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A POLYMORPHISM OF A PLATELET GLYCOPROTEIN RECEPTOR AS AN INHERITED RISK FACTOR FOR CORONARY THROMBOSIS

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Abstract  Background. Platelet glycoprotein IIb/IIIa is a membrane receptor for fibrinogen and von Willebrand factor, and it has an important role in platelet aggregation. It is known to be involved in the pathogenesis of acute coronary syndromes. Previously, we found a high frequency of a particular polymorphism, PlA2, of the gene encoding glycoprotein IIla in kindreds with a high prevalence of premature myocardial infarction.

Methods. To investigate the relation between the PlA2 polymorphism and acute coronary syndromes, we conducted a case–control study of 71 case patients with myocardial infarction or unstable angina and 68 inpatient controls without known heart disease. The groups were matched for age, race, and sex. We used two methods to determine the PlA genotype: reverse dot blot hybridization and allele-specific restriction digestion.

Results. The prevalence of PlA2 was 2.1 times higher among the case patients than among the controls (39.4 percent vs. 19.1 percent, P = 0.01). In a subgroup of patients whose disease began before the age of 60 years, the prevalence of PlA2 was 50 percent, a value that was 3.6 times that among control subjects under 60 years of age (13.9 percent, P = 0.002). Among subjects with the PlAl2 polymorphism, the odds ratio for having a coronary event was 2.8 (95 percent confidence interval, 1.2 to 6.4). In the patients less than 60 years of age at the onset of disease, the odds ratio was 6.2 (95 percent confidence interval, 1.8 to 22.4).

Conclusions. We observed a strong association between the PlAl2 polymorphism of the glycoprotein IIla gene and acute coronary thrombosis, and this association was strongest in patients who had had coronary events before the age of 60 years. (N Engl J Med 1996;334:1090–4.)

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STUDIES indicate that myocardial infarction and unstable angina result from the formation of a platelet aggregate at the site of a ruptured coronary atherosclerotic plaque.1,2 The formation of such aggregates requires the binding of fibrinogen and von Willebrand factor to the receptor, glycoprotein IIb/IIIa, on the platelet surface.9,20 Large trials have demonstrated a marked benefit of various inhibitors of platelet function both in preventing and reducing the mortality and morbidity associated with unstable coronary syndromes.21-23 Additional studies have linked ex vivo platelet reactivity to outcome in patients after myocardial infarction.25 In sum, there is strong evidence that platelets, and glycoprotein IIb/IIIa in particular, have an important role in the pathogenesis of acute coronary syndromes.

Platelet-membrane glycoproteins are highly polymorphic and can be recognized as alloantigens or autoantigens.26 The alloimmune thrombocytopenias are due to the incompatibility of epitopes on the various platelet-surface glycoproteins.27 The alloantigen referred to as PlA or Zw is the one most frequently implicated in syndromes of immune-mediated platelet destruction.28,31 Kunicki and Aster demonstrated that anti-PlA1 antiserum from PlA1-negative persons reacted with platelet glycoprotein IIIa.32 Subsequently, Newman et al. identified the molecular basis of this polymorphism: persons positive for PlA1 have a leucine at position 33 of mature glycoprotein IIIa; persons positive for PlA2 have a proline at this position, which is the result of the substitution of cytosine for thymidine at position 1565 in exon 2 of the glycoprotein IIIa gene.33 We recently determined the allelic frequencies of PlA1 and PlA2 in several racial and ethnic groups in the metropolitan Baltimore area.34

Previously, we found an unexpectedly high frequency of family members homozygous for PlA2 in kindreds with a high prevalence of acute coronary events at a relatively young age (under 60 years).35 This observation led us to postulate that the presence of at least one PlA2 allele may be related to the development of symptomatic unstable coronary heart disease. We conducted a case–control study to examine whether there was an association between the PlA2 allele and unstable coronary syndromes, especially in persons less than 60 years of age.

