The spectrum of premature atherosclerosis: from single gene to complex genetic disorder
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Platelet Gp IIIa PlA polymorphism; its relation to coronary artery disease and its response to aspirin

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Submitted for publication
Abstract

Background: The platelet glycoprotein (GP) IIa PIA variant is associated with platelet dysfunction, but the clinical relevance for coronary artery disease (CAD) is disputed. We therefore studied this polymorphism in relation to CAD, angiographic progression of CAD and the response to aspirin (ASA) in both CAD patients and controls.

Methods: Platelet GP IIa PIA1/A2 genotypes were determined in 753 of the 885 participants of the Regression Growth Evaluation Statin Study, a placebo-controlled lipid lowering regression trial and in 222 controls. CAD progression was measured by quantitative coronary angiography at baseline and after two years.

Results: The PIA2 allele frequencies in patients and controls were 0.17 and 0.14 respectively, in accordance with Hardy-Weinberg equilibrium. Angiographic progression was unrelated to the platelet GP IIa PIA genotype, but placebo treated patients receiving ASA therapy who carried the PIA2 allele showed a significantly greater loss of mean segment diameter than patients not on ASA therapy (0.17 versus 0.07, p=0.03). This difference was not observed in the statin treated patients.

Conclusions: The Gp IIa PIA polymorphism is not associated with CAD nor with CAD progression. However, CAD patients carrying the PIA2 allele did not benefit from ASA therapy, and may therefore be candidates for other antiplatelet treatment.

Introduction

Platelets adhere and aggregate at the site of ruptured coronary atherosclerotic plaques, resulting in the formation of a platelet-rich thrombus, with acute myocardial infarction as a possible clinical outcome. Increased platelet aggregability is associated with myocardial infarction and is an independent risk factor for recurrent myocardial infarction. The platelet glycoprotein IIb/IIa surface receptor plays a pivotal role in this process: the receptor is able to bind fibrinogen and von Willebrand factor, thereby linking platelets together into larger aggregates. It has been suggested that these platelet-membrane glycoproteins are involved in the pathogenesis of coronary artery disease (CAD) and they have become targets in the treatment of acute coronary syndromes. Clinical trials with agents blocking the glycoprotein IIb/IIa receptor demonstrated a reduction in
morbidity and mortality in patients with unstable angina and acute myocardial infarction, as well as a reduced restenosis rate after angioplasty.\textsuperscript{6,7}

The polymorphism of the platelet glycoprotein (GP) II\textsubscript{a} PIA1/A2 is associated with platelet dysfunction. Recent studies have focussed on the question whether this polymorphism is associated with either a higher risk of CAD or altered therapeutic responsiveness to platelet antagonists. The data, however, have been conflicting. In 1994, Weiss et al. reported that patients with AMI, in particular when they were young, were more likely to carry the PIA2 allele, which was later confirmed by other investigators.\textsuperscript{8,9} However, other studies failed to detect this association, including the largest prospective trial.\textsuperscript{10-12} Subsequently, several studies showed a significant association between the GP II\textsubscript{a} PIA polymorphism and the risk of restenosis after coronary stent placement,\textsuperscript{13,14} which again could not be confirmed by others.\textsuperscript{15}

Although the results so far have not provided a definite answer with regard to the relevance for platelet function of this receptor polymorphism in vivo, in vitro studies did show differences in the presence of a PIA2 allele, such as both increased platelet aggregability\textsuperscript{16} and fibrinogen binding.\textsuperscript{17} Therefore we hypothesised that, if indeed the PIA2 allele is associated with an altered platelet response in vivo, this could result in a differential effect of anti-platelet therapy in CAD patients. In the present study, we evaluated platelet GP II\textsubscript{a} PIA polymorphism in relation to the risk of CAD, the angiographic progression of CAD, and the response to ASA.

