %</td>
<td>43.7</td>
<td>41.4</td>
<td>0.75</td>
</tr>
<tr>
<td>White, %</td>
<td>73</td>
<td>82</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>5.5</td>
<td>5.0</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Probability value is from test of differences between genotypes after controlling for the confounding effects of age, sex, and race (except where stratified).
spective and not population-based and therefore were potentially subject to bias. Even though the stronger association of the PlA2 with coronary artery disease at a younger age argues for a pathogenic role of the PlA2 allele, such an age interaction was not observed in several other studies. The present prospective study is based on follow-up of a cohort of 15 792 healthy individuals who were selected from 4 communities in the United States. These individuals had been followed since enrollment in 1987 to 1989. Ascertainment of CHD was based on strict, standardized criteria. The frequency of the PlA1/PlA2 plus PlA2/PlA2 genotypes was not significantly different between cases and controls, indicating a lack of an association between the PlA2 allele and incident CHD. A vast majority of our cases were <60 years of age. Although the number of cases >60 years was too small for statistical analysis, our results are unlikely to show an age interaction with PlA genotypes.

It has been suggested that the relation of the PlA2 polymorphism could differ with different coronary phenotypes: acute coronary syndrome versus coronary atherosclerosis. The inclusion criteria for the CHD events in our study allow enrollment of subjects who had a coronary revascularization procedure. To estimate if the association of the PlA2 polymorphism with CHD incidence will change if cases were restricted to those with a proven acute coronary syndrome, the analysis was repeated after removing silent MI and procedure-related events (30.5% of events removed). It yielded similar results for the subset with acute CHD syndromes, that is, the association remained nonsignificant. No significant relation between established CHD risk factors and the PlA2 allele was noted either. Our results are similar to those reported for the prospective Physician’s Health Study (PHS) cohort, which did not show a statistically significant difference in PlA2 frequency between incident CHD cases and control subjects.

Notably, the PHS included only men. Observations of our study extend to both men and women and to whites and blacks.

The hypothesis that the PlA2 polymorphism is an important inherited risk factor for CHD implies an increase in platelet activation as a mediator of the thrombotic event. Our study did not find an enhancement of platelet activation in individuals with the PlA2 allele, as measured by plasma β-TG levels. In another study of patients with acute stroke and control subjects, levels of β-TG and platelet factor 4 were not associated with the PlA2 allele. In the PHS study, aspirin did not provide any more protection to individuals carrying at least 1 copy of the PlA2 allele compared with those with the PlA1/PlA1 genotype. On the other hand, the Framingham Offspring Study showed that PlA2 allele was associated with increased platelet aggregability by epinephrine, suggesting that molecular variants of the GPIIIa gene play a role in platelet reactivity in vitro. A higher prevalence of the PlA2 polymorphism has been reported in siblings of patients with premature CHD than in those of a race-matched and geographic area–matched control cohort, who remained asymptomatic. The occurrence of a genetic defect that could lead to an increase in CHD may also cluster within families with strong familial aggregation of CHD. However, in a study of 1292 adults from 515 such families, no increased frequency of the PlA2 allele was noted, and earlier onset of CHD prevalence was not influenced by the presence or absence of the PlA2 allele.

Taken together, the reported data indicate that healthy individuals carrying the PlA2 allele do not have an increased risk of CHD.

Our study has several advantages over the nonprospective case-control studies in that it is population based, prospective in nature, and contains a relatively large number of participants and incident events. A limitation of this study is the inclusion of subjects who were 45 to 64 years of age at entry into the study. If PlA2, like other genetic factors, were to play a more important role in increasing CHD risk in young subjects, we might have missed individuals dying young or having premature CHD who would be excluded from our study. Hence, the interpretation of results from this population-based prospective follow-up study should be applied to middle-aged men and women only.

Acknowledgments

The ARIC study was funded by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022. We appreciate the important contributions of the following ARIC personnel: Debbie Rubin Williams, Patsy H. Tacker, Lily Wang (University of North Carolina Coordinating Center, Chapel Hill); Leslie Angel-Potter, Dawn Scott, Nadine Shelton (University of North Carolina, Chapel Hill); Julia Flesham, Kathy Joyce, Charlene Kearney-Cah (Wake Forest University, Winston-Salem); Bobbie J. Alliston, Barbara Davis, Agnes Hayes (University of Mississippi Medical Center, Jackson); Maxine Dammen, Caryl DeYoung, Christine Dwight (University of Minnesota, Minneapolis); Sunny Harrell, Patricia Hawbaker, Joan Nelling (Johns Hopkins University, Baltimore); Willa Wang, Chrisye Moore, Johanna Kindaard, Susan Mitterling, (University of Texas Medical School, Houston); and Wanda R. Alexander, Christie M. Ballantyne, and Val Creswell (Methodist Hospital, Atherosclerosis Clinical Laboratory, Houston).

References


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Circulation. 2000;102:1901-1905
doi: 10.1161/01.CIR.102.16.1901
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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