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.1 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.2 Abciximab and other antagonists of fibrinogen binding to glycoprotein (GP)IIb-IIIa have been shown to reduce thrombotic complications after coronary angioplasty or atherectomy.3 Platelet hyperaggregability has often been detected in patients with myocardial infarction (MI).1 The mechanism by which platelet hyperaggregability arises is unclear, but both genetic and environmental factors probably contribute.5,6 The genetic polymorphism of GPIIb-IIIa has recently been proposed as a potential factor related to platelet hyperaggregability and increased risk of MI.7,8 Binding of fibrinogen and von Willebrand factor to GPIIb-IIIa is the final common pathway by which platelet aggregation occurs.9 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 al7 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.10–13 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

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communities. Details of the criteria for inclusion of subjects from the participating communities and relevant epidemiologic data have been presented elsewhere.14 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.15

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.16

Determination of $P^i$ Genotypes
PlA1A2 genotype was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism, according to a previously published procedure.7 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 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$^T$ polymorphism. We did a blind replicate DNA analysis for genotyping the PlA$^T$ polymorphism. Results from replicate pair analysis (n=45) showed that the $k$ value was 0.939, which indicates very strong agreement between blind duplicates in determining the PlA$^T$ polymorphism.

Plasma β-Thromboglobulin Assay
Blood samples were collected and processed according to standardized procedures and organizational plans described previously.17 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 0.83.18

Coeficient of 0.83. 18

Exclusions
We excluded participants with prevalent CHD, stroke, or transient ischemic attack. We also excluded very few participants who were neither white nor black. A total of 997 participants were in the cohort random sample or had CHD before December 31, 1993. Of these, 32 were excluded for missing GPIIIa results, because of either a missing DNA sample or failure of the PCR amplification. The final sample contained 965 individuals. The sample included 439 CHD cases and a reference cohort sample of 544 (of whom 18 were also CHD cases). For the purpose of correlating plasma level of β-TG with GPIIIa genotype, only 795 participants who had results for both GPIIIa genotype and plasma level of β-TG were analyzed.

Statistical Analysis
For data analysis, we combined A1A2 and A2A2 and compared them with A1A1 because the prevalence of A2A2 was very low. Therefore, the genetic model is one that considers A2 dominant over A1. To determine the relation of GPIIIa PlA1A2 polymorphism with other variables, including β-TG, some of which may be confounders in this analysis, we used ANCOVA to compute age-, race-, and sex-adjusted mean levels or percentages of the other variables for A1A2/A2A2 and A1A1. We also used ANCOVA to compute age-, race-, and sex-adjusted mean levels or percentage values of study variables for CHD cases versus noncases after appropriate weighting for the stratified case-cohort sampling design. For this descriptive analysis, we treated the cases as 1 stratum and divided the noncases into 8 strata as described for the cohort sample and weighted each stratum by the inverse of the sampling fraction. We computed the risk ratios and 95% confidence intervals for the time to the development of CHD by using a weighted, proportional hazards regression by Barlow’s method.19 In the weighted, proportional hazards regression models, we adjusted GPIIIa PlA1A2 estimates for sex, age, race, and other factors related to CHD in this sample: hypertension, diabetes mellitus, total cholesterol, HDL-cholesterol, LDL-cholesterol, fibrinogen, smoking, and ethanol intake.

Results
Since the proportion of the PlA2A2 genotype was too small (<2%) to determine its independent risk for CHD, the A1A2 heterozygotes and A2A2 homozygotes were combined for data analysis. The frequency of the A1A1 genotype in cases was 73.1% as compared with 74.1% in noncases, and the A1A2+A2A2 frequency was 26.9% in cases as compared 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 PlA1 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 *t* test. The minimal detectable difference was 18%, given an *α* error of 0.05 and a *β* 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 PlA2 allele. There was no significant difference in any of the variables considered. The weighted, proportional hazards regression analysis showed that PlA2 genotype was not significantly associated with the PlA2 allele, but an association of the PlA2 allele with high LDL-cholesterol concentrations, but the frequency of the PlA2 allele in subjects with CHD was not different from that of age- and sex-matched control subjects. All of these studies were nonpro-

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

<table>
<thead>
<tr>
<th>GPiIIa 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>
<tr>
<td><em>P</em></td>
<td>0.79</td>
<td>0.89</td>
<td>0.66</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In noncases there was no significant difference in age-, race-, and sex-adjusted mean plasma β-TG values between participants with the A1A1 genotype (22.6 ng/mL) and individuals with the A1A2+A2A2 genotypes (18.7 ng/mL, *P*=0.797) (Figure). Likewise, for CHD cases, the age-, race-, and sex-adjusted mean values of β-TG were similar: 22.1 ng/mL for A1A1 and 21.6 ng/mL for A1A2/A2A2 (*P*=0.667).

### 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 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. 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. The prevalence of the Pi^A2 allele was 50% in men having a MI before the age of 47 years. A statistical interaction between the Pi^A2 allele and cholesterol levels, as they combine to affect MI prevalence, has also been noted. In a retrospective study of 178 Spanish men <50 years of age, Batalla et al 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-

### TABLE 2. Baseline Characteristics of ARIC Cohort Random Reference Subjects With Respect to Presence or Absence of PlA2 Allele

| Variable                  | A1A1 (n=399) | A1A2/A2A2 (n=145) | *P*
<table>
<thead>
<tr>
<th></th>
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</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).*

Comparison of age-, race-, and sex-adjusted mean values of plasma β-TG levels (ng/mL) in cases and noncases with respect to Pi^A^ genotypes.
pective 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 suggests 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/PlA1 plus PlA2/PlA2 genotypes was not significantly different between cases and noncases, 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.

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References


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