Methods

Study population

The study subjects were male patients who had participated in the REGRESS trial.\textsuperscript{1} This trial was a double-blind, placebo-controlled multicenter study that assessed the effect of pravastatin on the progression of coronary atherosclerosis in 885 male subjects with average total cholesterol levels between 4 and 8 mmol/l. Quantitative Coronary Angiography (QCA) was performed at baseline and after two years. As described in the original article, for QCA the coronary tree was divided into 13 segments according to the American Heart Association classification. Obstructions within these segments were coded and analysed separately by an experienced cardiologist blinded to treatment allocation. The primary outcome measure was a comparison between the group treated with the cholesterol-lowering agent and the placebo group of changes in average mean segment diameter (MSD) and changes in average minimum obstruction diameter (MOD) per patient. To calculate average
MSD per patient, the MSD’s of all qualifying segments were added and divided by the number of contributing segments; segments that were occluded distal to and at either baseline or follow-up were not included because no meaningful MSD value can be calculated for these cases. Calculations for average MOD were done in the same manner, except that obstructions that had progressed to occlusion or occlusions that had recanalised were not excluded (MOD of occlusion equals 0). ASA was used by 427 of the 885 (57%) of the patients. DNA samples were available from 753 of the 885 patients.

The controls were healthy, normolipidemic males selected from the general population, without a history of angina, myocardial infarction or clinical signs of cerebrovascular or peripheral arterial disease. 222 control subjects were selected from this larger cohort according to the following criteria: men of Dutch ancestry, total cholesterol between 4 and 8 mmol/l, no diabetes, no medication for hypertension or hypercholesterolemia, a Body Mass Index between 20 and 30 kg/m², and alcohol intake less than 3 consumptions per day.

**DNA Analysis**

Genomic DNA was extracted from leukocytes as previously described. The PIA1/A2 polymorphism is the result of a cytosine/thymidine substitution at position 1565 in exon 2 of the Gp IIIa gene. It creates an MspI restriction site and therefore a restriction-fragment length polymorphism (RFLP). To detect this RFLP, a 279 bp fragment containing exon 2 was amplified from genomic DNA isolated from whole blood by polymerase chain reactions (PCR). The PCR product was digested with the restriction enzyme MspI; the fragments obtained were separated on a 3% agarose gel and visualised after staining with ethidium bromide.

**Statistical analysis**

PIA1/A2 and PIA2/A2 (PIA2 allele carriers) were combined for data analysis, and compared with PIA1/A1. The Hardy-Weinberg equilibrium of the GP IIIa PIA polymorphism was tested in each group studied, and subsequently the allele frequencies were compared using the chi-square test. Characteristics of patients with and without the PIA2 allele were compared using Students’ t-test, the Mann-Whitney test, or the chi-square test as appropriate. Angiographic changes during the REGRESS trial were evaluated with covariance analysis with baseline values as covariates. Changes in patients off or on ASA therapy were also compared using covariance analysis.
PLATELET GP IIIA PL<sup>A</sup> POLYMORPHISM

Table 1. PIA polymorphism in REGRESS patients and REGRESS controls

<table>
<thead>
<tr>
<th></th>
<th>pIA&lt;sup&gt;A1&lt;/sup&gt;/pIA&lt;sup&gt;A1&lt;/sup&gt;</th>
<th>pIA&lt;sup&gt;A1&lt;/sup&gt;/pIA&lt;sup&gt;A2&lt;/sup&gt;</th>
<th>pIA&lt;sup&gt;A2&lt;/sup&gt;/pIA&lt;sup&gt;A2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>REGRESS patients</td>
<td>527 (70)</td>
<td>207 (28)</td>
<td>19 (3)</td>
</tr>
<tr>
<td>REGRESS controls</td>
<td>162 (73)</td>
<td>58 (26)</td>
<td>2 (1)</td>
</tr>
</tbody>
</table>

Results

Frequency of the GP IIIa PIA polymorphism

PIA genotypes could be determined in 753 of the 885 REGRESS patients, in all 222 age- and sex- matched healthy normolipidemic controls. The PIA<sup>2</sup> allele frequencies in these three groups were 0.16 and 0.14 respectively (p=0.30), in accordance with Hardy-Weinberg expectations (Table 1).

