Defective platelet response to arachidonic acid and thromboxane A$_2$ in subjects with Pl$^{A2}$ polymorphism of $\beta_3$ subunit (glycoprotein IIIa)

GIUSEPPE ANDRIOLI, PIETRO MINUZ, PIETRO SOLORO, SILVER PINCELLI, RICCARDO ORTOLANI, SABRINA LUSSIGNOLI AND PAOLO BELLAVITE

Department of Morphological and Biomedical Sciences, Department of Biomedical and Surgical Sciences, and Department of Pathology, University of Verona, Verona, Italy

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Summary. The membrane complex $\alpha_{IIb}\beta_3$ is the major receptor for fibrinogen and is involved in platelet adhesion and aggregation. Evidence has been presented that the Pl$^{A2}$ allele of the $\beta_3$ Pl$^{A2}$/Pl$^{A2}$ gene polymorphism might be an independent risk factor for coronary thrombosis, but the matter is still controversial. We investigated the relationship between this polymorphism and possible alterations of platelet functions in vitro. The platelet adhesion to fibrinogen-coated microplate wells and the aggregation induced by several different agonists were tested in 63 healthy volunteers, among them, 49 subjects with Pl$^{A1}$/Pl$^{A1}$ polymorphism, 12 subjects with Pl$^{A1}$/Pl$^{A2}$ polymorphism and two subjects with Pl$^{A2}$/Pl$^{A2}$ polymorphism. Subjects with Pl$^{A1}$/Pl$^{A2}$ polymorphism or with Pl$^{A2}$/Pl$^{A2}$ polymorphism showed significantly lower platelet responses as compared with Pl$^{A1}$/Pl$^{A1}$ subjects when either arachidonic acid or the thromboxane A$_2$ analogue, U46619, were used as agonists. In resting condition and after thrombin or ADP stimulation, platelet function was normal in all the subjects. An increased sensitivity to the anti-aggregatory effect of acetylsalicylic acid was observed in platelets from subjects with the Pl$^{A2}$ allele. Finally, using a flow-cytometric evaluation and determining the $\beta$-thromboglobulin plasma levels, we did not find any evidence of a Pl$^{A2}$ platelet hyperreactivity ex vivo. Our findings are not consistent with the hypothesis that the purported increase of cardiovascular risk in these subjects may be as a result of platelet hyperactivation. On the contrary, the Pl$^{A2}$ allele is associated with a platelet functional deficiency, specifically linked to the activation of the fibrinogen receptor by thromboxane A$_2$.

Keywords: platelet, Pl$^{A1}$/Pl$^{A2}$ polymorphism, integrins, acetylsalicylic acid, thrombosis.

The membrane integrin complex $\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa) is a major receptor for platelet adhesion and aggregation. The gene encoding the subunit $\beta_3$ is polymorphic at exon 2 (position 1565). The more common allele encodes a leucine (Pl$^{A1}$) and the less common allele encodes a proline (Pl$^{A2}$). A high frequency of family members homozygous for Pl$^{A2}$ was found in kindreds with a high prevalence of acute coronary diseases under 60 years of age (Weiss et al., 1995). The same authors later reported an association between acute coronary thrombosis and Pl$^{A2}$ polymorphism (Weiss et al., 1996). These papers generated great interest and have given rise to various reports on this and related matters, some of which support the original findings (Carter et al., 1997; Walter et al., 1997; Zotz et al., 1998) and some of which do not (Hato et al., 1997; Herrmann et al., 1997; Ridker et al., 1997; Samani & Lodwick, 1997; Durante-Mangoni et al., 1998; Garg et al., 1998; Mamotte et al., 1998; Scaglione et al., 1998; Sperr et al., 1998; Laule et al., 1999). Recently, Gardemann et al. (1998) established an association between Pl$^{A2}$ polymorphism and coronary disease in a large population. However, they did not find a relationship between this genotype and myocardial infarction. This polymorphism was also investigated in relation to stroke and conflicting results have also been reported (Carlsson et al., 1997; Carter et al., 1998; Wagner et al., 1998).

