Associations Between a Polymorphism in the Gene Encoding Glycoprotein IIIa and Myocardial Infarction or Coronary Artery Disease

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OBJECTIVES

The purpose of this study was to determine whether a common variant (PI A2) of the membrane glycoprotein (GP) IIIa gene is associated with myocardial infarction (MI) or coronary artery disease (CAD).

BACKGROUND

Platelet GP IIb/IIIa is believed to play a central role in MI, binding fibrinogen, cross-linking platelets and initiating thrombus formation. Genetically determined differences in binding properties of GP IIb/IIIa might result in changes in platelet activation or aggregation and affect the risk of MI or CAD.

METHODS

To determine associations (odds ratios [OR] ≥1.5 to 2.0) of genotype with MI or CAD, blood was drawn from 791 patients (pt) undergoing angiography. A 266 base pair fragment of the GP IIIa gene was amplified by the polymerase chain reaction and digested with the MspI restriction enzyme. Genotypes were identified after electrophoresis of digestion products in 1.5% agarose gel.

RESULTS

Of the 791 pt, 225 had acute (n = 143) or previous MI, and 276 did not have MI or unstable angina. The PI A2 allele was carried by 33.8% of MI pt versus 26.9% of no-MI control subjects, OR = 1.39 (95% CI, 0.95 to 2.04, p = 0.09). Angiographically, 549 pt had severe (>60% coronary stenosis) CAD, and 170 had normal coronary arteries (<10% stenosis). The PI A2 allele was found in 31.0% of CAD pt versus 28.2% of no-CAD control subjects, OR = 1.14 (CI, 0.78 to 1.67, p = 0.50). When adjusted for six standard risk factors, ORs were 1.47 (CI, 0.98 to 2.20, p = 0.062) for MI and 1.20 (CI, 0.80 to 1.81, p = 0.38) for CAD.

CONCLUSIONS

The PI A2 variant of the gene encoding GP IIIa is modestly associated (OR ~ 1.5) with nonfatal MI but shows little if any association with CAD per se. (J Am Coll Cardiol 1999; 33:727–33) © 1999 by the American College of Cardiology
morphism PI\textsuperscript{A2} had a proline at this position. The change in amino acids was shown to be the result of a substitution of cytosine for thymidine at nucleotide position 1565, located in exon 2 of the glycoprotein III\textsubscript{a} gene (16).

Given the central role of platelets in vascular thrombosis, interest has grown in the possibility that mutations in platelet receptor glycoproteins, possibly including common polymorphisms such as PI\textsuperscript{A1/A2}, may represent independent risk factors for vascular thrombosis. Weiss and colleagues found an unexpectedly high frequency of PI\textsuperscript{A2} homozygosity in families with a high prevalence of early coronary events (21). Subsequently, they reported the same polymorphism to be a risk factor for unstable angina and MI in a general population, based on a case–control study (22). However, this finding could not be confirmed in a subsequent report from the Physicians Health Study (23). Neither study was angiographically controlled. To shed further light on an association of the PI\textsuperscript{A1/A2} polymorphism of GP III\textsubscript{a} with coronary heart disease, we studied a relatively large patient population whose coronary status was defined angiographically. The study was performed in compliance with the hospital's human studies committee and with subjects' written informed consent.

METHODS

Study hypotheses. We tested whether carriage of the GP III\textsubscript{a} PI\textsuperscript{A2} allele is associated with an increased risk for: 1) MI, and 2) coronary artery disease (CAD) in patients studied by coronary angiography.

Patient and control populations. Study patients and control subjects consisted of consecutive, stable, consenting subjects presenting for coronary angiography at LDS Hospital either because of symptoms relating to suspected CAD or because of unrelated conditions requiring angiographic evaluation (e.g., valvular disease, cardiomyopathy). Subjects were of unrestricted age and gender who gave written informed consent for a blood draw for deoxyribonucleic acid (DNA) extraction at the time of angiography, to be used in studies approved by the hospital’s institutional review board. An independent, free-living, volunteer population sample, without clinically evident disease, served as a further control group. Subjects were residents of Utah, southwestern Idaho or southeastern Wyoming, a population that is ethnically primarily of Northern European (Anglo-Scandinavian) descent that previously has been shown to be genetically representative of North American Caucasians (24).