Table 2. Baseline characteristics of REGRESS patients according to PIA genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>pIA&lt;sup&gt;A1&lt;/sup&gt;/pIA&lt;sup&gt;A1&lt;/sup&gt;</th>
<th>pIA&lt;sup&gt;A1&lt;/sup&gt;/pIA&lt;sup&gt;A2&lt;/sup&gt; + pIA&lt;sup&gt;A2&lt;/sup&gt;/pIA&lt;sup&gt;A2&lt;/sup&gt;</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=527</td>
<td>n=226</td>
<td></td>
</tr>
<tr>
<td>Age years</td>
<td>mean (SD): 56.0 (8.3)</td>
<td>56.6 (7.5)</td>
<td>0.28</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>mean (SD): 26.0 (2.6)</td>
<td>26.1 (2.8)</td>
<td>0.50</td>
</tr>
<tr>
<td>Ever smoking</td>
<td>n (%): 464 (88)</td>
<td>199 (88)</td>
<td>0.99</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>mean: (SD): 135 (19)</td>
<td>136 (18)</td>
<td>0.28</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>mean (SD): 81 (10)</td>
<td>82 (10)</td>
<td>0.78</td>
</tr>
<tr>
<td>History of MI</td>
<td>n (%): 255 (48)</td>
<td>106 (47)</td>
<td>0.71</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>mean (SD): 6.09 (0.85)</td>
<td>5.90 (0.90)</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>mean (SD): 0.93 (0.22)</td>
<td>0.90 (0.23)</td>
<td>0.09</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>mean (SD): 3.34 (0.78)</td>
<td>4.21 (0.80)</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>mean (SD): 1.65 (0.44)</td>
<td>1.59 (0.44)</td>
<td>0.31</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>n (%): 278 (53)</td>
<td>108 (48)</td>
<td>0.21</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>n (%): 57 (11)</td>
<td>20 (9)</td>
<td>0.41</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>n (%): 394 (56)</td>
<td>160 (71)</td>
<td>0.26</td>
</tr>
<tr>
<td>Aspirin</td>
<td>n (%): 304 (57)</td>
<td>122 (54)</td>
<td>0.63</td>
</tr>
<tr>
<td>MSD mm</td>
<td>mean (SD): 2.74 (0.37)</td>
<td>2.73 (0.39)</td>
<td>0.80</td>
</tr>
<tr>
<td>MOD mm</td>
<td>mean (SD): 1.76 (0.35)</td>
<td>1.76 (0.36)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

SD = standard deviation; BP = blood pressure; MI = myocardial infarction; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MSD = mean segment diameter; MOD = minimal obstruction diameter
Table 3. Change in angiographic parameters according to PIA genotype

<table>
<thead>
<tr>
<th>PIA1 / PIA1</th>
<th>PIA1 / PIA2 + PIA2 / PIA2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=527</td>
<td>n=226</td>
<td></td>
</tr>
<tr>
<td>MSD loss in mm (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo group:</td>
<td>0.10 (0.19)</td>
<td>0.12 (0.23)</td>
</tr>
<tr>
<td>Pravastatin group:</td>
<td>0.07 (0.19)</td>
<td>0.07 (0.17)</td>
</tr>
</tbody>
</table>

SD = standard deviation, MSD = mean segment diameter.

Baseline characteristics

Baseline characteristics of the patients group homozygous for the PIA1 allele and of patients carrying the PIA2 allele are shown in Table 2. Total cholesterol and HDL-cholesterol levels were higher in the PIA1 homozygous group than in the PIA2 allele carriers. Besides these, there were no other significant differences in baseline characteristics among patients within each genotype with regard to risk factors for CAD, medication or angiographic parameters. From the 855 patients of the REGRESS population DNA was available of 753 patients. The baseline characteristics and the parameters of disease progression in these 753 patients and in the 132 patient with no available DNA were comparable.