Even though there is strong evidence that the complex $\alpha_{IIb}\beta_3$ has a primary role in the pathogenesis of acute coronary syndromes (Kleiman et al., 1998), the role of the Pl$^{A2}$ polymorphism as a thrombotic risk factor is undefined and the mechanism(s) whereby this polymorphism could lead to an increased risk of thrombosis is unknown. To date,
there is little evidence of an association of Pl\textsuperscript{A2} with molecular markers of abnormal haemostasis: an association between this polymorphism and higher plasma Lp(a) concentration (Joven et al., 1998), and a reduced plasma fibrinogen concentration (Senti et al., 1998) compared with Pl\textsuperscript{A1} subjects have been reported. This may indicate that this polymorphism plays a role in platelet post-activation events, possibly leading to increased binding of fibrinogen (Goodall et al., 1999). However, this hypothesis appears to be negated by the finding of no effect on fibrinogen binding (Meiklejohn et al., 1997) or decreased fibrinogen binding to Pl\textsuperscript{A2} platelets (Weiss et al., 1997). Investigations of the possible effects of the Pl\textsuperscript{A1/A2} polymorphism on human platelet function in vitro have reported contradictory results: Feng et al. (1999) described an increased aggregability of the Pl\textsuperscript{A2} platelets to epinephrine and ADP whereas Corral et al. (1997) reported no difference in platelet aggregation between Pl\textsuperscript{A1} and Pl\textsuperscript{A2} genotypes, and Lasne et al. (1997) described a hypo-aggregability of Pl\textsuperscript{A2} platelets, compared with Pl\textsuperscript{A2} platelets, to thrombin and ADP.

No data have been published on the possible effect of Pl\textsuperscript{A1/A2} genotypes on platelet adhesion, one of the earlier events by which platelets perform their functions in the haemostatic process. Therefore, further investigations are required to investigate the relationship between this polymorphism of the fibrinogen receptor and the various functions of platelets that can be assessed in vitro.

The objective of the present report was to evaluate possible modifications of platelet adhesion to fibrinogen and of aggregation, using stimulants that act through different activation pathways, in subjects with different Pl\textsuperscript{A1} alleles. The starting hypothesis was that the presence of the Pl\textsuperscript{A2} allele was associated with a tendency to higher basal (resting) adhesion and/or higher responsiveness to agonists. We have tested this hypothesis in a well defined group of healthy volunteers.

PATIENTS AND METHODS

Subjects. A total of 63 healthy volunteers (35 men and 28 women), all non-smokers, aged from 25 years to 62 years, were studied. The participants had not taken any drugs for at least 3 weeks and were carefully interviewed at the time they donated blood to exclude known risk factors affecting platelet function and/or platelet dysfunction. All volunteers participating in this study had given their informed, written consent, according to Italian law. Blood samples were collected after overnight fasting and without venous occlusion, to avoid stasis and anoxia.

For the purpose of the Pl\textsuperscript{A} genotyping, 4 ml of venous blood was collected into EDTA-containing vacutainer tubes. DNA was extracted from whole blood using a standard extraction protocol (Sambrook et al., 1989). Pl\textsuperscript{A} genotypes were determined as previously described (Weiss et al., 1996). The sense oligonucleotide primer was 5'-TGGACTTCTTTTGCTGGCTGATTAC-3' and the antisense primer was 5'-CGATGGATCCGGGCAGCATATC-3' (M-Medical SrI) (Osborn et al., 1996). Template DNA was amplified in a final volume of 100 \( \mu l \) containing 20 pmol of each primer, 200 pmol/l of each dNTP, 2 U of Taq polymerase (Bioline), 50 mmol/l KCl, 1.5 mmol/l MgCl\(_2\) and 10 mmol/l Tris-HCl (pH 8.3). After initial denaturation at 95\(^\circ\)C for 2 min, amplification of DNA was performed in a thermal cycler (Perkin Elmer Cetus) for 39 cycles (denaturation: 2 min at 95\(^\circ\)C; annealing: 1 min at 58\(^\circ\)C; extension: 2 min at 72\(^\circ\)C). Amplified DNA was digested by 20 U MspI (16 h at 37\(^\circ\)C). The digested products of the Pl\textsuperscript{A1} and Pl\textsuperscript{A2} alleles were electrophoresed in 4% agarose gels and visualized by ethidium bromide staining. As the negative internal control, the polymerase chain reaction (PCR) procedure was also performed utilizing amplification products in samples containing no genomic DNA. The results of the genotyping assay were analysed by at least two investigators who were unaware of the origin of the DNA. Whenever there was any ambiguity in PCR, MspI digestion and scoring were repeated.

