PIA polymorphism of the glycoprotein IIIa and efficacy of reperfusion therapy in patients with acute myocardial infarction

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Summary
The PIA polymorphism of the platelet glycoprotein IIIa gene is associated with altered platelet function and response to antiplatelet drugs. We sought to assess whether the PIA polymorphism influences myocardial salvage achieved by reperfusion therapy in patients with acute myocardial infarction. We analyzed 292 patients enrolled in 2 randomized trials that compared stenting plus abciximab with thrombolysis (alteplase alone or alteplase plus abciximab) in acute myocardial infarction. Patients were genotyped for the PIA polymorphism using polymerase chain reaction with fluorogenic probes. Technetium-99m sestamibi was injected before and 1-2 weeks after reperfusion treatment. The scintigrams enabled the calculation of the initial perfusion defect, final infarct size, and the proportion of initial defect salvaged by reperfusion (salvage index). Clinical follow-up was done up to 18 months after primary treatment. The genotype distribution was as follows: PIA2/A2 in 3.4%, PIA1/A2 in 24.7% and PIA1/A1 in 71.9% of patients. There were no significant differences between PIA2 allele carriers and PIA1/A1 patients in salvage index (0.46±0.50 vs. 0.41±0.43, respectively, P=0.48), final infarct size (16.8±20.8% vs. 18.4±19.1% of left ventricle, respectively, P=0.46) as well as 18-month mortality (8.5% vs. 7.1%, respectively, P=0.69). The lack of relationship between PIA2 allele and myocardial salvage was observed for both reperfusion strategies, stenting and thrombolysis. Thus, these findings show that the functional PIA polymorphism of platelet glycoprotein IIIa has no influence on the degree of myocardial salvage achieved by reperfusion therapies in patients with acute myocardial infarction.

Keywords
PIA polymorphism, glycoprotein IIIa, platelets, acute myocardial infarction, reperfusion

Introduction
The success of reperfusion therapy in acute myocardial infarction (AMI) depends not only on the rapid and sustained restoration of blood flow in the epicardial artery but also on the achievement of the adequate perfusion in coronary microcirculation (1, 2). The failure to achieve a sufficient blood supply at this level has been associated with negative outcomes (greater infarct size, poor left ventricular function and higher mortality). despite the presence of blood flow in the epicardial infarct-related artery (3-5). One of the reasons for microcirculatory dysfunction after reperfusion is considered to be the obstruction of distal small vessels due to formation of platelet aggregates (6). Systemic activation of platelets has been found to occur after reperfusion treatment, which may limit its efficacy (7). The PIA polymorphism of the platelet fibrinogen receptor has been found to be associated with premature myocardial infarction and clinical thrombotic events after coronary interventions (8-
11), possibly caused by impaired platelet function (12) and enhanced thrombin formation (13). Thus, it could be suspected that PI^A^ polymorphism may influence myocardial salvage - the key result of reperfusion therapy. In this study, we investigated the relationship between the PI^A^ polymorphism and myocardial salvage after reperfusion therapy in patients with AMI.

**Methods**

Stent versus Thrombolysis for Occluded Coronary Arteries in Patients with Acute Myocardial Infarction (STOPAMI) 1 and 2 were two randomized trials that compared stenting with thrombolysis in 302 consecutive patients with AMI within the first 12 hours from symptom onset (14, 15). The details of inclusion criteria and reperfusion regimen have been published previously (14, 15). In brief, stenting was always combined with abciximab (152 patients) and thrombolysis consisted in either full-dose alteplase alone (67 patients) or half-dose alteplase plus abciximab (60 patients) (14, 15). A 6-month visit to the outpatient clinic and an 18-month phone contact were carried out in all patients. All patients had given written informed consent for the intervention and PI^A^ genotype determination. The study protocol conformed to the Declaration of Helsinki and was approved by the institutional ethics committee.