METHODS

Selection of Case Patients and Control Subjects

Studies on case patients and control subjects were approved by the Joint Committee for Clinical Investigation of Johns Hopkins University and Hospital. Genotypic analyses were performed on 71 consecutive case patients admitted to the Coronary Care Unit of Johns Hopkins Hospital with an established diagnosis of myocardial infarction or unstable angina as defined by World Health Organization criteria. Similar genotypic analyses were performed on control subjects matched with the case patients for age, race, and sex, but who had no documented history of either stable or unstable angina or myocardial infarction. The control subjects were selected by reviewing patient charts from a population of patients admitted to the general medical
PCR with primers flanking the exon, as previously described. Genomic DNA from case patients or controls in a polymerase chain reaction (PCR) was digested separately with both enzymes, and the digestion sites are generated as the result of the restriction-enzyme digestion with MspI and NcoI (New England Biolabs, Beverly, Mass.), which are able to distinguish the Pl42 allele from the Pl142 allele because new restriction sites are generated as the result of the Pl42 polymorphism. Exon 2 PCR products were digested separately with both enzymes, and the resulting fragments were analyzed on a 3 percent agarose gel. The results of both techniques were confirmed by at least two independent investigators who were unaware of the origin of the DNA.

**Statistical Analysis**

The size of the sample was established after pilot studies indicated that the prevalence of Pl42 among the case patients would be approximately 40 percent and the expected prevalence in control subjects would not exceed 20 percent. With the expectation that a difference of this magnitude would strongly support the concept that Pl42 is a significant genetic risk factor, and with a one-sided alpha error of 0.05, the size of the sample was set at 71 to limit the beta error to 0.1. Student’s t-test was used to compare established risk factors for coronary heart disease in the case patients and the control subjects, with a two-tailed test used for the continuous variables. A P value of 0.05 or less was considered to indicate statistical significance. Discrete data (including data on the Pl14 alleles) were analyzed by the chi-square test.

The strength of the association of the Pl42 genetic factor with the occurrence of acute coronary events was estimated by calculation of the odds ratios with Epilinfo software (version 6, Centers for Disease Control and Prevention, Atlanta) and the Cornfield method for the calculation of 95 percent confidence intervals. The relative strength of association of other risk factors was measured in a similar manner. The significance of the difference in the odds ratios was not tested, since the sample size was not designed for such analyses. The relation of the Pl4 allele to each of the remaining predictor variables was examined by bivariate chi-square analysis. Finally, the association of the Pl14 allele with coronary events, standardized for the other risk factors, was determined by the multiple logistic-regression method with the Stata statistical package (version 4.0, Stata, College Station, Tex.).

**RESULTS**

**Characteristics of the Study Population**

Table 1 shows the prevalence of selected risk factors for coronary heart disease among the case patients and controls. The two groups were matched according to age, race, and sex, and there were therefore no significant differences in these variables. Owing to the sample size, the only risk factor that differed significantly between the groups was smoking status (P = 0.05).

**Prevalence of Pl42**

Genotyping results for 3 of the 71 case patients are shown in Figure 1, demonstrating the three possible Pl43 allelic combinations. Table 2 summarizes the genotyping data for all case patients and controls. The prevalence of Pl42 among the case subjects was 39.4 percent (percentage of subjects who were either heterozygous [Pl142/Pl42] or homozygous [Pl42/Pl42]), a value that was significantly higher than the prevalence among the 68 controls (19.1 percent, P = 0.01). The association between Pl42 and coronary events was even stronger in patients who were less than 60 years of age when they had their first coronary event. Of the 42 such patients, 30 percent carried at least one Pl42 allele, as compared with 13.9 percent of the 36 controls who were less than 60 years of age (P = 0.002).