Platelet isolation and in vitro functional testing. For the aggregation and adhesion tests, platelets were harvested from blood by differential centrifugation (Andrioli et al., 1999) and utilized within 1 h. A final volume of 20 ml of blood was drawn by venepuncture in 3:32 ml of anticoagulant solution (11.8 mmol/l citric acid, 18 mmol/l dextrose, 14 mmol/l sodium citrate) and platelet-rich plasma was obtained by centrifugation at 300 g for 10 min. The platelet-rich plasma was recentrifuged at 700 g for 15 min and platelets were gently suspended (2 \( \times \) 10\(^8\) platelets/ml for aggregation test and 5 \( \times \) 10\(^7\) platelets/ml for adhesion test) in a buffer composed of 145 mmol/l NaCl, 5 mmol/l KCl, 10 mmol/l HEPES, 0.5 mmol/l Na\(_2\)HPO\(_4\), 6 mmol/l glucose and 0.2% human serum albumin, pH 7.4 (buffer A). The platelet suspensions were kept at room temperature and utilized within 1 h. Ten minutes before use, platelets were warmed to 37\(^\circ\)C.

A colorimetric procedure measuring the activity of acid phosphatase was used for the determination of the adhesion of human platelets to fibrinogen-coated culture microplates (Bellavite et al., 1994). Briefly, 96-well microtitre plates were coated overnight with 0.2 mg/ml human fibrinogen (Sigma Chemical Company) in phosphate-buffered saline (PBS) and washed twice with physiological saline. Immediately after coating and washing, the wells were supplemented with 25 \( \mu l \) of buffer A containing 3 mmol/l CaCl\(_2\) and 3 mmol/l of MgSO\(_4\) (for assay of unstimulated cells) or with 25 \( \mu l \) of the tested agonists (final concentrations: 3 mmol/l ADP, 0.02 U/ml thrombin, 5 mmol/l arachidonic acid, 100 mmol/l U46619, a thromboxane A\(_2\) analogue) in buffer A containing 3 mmol/l CaCl\(_2\) and 3 mmol/l MgSO\(_4\). Plates were then warmed to 37\(^\circ\)C and 50 \( \mu l \) of the platelet suspension (2 \( \times \) 10\(^8\) platelets) were added to each well. The incubation was carried out for 60 min and plates were then subjected to two washing cycles with PBS at room temperature. After washing, the wells containing adherent platelets were rapidly supplemented with 150 \( \mu l \) of 100 mmol/l citrate buffer, pH 5.4, containing 5 mmol/l \( p \)-nitrophenyl-phosphate and 0.1% Triton X-100. After incubation at room temperature for 60 min, the reaction was stopped and the colour was developed by the addition of 100 \( \mu l \) of 2 N NaOH. The
p-nitrophenol produced by the reaction was measured with a microplate reader (Reader 400, SLT Labs Instruments) at 405 nm against a platelet-free blank. The percentage of adherent cells was calculated on the basis of a standard curve obtained with a defined number of platelets from the same donor.

The rate of platelet aggregation was measured according to Born (1962) by using a four-channel aggregometer (Aggrecorder II PA-3220, Daiichi). Suspensions of washed platelets (0.5 ml, 108 platelets) were warmed in the (Aggrecorder II P A-3220, Daiichi). Suspensions of washed to Born (1962) by using a four-channel aggregometer same donor.

Curve obtained with a defined number of platelets from the adherent cells was calculated on the basis of a standard a microplate reader (Reader 400, SLT Labs Instruments) at p

Table I. General characteristics of the studied subjects. Data are means ± SD (ranges for age only).

<table>
<thead>
<tr>
<th></th>
<th>$\text{Pl}^{A1/A1}$</th>
<th>$\text{Pl}^{A1/A2}$ and $\text{Pl}^{A2/A2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>26/23</td>
<td>11/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46.6 ± 4.8 (25–62)</td>
<td>48.2 ± 5.3 (27–59)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7 ± 1.1</td>
<td>24.1 ± 1.0</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>119.7 ± 7.9/71.5 ± 6.5</td>
<td>122.4 ± 8.3/74.6 ± 5.0</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.02 ± 0.35</td>
<td>4.21 ± 0.29</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.72 ± 0.12</td>
<td>4.63 ± 0.16</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.01 ± 0.21</td>
<td>1.08 ± 0.26</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.22 ± 0.31</td>
<td>3.13 ± 0.27</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.54 ± 0.30</td>
<td>1.48 ± 0.24</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.78 ± 0.39</td>
<td>3.01 ± 0.51</td>
</tr>
<tr>
<td>Platelet count (x 10⁹/l)</td>
<td>237 ± 40</td>
<td>248 ± 31</td>
</tr>
</tbody>
</table>