Key demographic characteristics of subjects were recorded on computerized angiographic data forms, including age, gender and history of MI (25). Assessment of CAD was made by review of angiograms by the patient's cardiologist, who was uninformed as to GP III\textsubscript{a} genotype. Results were entered into the computer database in a format modified after the Coronary Artery Surgery Study protocol (25,26).

Patients were designated to have significant CAD if they had >60% stenosis of at least one coronary artery or major branch (n = 549) and no CAD if <10% stenosis was present (n = 170). Of CAD patients, 129 were hospitalized with an acute MI (203 had an MI at some time), 205 with unstable angina, 165 with other chest pain syndromes, 84 with valvular heart disease and 1 with cardiomyopathy, singly or in combination. Of the control subjects with normal coronary arteries, 107 presented with chest pain syndromes without CAD or MI, 47 with valvular heart disease and 6 with cardiomyopathy, singly or in combination, and the rest for other reasons. Patients with minor CAD (10% to 60% stenosis; n = 72) were designated as having “indeterminate” CAD status and were not included in CAD analyses.

The MI group consisted of 225 patients, 143 with acute (same hospitalization) MI, the rest with previous MI only. A history of MI was identified and entered on the cath-lab report form by the attending physician: old MI was defined on the basis of a current electrocardiogram (ECG) and historical data; recent MI (i.e., MI occurring during the index hospitalization) was, in addition, to be associated with positive cardiac serum markers. The no-MI control group (n = 276) was selected from other patients undergoing angiography, excluding those with a history of MI at any time as well as those with CAD associated with either unstable angina or other chest pain syndromes. Of no-MI control subjects, 146 presented with chest pain without severe CAD, 76 with valvular disease and 9 with cardiomyopathy, alone or in combination, and the rest for other reasons.

Designations of MI and CAD status were made by the attending physician without knowledge of DNA genotype after considering angiographic results together with patient history and ECG.

Deoxyribonucleic acid extraction. Approximately 20 to 30 ml of blood was withdrawn by venipuncture at the time of coronary angiography and collected in ethylenediaminetetraacetic acid (EDTA). The leukocyte buffy coat was separated by centrifugation. Recovered leukocytes were washed in TNE buffer (10 mmol/L Tris base, 10 mmol/L NaCl, 1 mmol/L EDTA) and resuspended in 2 ml of a solution of sodium chloride (75 mmol/L) and
Deoxyribonucleic acid genotyping. To identify the PI\textsuperscript{A} genotypes, the following primers were used: 5' TTC TGA TTG CTG GAC TTC TCT T 3' and 5' TCT CTC CCC ATG GCA AAG AGT 3'.

The polymerase chain reaction (PCR) amplification protocol consisted of an initial denaturation segment of 94°C for 5 min. After this, each cycle consisted of three segments (94°C for 60 s, 57°C for 45 s and 72°C for 60 s). This cycle was repeated 35 times followed by an additional extension cycle at 72°C for 15 min. The 266 base pair fragment amplified by PCR was digested with MspI. Polymorphic genotypes were identified after electrophoresis of digestion products in 1.5% agarose gel and staining with 1 \mu g/ml ethidium bromide. The gels were read blinded to clinical and angiographic results by a single experienced reader. A representative gel is shown in Figure 1.

Statistical planning and analysis. Power calculations indicated that to determine odds ratios (ORs) for CAD or MI of the PI\textsuperscript{A2} polymorphic allele carriage in the GP IIIa gene of between 1.5 and 2.0 with a power of 80% at a two-sided alpha level of 0.05 in a population with a carriage rate of the PI\textsuperscript{A2} allele of 25% would require samples of between 151 and 464 subjects per group (GB Stat for Windows). Accordingly, we assembled and studied a population of 791 subjects; 69% of the population samples had severe CAD, and 28% had a history of MI.