**Determination of the PI^A^ genotype**

Genomic DNA was extracted from 200 µl of peripheral blood leukocytes with the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) or the High Pure PCR Template Preparation Kit (Roche Applied Science Diagnostics, Mannheim, Germany). DNA was available in 292 patients who constitute the present study population. Genotype analysis was performed with allele-specific fluorogenic oligonucleotide probes in an assay combining the polymerase chain reaction (PCR) and the 5' nuclease reaction (TaqMan technique; Applied Biosystems, Darmstadt, Germany) (16). Primers and probes were established with the Primer Express software (version 2.0.0; Applied Biosystems) after download of the DNA sequence deposited under accession number U03881 (glycoprotein [GP] IIIa exon 2) in the GenBank or EMBL database. Primers 5' TTC TCT TTG GGC TCC TGT CCT ACA 3' and 5' ACA GTT ATC CTT CAG CAG ATT CTC ATT 3' were used to amplify a 85-bp portion of exon 2 containing the polymorphic site. The sequence of the PI^A1^-specific probe was 5' FAM-CCT GCC TCT GGG CTC ACC TC 3', and the sequence of the PI^A2^-specific probe was 5' VIC-TGC CTC GGG GCT CAC CTC G 3'. FAM, i.e. 6-carboxy-fluorescein, and VIC (proprietary dye of Applied Biosystems) were the fluorogenic dyes used to accomplish allelic discrimination. The two-step thermocycling procedure consisted of 40 cycles of denaturation at 95°C for 15 sec and primer annealing and extension at 60°C for 1 min. As a control, genotyping was repeated for 20% of the samples using DNA prepared separately from the original blood sample. The operators who performed the PI^A^ genotype determination were unaware of the patients’ clinical and angiographic characteristics.

**Scintigraphic evaluation**

All patients received an intravenous injection of 27 mCi (1000 MBq) of technecium Tc 99m sestamibi immediately after randomization. Single-photon-emission computed tomography was done within 6-8 hours of injection of the radionuclide to calculate the initial perfusion defect representing the area at risk. A second scintigraphic study was performed 7-14 days after primary treatment for the calculation of the final infarct size. Salvage index was calculated as the proportion of the initial area at risk salvaged by reperfusion. Paired scintigraphic studies necessary for the calculation of salvage index were available in 260 of the 292 patients (89 %) included in this study.

The table below shows the baseline characteristics according to the PI^A2^- allele presence.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PI^A2^- allele carries</th>
<th>PI^A1/A1^ genotype patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62.1±12.7</td>
<td>60.6±12.8</td>
<td>0.37</td>
</tr>
<tr>
<td>Female</td>
<td>27 (32.9)</td>
<td>46 (21.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Current smoker</td>
<td>37 (45.0)</td>
<td>106 (50.0)</td>
<td>0.41</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>16 (19.5)</td>
<td>40 (19.0)</td>
<td>0.93</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>58 (70.7)</td>
<td>148 (70.5)</td>
<td>0.97</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>53 (64.6)</td>
<td>132 (62.9)</td>
<td>0.78</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>7 (8.5)</td>
<td>29 (13.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>Previous bypass surgery</td>
<td>2 (2.4)</td>
<td>10 (4.8)</td>
<td>0.37</td>
</tr>
<tr>
<td>Killip class &gt;2</td>
<td>5 (6.1)</td>
<td>11 (5.2)</td>
<td>0.97</td>
</tr>
<tr>
<td>Anterior infarction</td>
<td>44 (54.0)</td>
<td>94 (45.0)</td>
<td>0.17</td>
</tr>
<tr>
<td>Assigned treatment strategy</td>
<td>44 (53.7)</td>
<td>108 (51.4)</td>
<td>0.73</td>
</tr>
<tr>
<td>Stenting</td>
<td>38 (46.3)</td>
<td>102 (48.6)</td>
<td></td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>274.3±183.3</td>
<td>249.9±154.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Time-to-treatment interval, min</td>
<td>31.0±20.8</td>
<td>31.2±19.7</td>
<td>0.94</td>
</tr>
</tbody>
</table>

1Data presented are mean ± SD or number of patients (%). LV indicates left ventricle.
Gorchakova, et al.: PI^A polymorphism and reperfusion efficacy in AMI

**Statistical analysis**
The data are presented as mean ± SD, counts or percentages. The analysis consisted in the comparison between PI^A2 allele carriers and PI^A1 homozygotes. For the comparison of categorical variables we used the two-sided $\chi^2$ or Fisher’s exact test (in the presence of expected cell values <5). Student’s t-test was used for the comparison of continues variables. P values < 0.05 were considered statistically significant.