RESULTS

Population characteristics

Sixty-three subjects, 35 men and 28 women, with a mean age of 47 years (range 25–62 years), were studied. In this group, we found 49 subjects with $\text{Pl}^{A1/A1}$ polymorphism (77.8%), 12 subjects with $\text{Pl}^{A1/A2}$ polymorphism (19%) and two subjects with $\text{Pl}^{A2/A2}$ polymorphism (3.2%). Owing to the small number of $\text{Pl}^{A2/A2}$ subjects, they were grouped together with the $\text{Pl}^{A1/A2}$ subjects and all these 14 subjects are referred to as $\text{Pl}^{A2}$ subjects. The most relevant characteristics of the volunteers and their plasma parameters are summarized in Table I. As shown, no significant differences were observed between the $\text{Pl}^{A1}$ and $\text{Pl}^{A2}$ subjects. Metabolic indices and body mass were remarkably similar in the two groups. There was a difference in sex distribution. However, platelet activation did not differ
between men and women in both groups. The clinical interviews and these baseline parameters excluded the presence in our studied subjects of the most known cardiovascular risk factors (smoking, hypertension, diabetes, hyperlipidaemia, increased plasma fibrinogen levels) associated with modified platelet activity.

**Platelet adhesion and aggregation**

The platelet adhesion on fibrinogen in the resting state and after different stimuli was tested *in vitro* under static conditions. We found no differences between the Pt^A1^ and the Pt^A2^ genotypes in resting adhesion and after 3 μmol/l ADP or 0.02 U/ml thrombin stimulation (Table II). On the other hand, using 5 μmol/l arachidonic acid or 100 nmol/l U46619 as agonists, the Pt^A2^ platelets showed a significantly reduced adhesion compared with the Pt^A1^ platelets.

**Platelet aggregation**

The platelet aggregation (Table II) was also performed with different stimuli: 0.06 U/ml thrombin, 10 μmol ADP, 15 μmol arachidonic acid and 1 μmol U46619. No differences were found between Pt^A1^ and Pt^A2^ platelet aggregation using thrombin or ADP as stimulants. Pt^A2^ platelets aggregated with a significantly reduced rate when compared with Pt^A1^ platelets using arachidonic acid or U46619.

The results of aggregation tests in response to arachidonic acid and to U46619 performed in each donor are reported in Fig 1. Two Pt^A2^ subjects were non-responders to arachidonic acid and to U46619, and four Pt^A2^ subjects were markedly low-responders (< 30% aggregation). There was a good correspondence in the aggregation responses to the two different agonists in the different individuals, with the exception of one Pt^A1/A1^ subject, whose platelets showed

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**Table II.** Pt^A1^ and Pt^A2^ platelet adhesion to fibrinogen and aggregation induced by various stimuli. Data are expressed as means ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Resting</th>
<th>Thrombin</th>
<th>ADP</th>
<th>Arachidonic acid</th>
<th>U46619</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt^A1^</td>
<td>4.9 ± 1.6</td>
<td>20.0 ± 3.8</td>
<td>16.1 ± 1.9</td>
<td>18.5 ± 1.4</td>
<td>18.3 ± 1.7</td>
</tr>
<tr>
<td>Pt^A2^</td>
<td>4.8 ± 1.8</td>
<td>18.8 ± 1.8</td>
<td>16.0 ± 1.4</td>
<td>13.5 ± 2.9</td>
<td>16.0 ± 1.2</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt; 0.003</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Aggregation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt^A1^</td>
<td>–</td>
<td>76.3 ± 3.4</td>
<td>56.6 ± 8.3</td>
<td>65.2 ± 12.7</td>
<td>68.5 ± 9.4</td>
</tr>
<tr>
<td>Pt^A2^</td>
<td>–</td>
<td>77.4 ± 2.6</td>
<td>57.2 ± 5.0</td>
<td>28.4 ± 17.2</td>
<td>37.9 ± 23.1</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

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**Fig 1.** Distributions of Pt^A1^ (○) and Pt^A2^ (●) platelet aggregation to arachidonic acid and U46619. Each Pt^A2^ subject is identified by their identification number, in order to compare the response to the two agonists. Subjects numbers 12 and 61 were homozygous for the Pt^A2^ trait.