Allelic and genotypic frequencies were determined from observed genotype counts, and the expectations of the Hardy–Weinberg equilibrium were evaluated by chi-square analysis. Comparisons between allelic and genotypic frequencies used chi-square analysis. Associations were assessed as ORs; 95% confidence intervals (CIs) were calculated as previously described (27). Exploratory analyses were prospectively planned in subgroups of interest. Univariate and multivariate logistic regression, used to determine crude and adjusted ORs for associations with the genetic marker, respectively, were done using SPSS 6.1 (Chicago, IL). Multivariate analyses used a backward, conditional approach, entering simultaneously genotype and six major CAD risk factors: age, gender, cholesterol concentration, smoking status, diabetic status and history of hypertension (see Table 3 for details).

RESULTS

Characteristics of the patient groups. A total of 791 angiographic subjects were studied. Their age averaged 64 years (range 17 to 89); 225 had a history of MI, and 549 had severe CAD. Key patient characteristics are summarized by disease subgroup in Table 1. The control and diseased groups differed in that those with CAD were older, and more frequently male, smokers and (trend) diabetic. Those with MI were more frequently male and smokers, and more likely (trend) to have a history of hypertension (although post-MI, treated systolic blood pressure was slightly lower).

Genotypic and allelic frequencies in the control groups. Genotypic and allelic frequencies for the study groups are shown in Table 2. The PI\textsuperscript{A2} allelic frequency was 15.0% in the no-MI angiographic control group (n = 276), 15.9% in the no-CAD control group (n = 170) and 12.8% in the smaller group (n = 94) of normal volunteers. Genotypic distributions in the control groups conformed with Hardy–Weinberg expectations. The homozygotic PI\textsuperscript{A2/A2} genotype appeared in 3.3% and 3.5% of the no-MI and no-CAD control groups and PI\textsuperscript{A2} allele carriage (homo- or heterozygous) in 26.9% and 28.2%, respectively.

Association between the PI\textsuperscript{A2} polymorphism and MI. Among patients with a diagnosis of MI (n = 225), the PI\textsuperscript{A2} polymorphic allelic frequency was 17.8% (OR = 1.22, CI 0.87 to 1.71, p = 0.24 vs, no-MI control subjects) (Table 2). The homozygous or heterozygous carriage rate of the PI\textsuperscript{A2} allele of 33.8% was associated with an OR for MI in the entire angiographic cohort of 1.39 (CI 0.95 to 2.04, p = 0.09). Simultaneously adjusting the OR for the presence of
six standard risk factors gave an adjusted OR of 1.47 (CI 0.97 to 2.22, \( p = 0.062 \)) (Table 3). Other independent associates, in order of strength of association, included: gender, smoker, genotype, hypertension and age (Table 3). Using the smaller normal volunteer group as control gave a crude OR of 1.67 (CI 0.88 to 2.45, \( p = 0.14 \)).

### Association between the PI\(^{A2}\) polymorphism and CAD.

Among patients with severe CAD \((n = 549)\), the PI\(^{A2}\) allelic frequency was 15.9% (OR = 1.06, CI 0.76 to 1.48, \( p = 0.73 \)) vs. no-CAD control subjects (Table 2). Carriage of the PI\(^{A2}\) allele occurred in 31.0% and was associated with an OR for CAD of 1.14 (CI 0.78 to 1.67, \( p = 0.50 \)). Adjustment of the OR for the standard risk factors did not significantly improve the association of PI\(^{A2}\) carriage with CAD (OR = 1.20, CI 0.80 to 1.81, \( p = 0.38 \)). Indeed, genotype was not selected as a multivariate associate of CAD; these included, in order of strength of association, gender, age, smoking and diabetes. Using the smaller normal volunteer group as control gave a crude OR of 1.47 (CI 0.88 to 2.45, \( p = 0.14 \)).