**Results**

**Baseline characteristic of patients**
All patients were Caucasians and 261 (89.4%) of them were Germans. The PI^A genotype was PI^A1/A1 in 210 (71.9%), PI^A1/A2 in 72 (24.7%) and PI^A2/A2 in 10 (3.4%) patients. Thus, the frequency of the PI^A2 allele was 15.7%. Main baseline characteristics of the patients are listed in Table 1. There were no significant differences between the 2 groups with respect to baseline characteristics except for the proportion of women, which was higher in the group of PI^A2 allele carriers (32.9% vs. 21.9%; P=0.05).

**PI^A genotype, myocardial salvage and clinical outcome**
There were no significant differences between PI^A2 allele carriers and PI^A1/A1 patients with respect to salvage index (0.46±0.50 vs. 0.41±0.43, P=0.48). More specifically, it was 0.40±0.30 in PI^A2/A2 patients, 0.47±0.53 in PI^A1/A2 patients and 0.41±0.43 in PI^A1/A1 patients (P=0.72). The final infarct size was 16.8±20.8% of the left ventricle among PI^A2 allele carriers and 18.4±19.1% of the left ventricle among PI^A1/A1 patients (P=0.46). At 18 months, the mortality rates were similar between the two groups of patients (8.5% for patients carrying the PI^A2 allele and 7.1% for patients of the PI^A1/A1 genotype, P=0.69).

**PI^A genotype, reperfusion strategy and myocardial salvage**
There were no significant differences regarding the reperfusion strategy assigned to the PI^A2 allele carriers and PI^A1/A1 patients (Table 1). No association was seen between PI^A2 allele presence and myocardial salvage in both stenting and thrombolysis groups (Fig. 1). In the group of stenting, salvage index was 0.57±0.33 for patients carrying the PI^A2 allele and 0.54±0.27 for patients carrying the PI^A1/A1 genotype (P=0.49). In the group of thrombolysis, salvage index was 0.32±0.62 for patients carrying the PI^A2 allele and 0.29±0.52 for patients carrying the PI^A1/A1 genotype (P=0.76, Fig. 1).

**Discussion**
Reperfusion therapy is the recommended strategy in most of the patients with AMI (17). Myocardial salvage is the key mechanism of benefit from reperfusion therapy in these patients. Although restoration of anterograde blood flow in the infarct-related artery is of primary importance in salvaging ischemic myocardium, an increasing role in the success of reperfusion therapy has been ascribed to the integrity of microcirculation (1, 2). Microvascular dysfunction is considered to result from both ischemic injury and atheroembolism, which develop in the first 1 to 2 hours of AMI, and reperfusion injury, which initiates after the restoration of anterograde blood flow (18). Embolization of platelet aggregates into the peripheral vascular bed represents a major mechanism of microvascular dysfunction (6). In an in vivo model of experimental ischemia/reperfusion, thrombocytes were found to adhere on endothelium and aggregate in capillaries, thus reducing the normal flow (19, 20). The microvascular dysfunction has in part been explained by enhanced accumulation of fibrinogen on endothelium surface (19) and subsequent formation of fibrinogen bridging between endothelium and platelets. Both thrombolysis and primary coronary interventions may lead to platelet activation and increased density of GP IIb/IIIa (α_{IIb}β_{3}) receptors in patients with AMI (7, 21-23).
Platelets obtained from patients with AMI after reperfusion show an enhanced adhesion to the intact endothelium (24). This interaction is mediated by vitronectin ($\alpha_\beta$) and fibrinogen receptors, both of which have the GP IIIa subunit ($\beta_3$-integrin) in their structure (24). Therefore, the GP IIb/IIIa receptor is required for platelet adhesion to endothelium as well as for platelet aggregation and its modifications may impact on the flow in the microvascular bed.

