Platelet Glycoprotein IIIa $P^A$ Polymorphism, Fibrinogen, and Platelet Aggregability
The Framingham Heart Study

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Background—Recent data suggest that the $P^{A2}$ allele of the platelet glycoprotein IIIa receptor may be a genetic risk factor for cardiovascular disease. We previously reported that the $P^{A2}$ allele was associated with increased platelet aggregability, as indicated by lower epinephrine threshold concentrations. Paradoxically, however, it has been reported that $P^{A2}$-positive platelets have reduced fibrinogen binding. Because fibrinogen mediates platelet aggregability, we hypothesized that plasma fibrinogen levels may interact with $P^A$ genotype in modulating platelet aggregability.

Methods and Results—Glycoprotein IIIa $P^A$ genotype, fibrinogen level, and platelet aggregability were ascertained in 1340 subjects enrolled into the Framingham Offspring Study. Platelet aggregability was evaluated by the Born method. Higher fibrinogen levels were associated with increased epinephrine-induced aggregation ($P=0.002$) and a trend for ADP-induced aggregation ($P=0.07$). The fibrinogen effect was genotype specific, however, in that the increase in platelet aggregability with higher fibrinogen was present for the $P^{A1/A1}$ genotype ($P=0.0005$ and $P=0.03$ for epinephrine- and ADP-induced aggregation, respectively) but not for the $P^{A2/A2}$-positive genotype ($P>0.90$).

Conclusion—Higher fibrinogen levels were associated with increased platelet aggregability. However, the association between fibrinogen and platelet aggregability was genotype specific. This interaction may be responsible for the conflicting findings regarding $P^A$ genotype and platelet aggregability. Further study of this gene-environment interaction may provide insight into cardiovascular disease risk. (Circulation. 2001;104:140-144.)

Key Words: platelets • genetics • glycoproteins • fibrinogen

Myocardial infarction results from the formation of a platelet-rich thrombus at the site of a ruptured coronary atherosclerotic plaque. The platelet surface receptor glycoprotein IIb/IIIa (GP IIb/IIIa) plays a key role in the formation of such thrombi by binding with fibrinogen and other ligands, including von Willebrand factor (vWF). The importance of the GP IIb/IIIa receptor has been further supported by recent clinical trials in which GP IIb/IIIa antagonists have been shown to reduce the morbidity and mortality associated with unstable angina, high-risk coronary angioplasty, and acute myocardial infarction.

Weiss and colleagues first reported that patients with acute coronary syndromes were more likely to carry the $P^{A2}$ variant of the GP IIIa receptor than were controls. The risk associated with $P^{A2}$ was especially high for those aged $\leq 60$ years at the time of infarction. Recently Walter and colleagues reported that patients with the $P^{A2}$ variant had an increased risk of coronary stent thrombosis compared with $P^{A1/A1}$-homozygous individuals. In addition, $P^{A2}$ has been associated with restenosis after coronary stent placement. However, an association between the $P^{A2}$ and cardiovascular disease (CVD) has not been a consistent finding.

Recently, we found that the presence of the $P^{A2}$ variant was associated with increased platelet aggregability as indicated by a lower epinephrine threshold concentration. There was also a trend toward $P^{A2}$ being associated with a decreased threshold concentration for ADP, which was directionally consistent with the results seen with epinephrine-induced aggregation. Similar results have been reported with thrombin receptor–activating peptide. Paradoxically, however, Goldschmidt-Clermont et al showed that platelets with the $P^{A2}$ variant bound less exogenous fibrinogen than did the $P^{A1}$-homzygous platelets when stimulated with ADP. Fibrinogen mediates platelet aggregation by binding to...
GP IIb/IIIa, and fibrinogen levels influence platelet aggregability. In addition, vWF is a glycoprotein that mediates platelet adhesion by binding with platelet surface receptor GP IIb/IX. This receptor is particularly important in hemostasis and thrombosis at high shear rate. vWF also binds with the platelet GP IIb/IIIa receptor, mediating platelet aggregation.

In the current study, we hypothesized that platelet GP IIIa platelet GP IIb/IIIa receptor, mediating platelet aggregation. vWF also binds with the Ib/IX. This receptor is particularly important in hemostasis.