### Table 1. Characteristics of Diseased and Control Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI Number</td>
<td>225</td>
<td>276</td>
</tr>
<tr>
<td>Age (yr) (mean ± SD)</td>
<td>63 ± 11</td>
<td>62 ± 11</td>
</tr>
<tr>
<td>Range</td>
<td>31 to 89</td>
<td>17 to 89</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>79</td>
<td>57*</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>51</td>
<td>44</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>132 ± 22</td>
<td>138 ± 23*</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>71 ± 10</td>
<td>72 ± 11</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>36</td>
<td>20*</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Cholesterol (mg/dl) (n)</td>
<td>205 ± 41</td>
<td>206 ± 53</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>31</td>
<td>36</td>
</tr>
</tbody>
</table>

\( ^{*} p < 0.001, ^{†} p < 0.01, ^{‡} p < 0.05 \) control subjects versus patients.

BP = blood pressure; CAD = coronary artery disease; MI = myocardial infarction.

### Table 2. Genotypic Distributions and Allelic Frequencies of PI\(^{A1}/A2\) Polymorphism Among Patients and Control Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>PI(^{A1}/A1), n (%)</th>
<th>PI(^{A1}/A2), n (%)</th>
<th>PI(^{A2}/A2), n (%)</th>
<th>%PI(^{A1}) Allele</th>
<th>%PI(^{A2}) Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI (n = 225)</td>
<td>149 (66.2)</td>
<td>72 (32.0)</td>
<td>4 (1.8)</td>
<td>82.2</td>
<td>17.8</td>
</tr>
<tr>
<td>No MI (n = 276)</td>
<td>202 (73.2)</td>
<td>65 (23.6)</td>
<td>9 (3.3)</td>
<td>85.0</td>
<td>15.0</td>
</tr>
<tr>
<td>CAD/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD (n = 549)</td>
<td>379 (69.0)</td>
<td>157 (28.6)</td>
<td>13 (2.4)</td>
<td>83.3</td>
<td>16.7</td>
</tr>
<tr>
<td>No CAD (n = 170)</td>
<td>122 (71.8)</td>
<td>42 (24.7)</td>
<td>6 (3.5)</td>
<td>84.1</td>
<td>15.9</td>
</tr>
<tr>
<td>Free-living normals</td>
<td>72 (76.6)</td>
<td>20 (21.3)</td>
<td>2 (2.1)</td>
<td>87.2</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.
Homzygotic carriage of PI\textsuperscript{A2} was infrequent and not significantly predictive of either MI or CAD.

**Association between the PI\textsuperscript{A1/A2} polymorphism and MI in prespecified subgroups.** The PI\textsuperscript{A1/A2} polymorphism might affect risk primarily in a certain patient subgroup (e.g., in younger patients). We performed stratified analyses of associations of PI\textsuperscript{A2} carriage in the subgroups defined by presence or absence of age \(\geq 60\) years, male gender, smoking, diabetes, hypertension or cholesterol \(\geq 220\) mg/dl. A proposed increase in disease association in younger (<60 years) MI patients could not be confirmed: OR = 0.90, CI 0.48 to 1.70, age <60 years (n = 182); OR = 1.80, CI 1.11 to 2.93, age \(\geq 60\) years (n = 319). Analyses in other stratified subgroups did not point to notable heterogeneities.

**DISCUSSION**

**Summary of study results.** In a moderately large, angiographically defined population, we found a modest association between the variant GP IIIa allele, PI\textsuperscript{A2}, and nonfatal MI (OR \(\approx 1.5, \ p = 0.06\), adjusted for six other baseline risk factors). Comparison with the smaller normal volunteer population lent further support to an association of the polymorphism with MI. However, an association between allele carriage and CAD per se was not established (OR \(\approx 1.2\), with an OR of >1.8 excluded with 95% confidence). Overall, the results support the hypothesis that the PI\textsuperscript{A2} polymorphic allele may facilitate coronary thrombosis and hence MI through an alteration in platelet reactivity. Stratified analyses of the MI association revealed a notable heterogeneity only for age, with a stronger association in elderly than younger MI survivors.