The PI A polymorphism of the GP IIIa gene leads to the substitution of a leucine for a proline at the 33 residue of the protein amino acid sequence and induce conformational changes within the GP IIb/IIIa receptor (25). There is evidence of a pro-thrombotic effect of this polymorphism. Significantly more PI A2-positive cells bind to immobilized fibrinogen than did PI A1-positive cells (26). PI A2 cells demonstrated increased adhesive functions compared with their PI A1 counterparts (26). Platelets from patients carrying the PI A2 allele showed an increased tendency to bind fibrinogen (27). Compared to PI A1 homozygotes, PI A2 carriers showed increased platelet reactivity as assessed by adrenalin-induced platelet aggregation in 1422 subjects from the Framingham Offspring Study (12). Undas and colleagues found enhanced thrombin generation and altered sensitivity to aspirin inhibition in healthy PI A2 carriers (13, 28).

In accordance with the functional findings related to PI A polymorphism, several clinical studies have provided data in support of the clinical relevance of this polymorphism. The presence of the PI A2 allele has been found to correlate with an increased risk for myocardial infarction in some (10, 11) but not all studies (29), for recurrent events after myocardial infarction (30), and restenosis (8) after coronary artery stenting, as well as for more unfavorable outcome after aorto-coronary bypass surgery (31).

Although no concordant data exist on the relation between this polymorphism and thrombotic complications after percutaneous coronary interventions (9, 32, 33), an overview of the published findings pointed out to the prevalence of the negative impact of the PI A2 allele (34). In addition, there was a trend to more adverse events among PI A2 allele carriers in a study including 106 young patients with AMI (35). There are also data in support of interactions between this polymorphism and drugs used in clinical practice. Walter and colleagues found that the use of statins might eliminate the excess risk of in-stent restenosis in PI A2 patients (36). On the other side, platelets from PI A1/A2 patients undergoing coronary angioplasty were less completely inhibited after abciximab therapy than platelets from PI A1/A1 homozygotes suggesting a relative resistance to the therapy associated with PI A2 carriage (37).

In patients with AMI, we assessed whether the PI A allele of the GPIIIa gene has an influence on myocardial salvage achieved with interventional or thrombolytic reperfusion therapies. Both therapies represent current mainstays of the primary treatment of patients with AMI. The patients included in this study represent the largest series of patients with AMI evaluated so far for the clinical significance of the PI A polymorphism. They showed a PI A2 allele frequency similar to that usually reported in Europe (38-40) and United States (29). Despite the considerable total size of the study population, the number of PI A2/A2 patients is small due to the known rarity of this genetic variant; this limits the ability of our study to offer meaningful information on a subset of patients found to carry a particularly high risk (9).

The amount of data in favor of a relevant role of the PI A polymorphism in platelet function and of the platelets in the microcirculatory integrity after the use of reperfusion therapy was the basis for the hypothesis evaluated in the present study. The identification of possible interferences of this genetic variant with the effectiveness of the primary treatment in patients with AMI would have important implications for the clinical practice. The major findings of this study are that in the current clinical and therapeutical settings of AMI, the PI A polymorphism has no measurable influence on the efficacy of reperfusion treatment and that the lack of influence is not dependent on the type of reperfusion therapy applied. More than a negation of the role of this polymorphism, these findings should be interpreted as reflecting the complexity of the mechanisms involved in microcirculatory recovery and/or the contribution of current antiplatelet drugs used as an adjunct therapy in our patient population. Aspirin, thienopyridines and GPIIb/IIIa inhibitors used in most of the patients may have prevented the potential negative influence of the PI A2 allele. The present findings suggest, however, that genotyping for the PI A polymorphism does not actually provide relevant predictive information on the success of current reperfusion therapies in patients with AMI.

References