Table 4. Influence of aspirin on angiographic parameters according to PIA genotype

<table>
<thead>
<tr>
<th>MSD loss mm (SD)</th>
<th>n=327</th>
<th>n=426</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo group:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIA1 / PIA1</td>
<td>108</td>
<td>141</td>
<td>0.10 (0.20)</td>
</tr>
<tr>
<td>PIA1 / PIA2 + PIA2 / PIA2</td>
<td>58</td>
<td>60</td>
<td>0.17 (0.24)</td>
</tr>
<tr>
<td>Pravastatin group:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIA1 / PIA1</td>
<td>115</td>
<td>163</td>
<td>0.08 (0.20)</td>
</tr>
<tr>
<td>PIA1 / PIA2 + PIA2 / PIA2</td>
<td>46</td>
<td>62</td>
<td>0.05 (0.22)</td>
</tr>
</tbody>
</table>

SD = standard deviation, MSD = mean segment diameter, ASA = aspirin
Angiographic Assessment
Quantitative Coronary Angiography (QCA) was performed at baseline and after two years of follow up. At baseline MSD and MOD were similar in both genotypes (Table 2). Atherosclerosis progression, measured by QCA and expressed as a loss of MSD or MOD, was not related to PIA polymorphism, neither in the group of patients treated with placebo nor in the group treated with pravastatin (Table 3). In the pravastatin-treated group, the MSD loss was significantly less than in the placebo-treated group as described earlier.\textsuperscript{18} In the placebo-treated group, there was no difference in MSD loss in the patients homozygous for the PIA1 allele off or on ASA therapy. However, patients with a PIA2 allele on ASA therapy showed a significantly greater loss of MSD than patients with a PIA2 allele without ASA therapy (0.17 mm and 0.07 mm respectively, \(p=0.039\)). In the pravastatin-treated group there were no differences in MSD in the patients off or on ASA therapy according to PIA genotype (Table 4). There was no difference observed in MOD in the patients related to PIA genotype and ASA or pravastatin treatment.

At the end of the follow-up period, 65 of the 367 (18\%) placebo-treated and 41 of the 386 (11\%) pravastatin-treated patients in the study group had experienced a new cardiovascular event (\(p=0.008\)). This difference in cardiovascular event rate was not related to the PIA polymorphism. Influence of ASA treatment on clinical outcome could not be demonstrated in the different genotypes.

Discussion
Our results indicate that the PIA polymorphism of GP IIIa is not associated with the development of CAD nor with the progression of CAD as measured by QCA in our study population. Interestingly, the placebo treated patients with a PIA2 allele showed twice the rate of atherosclerosis progression on ASA therapy, as measured by QCA and expressed as a decrease in MSD, as patients not on ASA therapy (\(p=0.039\)). This subgroup of patients does apparently not benefit from ASA therapy and in the absence of lipid lowering medication shows clear progression. If these results are confirmed, PIA genotyping may select patients who are candidates for other antiplatelet treatment, if these are shown to be more affective than ASA. Weiss et al.\textsuperscript{8} showed in 1996 that the incidence of the PIA2 polymorphism was twice as high in CAD patients as in controls (39.4\% versus 19.1\%, \(p=0.01\)). Subsequently, larger studies have failed to confirm that the PIA2 allele carries an additional risk.\textsuperscript{11,15,20,21}
The results of our study are in contrast with the first reports on this issue. It should be noted, however, that studies observing differences tend to contain small numbers of patients and usually show a low prevalence of the allele involved.22,23 The most striking finding of our study is that the patients in the placebo group with a PIA2 allele on ASA therapy showed a higher rate of atherosclerosis progression, as compared to those not receiving ASA. However, we only found a significant difference for MSD and not for MOD. A possible explanation could be that MSD and MOD reflect two different aspects of progression of coronary atherosclerosis. Changes in MSD mainly reflect diffuse changes, whereas changes in MOD mainly reflect focal narrowing of atherosclerotic lesions, and the development of new lesions.