**Comparison with recent literature reports.** Previous studies have reported divergent results for the association between coronary events and the PI\textsuperscript{A1/A2} polymorphism (21–23). In an initial report, Weiss et al. found an unexpectedly high frequency of homozygosity for PI\textsuperscript{A2} among family members in kindreds with a high prevalence of coronary events at a younger age (<60 years) (21). Weiss and coworkers subsequently conducted a case–control study of 71 patients with MI or unstable angina and 68 inpatient control subjects matched for age, race and gender (22). They found the prevalence of PI\textsuperscript{A2} to be 2.1 times greater among case patients than control subjects (39% vs. 19%, \(p = 0.01\)). In patients with onset of disease before age 60 years, polymorphic allelic frequency was 3.6 times greater than in age-matched control subjects (50% vs. 13.9%, \(p = 0.002\)). Odds ratios for a coronary event were 2.8 (CI 1.2 to 6.4) for all patients and 6.2 (CI 1.8 to 22.4) for those <60 years of age, suggesting a strong association between the PI\textsuperscript{A1/A2} polymorphism and acute coronary thrombosis, especially for events occurring before the age of 60 years.

More recently, Ridker et al. evaluated the predictive value of the PI\textsuperscript{A1/A2} polymorphism for MI, stroke and venous thrombosis among 704 patients with events and 704 matched subjects remaining free of thrombotic events during a prospective follow-up averaging 8.6 years in men participating in the Physician’s Health Study (23). The frequency of the PI\textsuperscript{A2} allele was found to be similar to the control frequency (14.8%) among men who had MI (13.5%, \(p = 0.4\)), stroke (13.4%, \(p = 0.5\)) or venous thrombosis (14.5%, \(p = 0.9\)). Carriage rates of the PI\textsuperscript{A2} allele were 26.4% and 25.2% in control subjects and MI patients, respectively. The relative risk associated with carriage of PI\textsuperscript{A2} was 0.96 (CI 0.8 to 1.2) for any vascular events and 0.93 (CI 0.7 to 1.2) for MI, neither a significant difference. Similarly, there was no evidence for an association with the polymorphism in subgroups analyzed by age, smoking status, family history of disease, hypercholesterolemia, hy-
pertension or diabetes. Aspirin use had no effect on the findings.

Our study went beyond these two in defining CAD angiographically. Overall, the findings are intermediate between the negative result of the large, prospective Physician’s Health Study (23) and the positive result of the smaller, earlier study of Weiss et al. (22); in our population, an association of modest degree (OR = 1.5) was found for MI but not for CAD per se, when adjusted for baseline variables or when using the normal volunteer group as control subjects.

Explanation for differences among studies and analysis of results. There may be several explanations for differences in results among the three studies. Differences may be due to chance. The study of Weiss et al. was substantially smaller than the other two and had wider confidence intervals (22). Thus, chance is more likely to have played a role in that result. Indeed, control carriage rates for the PI A2 allele were lower in the Weiss study (19%) than in ours or the Ridker study, which were similar (~26% to 28%).

Results of the studies may also differ because of differences in the selection of control patients. Allelic frequencies may differ among populations of different ethnic, genetic or gender background. Weiss selected control subjects from a population of patients admitted to the hospital for nonischemic disorders (22); Ridker selected control subjects from a cohort of healthy male physicians with a low frequency of coronary risk factors (23); we selected subjects coming to angiography for suspected cardiac disease (e.g., undefined chest pain, valvular disease or cardiomyopathy) but shown to be free of MI or CAD, as well as a smaller free-living sample. Finally, differences may arise because of differences in study design (prospective [23] vs. retrospective [22]) or in the way coronary events were defined (MI, [unstable] angina, angiographic CAD, coronary interventions, death or various combinations).