Determination of Platelet Aggregability, Fibrinogen, and vWF Levels
Blood samples were obtained in the morning to avoid circadian variation in platelet aggregability. Blood was drawn in 3.8% sodium citrate solution (9:1 vol/vol). Platelet-rich plasma was separated by centrifugation for 10 minutes at 160g. Platelet aggregation was measured according to the method of Born. The aggregation agents tested were epinephrine and ADP in varying concentrations (0.01 to 30 μmol/L) and a fixed concentration of arachidonic acid (1.6 μmol/L). The lowest concentrations of ADP and epinephrine required to produce a biphasic response with >50% aggregation (threshold concentration) were determined. Therefore, a lower threshold concentration indicates an increase in platelet aggregability.

Blood for fibrinogen was drawn into 3.8% sodium citrate (9:1, vol/vol), and blood for vWF was collected in EDTA. Plasma was separated by centrifugation at 20 minutes at 2000g and stored at −80°C for later analysis. Fibrinogen was determined by the Clauss method. vWF antigen was measured by ELISA. The intra-assay coefficients of variation for fibrinogen and vWF in our laboratory were 2.6% and 8.8%, respectively, and the inter-assay coefficients of variation were 4.7% and 10.6%, respectively.

Genotyping
Detailed methods have been reported previously. Polymerase chain reaction results were scored without knowledge of platelet aggregation results. Genotyping was successful in 97% of the samples.

Table 1. Sample Characteristics

<table>
<thead>
<tr>
<th>Sample Characteristic</th>
<th>PlA1/PlA1</th>
<th>PlA1/PlA2 or PlA2/PlA2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>961</td>
<td>379</td>
<td>. .</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>47</td>
<td>46</td>
<td>0.98</td>
</tr>
<tr>
<td>Age, y</td>
<td>53±10</td>
<td>54±11</td>
<td>0.45</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>33</td>
<td>29</td>
<td>0.13</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>6</td>
<td>6</td>
<td>0.85</td>
</tr>
<tr>
<td>Smoker, %</td>
<td>20</td>
<td>16</td>
<td>0.04</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.5±5.1</td>
<td>27.8±5.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.67±1.22</td>
<td>1.58±0.97</td>
<td>0.16</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.28±0.96</td>
<td>5.31±0.93</td>
<td>0.69</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.27±0.39</td>
<td>1.30±0.39</td>
<td>0.30</td>
</tr>
<tr>
<td>Alcohol, oz/wk</td>
<td>2.9±4.2</td>
<td>2.4±3.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Menopause, %</td>
<td>66</td>
<td>62</td>
<td>0.29</td>
</tr>
<tr>
<td>Estrogen therapy, %</td>
<td>12</td>
<td>11</td>
<td>0.90</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>302±58</td>
<td>305±57</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD or percentages.

Statistical Analysis
Demographic and clinical characteristics were compared by Student’s t tests or χ² tests between genotype groups defined by the presence or absence of the PlA2 allele. Observed genotype frequencies were compared with the Hardy-Weinberg equilibrium prediction, also using χ² tests. Data on epinephrine and ADP threshold concentrations were log-transformed. Multiple linear regression models were used to analyze platelet aggregability, adjusting for age, sex, body mass index, diabetes, triglycerides, HDL cholesterol, and estrogen replacement status, together with the presence or absence of the PlA2 allele and fibrinogen quartile (determined separately for each sex). Generalized estimating equation algorithms were used to correct for intra-family correlation. Data on platelet aggregation were expressed as geometric mean±95% CI.

Results
Subject Characteristics
The characteristics of the study subjects are shown in Table 1. Subject characteristics with and without the PlA2 allele were quite similar, with the exception that cigarette smoking was slightly less common among the PlA2-positive genotype subjects (16% versus 20%, P = 0.04). Fibrinogen levels were also similar between the 2 groups. The allele frequencies of PlA1 and PlA2 were 0.85 and 0.15, respectively. Genotype frequencies were 71.7% for PlA1-homozygous, 25.8% for the PlA1/PlA2 genotype, and 2.5% for the PlA2-homozygous. The genotype frequencies are in accord with the Hardy-Weinberg equilibrium (P = 0.65).