Although our observation is based on a relative small number of patients and therefore should be interpreted with caution, it suggests that platelet PIA2 polymorphism may have an important influence on the response to antiplatelet agents and the subsequent atherosclerotic process. Is there a plausible biological mechanism to explain this finding? Feng et al.16 demonstrated that the presence of one or two PIA2 alleles was associated with increased platelet aggregability as indicated by incrementally lower threshold concentrations for epinephrine and ADP. Since ASA only inhibits the arachidonic acid activation pathway of the GPIIb/IIIa receptor, this therapy could be insufficient when platelet hyperreactivity exists in the presence of a PIA2 allele. Although ASA has been shown to be beneficial in both primary and secondary prevention of CAD24,25 there is growing evidence of a substantial heterogeneity in the response of patients to ASA therapy.26-30 Our results might point to a useful marker to predict such a lack of response to therapy. It is conceivable that combination therapy of ASA with other antiplatelet drugs, such as clopidogrel, which blocks another (ADP) pathway of activation of the GPIIb/IIIa receptor, might improve the therapeutic benefit in such patients.

In our study, pravastatin-treated patients on ASA therapy did not exhibit increased atherosclerosis progression as was observed in the placebo-treated group. It is well known that platelets from patients with elevated LDL-cholesterol levels are more sensitive to aggregating agents than platelets from normocholesterolemic patients.31 Nurden hypothesized that these effects result from different cholesterol concentrations in the microenvironment of the cell membrane around the GPIIb/IIIa complex, which might alter receptor function.32 Furthermore, pravastatin is known to decrease platelet aggregability33 and to inhibit thrombus formation on an injured artery.34 The present study demonstrated that the increase in
atherosclerosis progression as observed in the placebo group of patients on ASA with a PIA2 allele could be counterbalanced by pravastatin therapy. This supports the study of Walter et al., who demonstrated that the increased restenosis rate in patients with a PIA2 allele could completely be reversed by statin therapy and supports the recent study of Bray et al., who observed in the CARE study population that the PIA1/A2 genotype was associated with an excess of recurrent coronary events in patients after MI, who did not receive pravastatin.

We realize that the aspirin treatment was not randomised in our study. Since ASA therapy was not yet consequently given to all CAD patients in the study period, we do not think that clinical criteria for not giving ASA were confounders. This study confirms the absence of an association of the platelet GP IIIa PIA polymorphism with CAD as seen in the larger studies and is the first report that indicates that the PIA polymorphism is not associated with progression of CAD. We do not think this is due to insufficient power. First, and foremost, the differences in mean MSD and MOD loss between the genotypes are small, both in treated and in untreated patients. Secondly, the sample sizes are such that there is 78% power or more for diameter differences between variants of 0.05 or more. Naturally, for smaller differences (≤0.04 mm) there is less power, but we consider such differences as clinically not relevant.

In our study the difference in cardiovascular event rate was not related to the PIA polymorphism. Influence of ASA treatment on clinical outcome could not be demonstrated in the different genotypes. However it should be remembered that the REGRESS trial was not designed nor powered to evaluate clinical events occurrence.

Conclusions

The platelet glycoprotein (GP) IIIa PIA variant is associated with platelet dysfunction. The clinical relevance in coronary artery disease (CAD) is, however, debated. In this study platelet GP IIIa PIA1/A2 genotypes proved not to be associated with CAD or the progression of CAD. A subgroup of CAD patients on ASA therapy who carried the PIA2 allele, however, showed a significantly greater loss of mean segment diameter of the coronary arteries after two years than patients not on ASA therapy. This indicates that patients carrying the PIA2 allele may not benefit from ASA therapy alone and should possibly be prescribed different antiplatelet medication. Before our findings could have an impact on therapeutic decisions, they should be verified and extended in larger, prospective studies.
Acknowledgements

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