**Comparison of Major Risk Factors for Coronary Heart Disease**

Among the major risk factors for coronary heart disease examined in this study, the risk factor associated with the highest estimated odds ratio was carriage of the Pl42 allele (odds ratio, 2.8; 95 percent confidence interval, 1.2 to 6.4), followed in order by smoking (odds ratio, 2.2; 95 percent confidence interval, 1.0 to 4.8), hypertension (systolic blood pressure, ≥140 mm Hg) (odds ratio, 1.9; 95 percent confidence interval, 0.9 to 3.9), and hypercholesterolemia (total serum cholesterol level, ≥200 mg per deciliter [5.2 mmol per liter]) (odds ratio, 1.3; 95 percent confidence interval, 0.5 to 3.0) (Table 3). The prevalence of diabetes mellitus was sim-
Panel A shows the restriction map of exon 2 of the glycoprotein IIIa gene, undigested fragments, and fragments resulting when PlA1 or PlA2 PCR products were digested with the restriction enzymes MspI and NciI. Sizes (in base pairs) are indicated below the horizontal lines. The vertical bars indicate the ends of PCR fragments or digestion products. For clarity and because it does not cause a detectable change in the fragment sizes, an additional MspI site (present in both PlA alleles) 7 bp from the 3' MspI site is not shown. Panel B shows a representative ethidium-stained 3 percent agarose gel containing undigested PCR products (lanes 1, 5, and 8) and PCR products digested with MspI (lanes 2, 6, and 9) and NciI (lanes 3, 7, and 10) corresponding to the three possible allelic combinations: PlA1/PlA1 (lanes 1, 2, and 3), PlA1/PlA2 (lanes 5, 6, and 7), and PlA2/PlA2 (lanes 8, 9, and 10). Lane 4 shows λX174 DNA cut with HaeIII, which was used as a size marker. Panel C shows the results of reverse dot blot hybridization with the DNA shown in Panel B. Filters are shown with PlA1/PlA1-allele–specific oligonucleotides at the top and PlA2/PlA2-allele–specific oligonucleotides at the bottom.

Figure 1. Genotyping of PlA Loci by Allele-Specific Restriction Digestion and Reverse Dot Blot Hybridization.
states and Europe, the earlier studies of European prevalence of the $PI^{A2}$ allele are the most likely explanation for differences in the prevalence of younger patients (age less than 60 years at first acute coronary event), was the $PI^{A2}$ allele (Table 3). In addition, we conducted a multiple logistic-regression analysis adjusting for the presence of smoking, hypertension, hypercholesterolemia, and an age greater than 60 years, which demonstrated an independent association between $PI^{A2}$ and acute coronary events. All the risk factors tested yielded odds ratios consistent with what one would expect for a major risk factor for coronary heart disease, except for diabetes. However, we believe that the low odds ratio associated with diabetes is a result of the selection of control subjects from a population of inpatients with a high prevalence of diabetes.

One theoretical explanation for the increased prevalence of $PI^{A2}$ among the case patients could be a higher survival rate after myocardial infarction in this group than in $PI^{A2}$-negative patients. However, if $PI^{A2}$ did provide a survival advantage, the prevalence of $PI^{A2}$ would be unlikely to be increased among the case patients in whom disease occurred before the age of 60 years. Instead, our hypothesis is that $PI^{A2}$ is a risk factor for coronary heart disease events, particularly among younger persons. In fact, among the case patients, there was a significant difference of more than 7 years in the age at onset of disease between the $PI^{A2}$-positive patients and the $PI^{A2}$-negative patients (51.8 vs. 59.2 years, $P = 0.02$).