Plasma Fibrinogen Levels and Platelet Aggregability
Increased plasma fibrinogen levels were associated with incrementally increased platelet aggregability for epinephrine-induced aggregation and a trend for ADP-induced aggregation, as indicated by decreases in the threshold concentrations for epinephrine and ADP (P = 0.002 and 0.07, respectively) (Table 2), after adjusting for covariates and the presence of PlA2 allele.
Interaction Between Plasma Fibrinogen and $P_I^A$ Genotype on Platelet Aggregability

The threshold concentrations for epinephrine-stimulated aggregation decreased with higher fibrinogen levels among the $P_{IA}^A$-homozygous subjects, with mean epinephrine threshold concentrations of 1.11 (range, 0.95 to 1.30) μmol/L in the lowest fibrinogen quartile, 1.02 (0.87 to 1.19) μmol/L in the second quartile, 0.85 (0.72 to 1.00) μmol/L in the third quartile, and 0.72 (0.60 to 0.86) μmol/L in the highest fibrinogen quartile (trend $P=0.0005$) (Figure 1). However, the threshold concentrations were not related to fibrinogen levels among the $P_{IA}^{A2}$-positive genotype, with respective mean epinephrine threshold concentrations of 0.67 (0.52 to 0.86) μmol/L, 0.81 (0.63 to 1.04) μmol/L, 0.80 (0.62 to 1.05) μmol/L, and 0.63 (0.48 to 0.83) μmol/L (trend $P=0.91$). The interaction term describing the fibrinogen-related alterations in epinephrine threshold concentrations between genotypes was of borderline statistical significance ($P=0.06$, multivariate analyses).

For ADP-induced aggregation, the threshold concentrations also decreased with increased fibrinogen levels in the $P_{IA}^A$-homozygous group, with ADP threshold concentrations of 3.20 (range, 3.02 to 3.39) μmol/L, 3.18 (3.01 to 3.37) μmol/L, 2.97 (2.80 to 3.16) μmol/L, and 2.89 (2.70 to 3.09) μmol/L from the lowest fibrinogen quartile to the highest quartile (trend $P=0.03$) (Figure 2). As seen with epinephrine-induced platelet aggregation, there was no relation found between fibrinogen level and ADP threshold concentration in the subjects with $P_{IA}^{A2}$-positive genotype. The respective ADP threshold concentrations were 2.83 (2.58 to 3.10) μmol/L, 2.97 (2.71 to 3.26) μmol/L, 3.17 (2.87 to 3.49) μmol/L, and 2.88 (2.61 to 3.19) μmol/L (trend $P=0.97$). The interaction term assessing fibrinogen-related differences in ADP-induced aggregation between genotypes was statistically significant ($P=0.05$, multivariate analyses).

Similar results were obtained when we analyzed the interaction between fibrinogen levels and platelet aggregability and the interaction between fibrinogen and $P_{IA}^A$ genotype on platelet aggregability with fibrinogen as a continuous variable (data not shown).

Platelet aggregation thresholds were modestly but statistically significantly correlated between siblings for epinephrine threshold concentration ($r=0.245, P<0.0001$) and for ADP threshold concentration ($r=0.235, P<0.0001$). We obtained very similar results for the regression interaction terms relating $P_{IA}^A$ genotype with fibrinogen quartile to aggregation thresholds whether the sibship information was included or excluded (data not shown).

There was no significant association between plasma vWF antigen levels and platelet aggregation induced by ADP or epinephrine ($P=0.70$ and 0.96, respectively; multivariate analyses). Furthermore, vWF did not modify the association between the $P_{IA}^A$ genotype and platelet aggregability (data not shown).

Discussion

In a prior Framingham Heart Study investigation, we reported that the $P_{IA}^{A2}$ allele of the platelet GP IIIa receptor was associated with increased platelet aggregability. In this investigation, we found that higher fibrinogen levels were associated with increased platelet aggregability. In addition, there was an interaction between the $P_{IA}^A$ genotype and fibrinogen levels such that the association of greater platelet aggregability with increased fibrinogen levels was significant for the $P_{IA}^{A2A2}$ genotype but not for the $P_{IA}^{A2}$-positive genotype. Despite these differences, subjects with the $P_{IA}^{A2}$-positive
Plasma Fibrinogen Levels Interact With PI^A Polymorphism on Platelet Aggregability