The extent to which chance, selection biases and study design contributed to differences in results among these studies is uncertain. Additional observations from larger samples testing prospective subgroup (and overall) hypotheses will be needed to resolve these issues. However, if the results of the three studies to date are combined in an informal meta-analysis, an OR for MI of ~1.2 is found for carriers of PI A2 (p = NS). However, additional studies would be welcome, especially those with prospective follow-up in populations with a greater prevalence of potentially interacting risk factors than was present in the Physician’s Health Study.

Pathophysiologic considerations. The stronger association of PI A2 carriage with MI than CAD is consistent with the hypothesis that the polymorphism affects primarily thrombotic (platelet-dependent) rather than other mechanisms of ischemic heart disease pathogenesis. However, the association with MI observed was modest.

Why is the effect of the PI A1/A2 polymorphism of a critical platelet receptor protein (GP IIIa) on MI risk difficult to ascertain when a wealth of experimental and clinical evidence is consistent with a central role for platelets (2–14)? One explanation is that the polymorphism is unassociated with significant differences in the function of the GP IIb/IIIa fibrinogen receptor. Another possibility is that the PI A2 allele is in linkage disequilibrium with a disease-associated locus on some but not all PI A2-bearing haplotypes. Although the polymorphism clearly has been implicated in immune-mediated platelet dysfunction syndromes [15,16], its functional role in non–immunemediated processes, if any, has not been defined.) Another possibility is that the polymorphism acts within a polygenetically (rather than monogenetically) determined model. Indeed, there is much evidence to suggest that the pathogenesis of ischemic heart disease is complex and polygenic in origin in the vast majority of patients, with important environmental factors interacting with multiple, interacting and counteracting genetic determinants, many with low penetrance. Consistent with current understanding of pathophysiology, we did find the risk of the GP IIIa polymorphism to be more clearly associated with transitional events to MI, which include coronary thrombosis, than CAD per se. Thus, further research on platelet-associated genetic factors is warranted, particularly for MI.

Study strengths and limitations. This study has the advantage of being of relatively large size and angiographically controlled. The possibility exists of inadvertent genetic selection bias appearing in “normal” subjects that are selected for coronary angiography. Comparisons with the free-living population sample in our study puts this possibility into perspective. Also, our PI A2 allelic carriage rates for the volunteer and angiographic control samples (23%, 28%) are similar to (straddle) the carriage rate of controls in the Physician’s Health Study (26%). Finally, we performed conditional logistic regression to adjust for differences in baseline factors (resulting in only minor changes in the associated ORs).

Mistyping is a theoretical concern and was dealt with by retyping ~10% of our samples, with identical results. Our study was “retrospective” with respect to MI events, raising the possibility of changes in prevalence of PI A2 among cases and control subjects due to differential survival rates after MI based on PI A2 carrier status. A similar concern applies to the study of Weiss et al. (22), but they found a greater relative risk for the PI A2 allele in younger coronary event survivors, unlike our results or those of the Physician’s Health Study. The Physician’s Health Study determined relative risk associations during prospective follow-up. However, our sample may have an advantage over that of Ridker et al. (23) in being drawn from a population with a greater prevalence of coronary risk factors, more typical of the overall United States population than the unusually low risk sample recruited into the Physician’s Health Study; low
penetrant genetic variants may require the presence of interacting environmental factors to achieve expression.

Conclusions. In an angiographically defined North American population of European extraction, we found a modest association (OR = 1.5, p = 0.06) between a relatively common variant (PI A2) of the gene encoding GP IIIa and nonfatal MI but not CAD per se. This supports the hypothesis that the polymorphism may facilitate coronary thrombosis through effects on platelet reactivity. These angiographically defined results address inconsistencies between two earlier studies (22,23). Because the pathogenetic mechanisms of ischemic heart disease are complex and multifactorial, with environmental factors interacting with multiple genetic determinants that have low penetrance, further research on platelet-associated genetic factors is warranted.

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REFERENCES