In addition to our findings related to the prognostic potential of $PI^{A2}$, our results could directly affect the treatment of acute coronary events. For example, although aspirin has been the platelet inhibitor of choice for the treatment of unstable coronary syndromes, re-

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### Table 2. Genotypes of the Case Patients and Controls According to Age.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Case Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ages</td>
<td>60.6</td>
<td>80.9</td>
<td>0.01</td>
</tr>
<tr>
<td>$PlA1/PlA1$</td>
<td>23 (50)</td>
<td>12 (19.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>42 (100)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Age &lt;60 yr</td>
<td>21 (50)</td>
<td>31 (66.1)</td>
<td></td>
</tr>
<tr>
<td>$PlA1/PlA1$</td>
<td>19 (25)</td>
<td>5 (13.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Total</td>
<td>42 (100)</td>
<td>36 (100)</td>
<td></td>
</tr>
</tbody>
</table>

*Subjects with the $PlA1/PlA1$ genotype were classified as $PlA1$-negative, and subjects with the $PlA1/PlA2$ and $PlA2/PlA2$ genotype were classified as $PlA2$-positive. For the case patients, age refers to the age at onset of myocardial infarction or unstable angina, not the current age.

†The chi-square test was used to compare the prevalence of $PlA1$ in case patients and controls.

### Table 3. Odds Ratios for Selected Risk Factors, According to Age.

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>CLASS</th>
<th>NO. OF CASE PATIENTS</th>
<th>NO. OF CONTROLS</th>
<th>ODDS RATIO (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking status</td>
<td>Current or former</td>
<td>50</td>
<td>36</td>
<td>2.2 (1.0–4.8)</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>19</td>
<td>30</td>
<td>1.0 (0.5–1.9)</td>
</tr>
<tr>
<td>Systolic blood pressure on admission</td>
<td>≥140 mm Hg</td>
<td>36</td>
<td>25</td>
<td>1.9 (0.9–3.9)</td>
</tr>
<tr>
<td></td>
<td>&lt;140 mm Hg</td>
<td>33</td>
<td>43</td>
<td>1.9 (0.9–3.9)</td>
</tr>
<tr>
<td>Total serum cholesterol on admission</td>
<td>≥200 mg/dl</td>
<td>18</td>
<td>15</td>
<td>1.3 (0.5–3.0)</td>
</tr>
<tr>
<td></td>
<td>&lt;200 mg/dl</td>
<td>50</td>
<td>53</td>
<td>1.3 (0.5–3.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Type I or II</td>
<td>13</td>
<td>15</td>
<td>1.0 (0.5–2.0)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>58</td>
<td>53</td>
<td>1.0 (0.5–2.0)</td>
</tr>
</tbody>
</table>

*The total number of patients and controls for individual risk factors may not equal 71 and 68, respectively, since data were missing for some subjects. For the case patients, age refers to the age at onset of myocardial infarction or unstable angina, not the current age. CI denotes confidence interval.

†Subjects with the $PlA1/PlA2$ or $PlA2/PlA2$ genotype were classified as $PlA2$-positive.

‡The odds ratio was not calculated because the control subjects were selected from a population of inpatients with a high prevalence of diabetes.
cent studies indicate that treatment with specific inhibitors of glycoprotein IIb/IIIa leads to better outcomes than does aspirin therapy. It is conceivable that PlA2-positive patients would receive extra benefit from direct therapy with anti–glycoprotein IIb/IIIa, providing a rationale for decisions regarding the choice of antiplatelet therapy for patients with unstable coronary syndromes.

We are indebted to the members of Kindred PT1 for their invaluable contribution to this study, to Hyun Kim, Emily E. Milliken, Ying Jin, Williams S. Shear, and Lindsay D. Coleman for excellent technical support; to Dr. Pamela Ouyang, Lydia Nelson, and members of the medical house staff and nursing staff of Johns Hopkins Hospital for assistance in the recruitment of patients and the procurement of blood samples; to Drs. Kenneth L. Baughman and Roger Blumenthal for providing medical records and scientific expertise; and to Dr. Paul M. Ridker for thoughtful discussions.

**REFERENCES**


16. Newman PJ, Derbes RS, Aster RH. The human platelet alloantigens, PlA\textsuperscript{1} and PlA\textsuperscript{2}, are associated with a leucine\textsuperscript{14} proline\textsuperscript{15} amino acid polymorphism in membrane glycoprotein IIIa, and are distinguishable by DNA typing. J Clin Invest 1983;63:1778-81.