Our results show an interaction between the PI^A genotype and fibrinogen levels on platelet aggregability such that the enhanced platelet aggregability with increased fibrinogen levels were observed in the PI^A1A1 genotype but not in the PI^A2- negative genotype. However, the PI^A2- positive genotype still had an overall higher platelet aggregability compared with the PI^A1A1-homozygous even though the PI^A2- positive genotype platelets bind a smaller 14 or a similar 29 amount of exogenous fibrinogen with the GP IbIIa/IIa receptor than do the PI^A1A1 platelets. Our findings partially explain the conflicting findings regarding the PI^A2 allele and platelet function.11–14,27–29,31 Plasma fibrinogen level should be taken into account when platelet reactivity studies are interpreted because fibrinogen level is an important determinant for platelet reactivity and a modulator of platelet aggregability associated with the PI^A genotype.15,16,32

The mechanism for this interaction is unknown. The PI^A antigen system is not in the 2 putative RGD (Arg-Gly-Asp)-binding regions of the GP IIIa (PI^A1 is a leucine and PI^A2 a proline at amino acid position 33). However, according to Calvete,33 the PI^A system could be brought into immediate proximity with the binding regions by 2 disulfide bonds. Because of the unique structure of proline and its propensity for inducing conformational change, the amino acid substitution could influence the affinity of GP IbIIa/IIa receptor for fibrinogen. Thus, the platelet with the PI^A2 variant might have an increased binding with endogenous plasma fibrinogen at basal conditions. If that is the case, fewer GP IbIIa receptors will be available for exogenous fibrinogen after being stimulated as reported by Goldschmidt-Clermont et al.14 Further study of endogenous and exogenous fibrinogen binding is needed to test the hypotheses. Alternatively, platelets with the PI^A2 allele may have intrinsically increased aggregability as indicated by a lower epinephrine threshold concentration and therefore may be less dependent on plasma fibrinogen levels to achieve a similar degree of platelet aggregation as measured by the Born method.11–13

Strengths and Limitations of the Study

In a large number of subjects, we obtained genotype status, fibrinogen levels, and platelet aggregability data to evaluate the relation between the PI^A genotype and platelet aggregability and the interaction between the genotype and fibrinogen on platelet aggregability. A detailed risk factor profile was collected and adjusted for potential confounders. However, this cross-sectional study had several limitations. First, our analysis was based on the subset of Framingham subjects in whom both genotype and phenotype data were available. However, the genotype distribution and subject characteristics were similar among subjects excluded from analysis or included in the present analysis (data not shown). Second, we used an in vitro method to evaluate the relation between the PI^A polymorphism, fibrinogen levels, and platelet aggregability. The in vivo significance of our in vitro findings and their clinical relevance need to be evaluated further. Finally, we used epinephrine and ADP threshold concentrations to determine platelet aggregability. Many different methods and
parameters have been used to evaluate platelet aggregability, which may influence the results. For example, in one study, increased fibrinogen concentrations led to increased platelet fibrinogen bonding but reduced aggregation velocity. Meade et al. found that increased fibrinogen levels were associated with a reduced median effective dose of ADP, a finding similar to our current result, indicating increased platelet aggregability. However, they also reported that increased fibrinogen was associated with a lower maximal response when higher ADP concentration was used, suggesting decreased aggregability.

Implications of the Study
We found that increased plasma fibrinogen levels were associated with increased platelet aggregability, although this association was present for the PlA1/A1 but not the PlA2 genotype. Prospective studies are warranted to examine the combined effect of fibrinogen levels and the PlA gene polymorphism on incidence of CVD. If individuals with the T-of-T allele (who have increased platelet aggregability irrespective of fibrinogen levels) or PlA2 variant (who have increased platelet aggregability irrespective of fibrinogen levels) or PlA1 homozygotes with high fibrinogen levels have a higher incidence of CVD, they may benefit from more aggressive measures for prevention and treatment, including therapy with antiplatelet agents.

Acknowledgments
This study was supported by a grant from the National Institutes of Health National Heart, Lung, and Blood Institute (R01-HL-48157) to Dr. Tofler and by a National Heart, Lung, and Blood Institute Research Development Award (K04-HL-03138-01) to Dr. Lindpaintner.

References
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Circulation. 2001;104:140-144
doi: 10.1161/01.CIR.104.2.140
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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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