**Fig 2.** Effects of acetylsalicylic acid on arachidonic acid-induced platelet aggregation. After the incubation for 15 min of the platelet suspensions with scalar doses of acetylsalicylic acid, the aggregation test was performed as described in the Patients and methods section, using 15 μmol/l arachidonic acid. This series of assays included eight randomly chosen Pt^A2^ subjects (○) and the eight Pt^A1^ subjects (●) who showed normal or borderline responses to arachidonic acid in the absence of acetylsalicylic acid (subjects 4, 18, 21, 26, 28, 35, 37, 48 in Fig 1). Data are expressed as means ± SD.
a very low response to arachidonic acid (12%) and a normal response to U46619. Subjects numbers 12 and 61 were homozygous for the PlA2 allele; probably because of the small number of subjects, this analysis did not discriminate PlA1/A2 from PlA2/A2 platelets. Figure 1 also shows that six out of seven subjects with markedly defective aggregation by arachidonic acid and six out of six subjects with markedly defective aggregation by U46619 belonged to the PlA2 group.

Sensitivity of PlA1 and PlA2 platelets to acetylsalicylic acid

To investigate the contribution of the endogenous generation of thromboxane A2 in subjects with different PlA1 and PlA2 polymorphisms, platelet aggregation in response to arachidonic acid was tested after incubation with increasing doses of acetylsalicylic acid. For technical reasons, this comparison could be performed only in PlA1 and PlA2 subjects that showed normal or near normal aggregation. Therefore, the six PlA2 subjects with markedly reduced (< 30%) aggregation responses were excluded from this series of experiments. Dose–effect curves (Fig 2) showed that PlA2 platelets are significantly more sensitive to inhibition by acetylsalicylic acid than PlA1 platelets. The concentration of the drug able to reduce the aggregation response by 50% was 2.7 ± 0.6 μmol/l and 23.4 ± 3.3 μmol/l for PlA2 and PlA1 platelets respectively (P < 0.005).

Flow cytometric platelet evaluation and β-thromboglobulin determination

To evaluate a possible different activated state of PlA2 platelets with respect to PlA1 platelets ex vivo, we studied the CD41 and CD62P expression on platelet membranes and determined the plasma β-thromboglobulin levels of PlA2 vs. PlA1 groups. The expression of the selected platelet antigens did not differ significantly in the two groups (% of CD41+ platelets: PlA1 68 ± 4, PlA2 70 ± 5, NS; % of CD62P+ platelets: PlA1 4 ± 1%, PlA2 5 ± 2, NS). There was also no difference between the plasma β-thromboglobulin levels of the two groups (PlA1 26.5 ± 1.8 ng/ml, PlA2 subject 29.2 ± 2.2 ng/ml, P NS).

DISCUSSION

Integrins containing a β1 subunit include αIIbβ3, the most abundant and functionally important integrin on platelets, and αIbβ1, which is prominently expressed on endothelial cells, monocytes/macrophages and vascular smooth muscle cells. The glycoprotein αIIbβ3 is the major fibrinogen receptor of the human platelet membrane and is activated after cell stimulation by a number of agonists, thus participating in adhesion and aggregation. The interest in this protein in clinical pathology was further increased when an association between the myocardial infarction with the PlA2 allele of the PlA1/A2 β3 gene polymorphism was reported (Weiss et al. 1996). However, the relationship between this molecular variance, cellular functions and pathological effects remains to be clarified.

The design of this in vitro study did not enable us to define whether or not the PlA2 genotype is clinically relevant, because all the subjects of the population under study were of healthy status. However, our data may allow better understanding of the molecular pathophysiology of cardiovascular risk in relation to the PlA1 phenotype.

In the present study, we analysed whether β3 genotypes have any association with two major platelet functions, i.e. adhesive and aggregative reactions. Using two different tests of platelet function and four different agonists, we provided the new finding that an abnormal platelet response to thromboxane A2 (either exogenously or endogenously generated from arachidonic acid) exists in PlA2 subjects. Thus, not only the hypothesis of a hyper-responsiveness of platelets as a possible mechanism for increased cardiovascular risk in these subjects was rejected, but a paradoxical defect in these cells was identified.

There are several possible explanations for the agonist specificity of such a defect. The most direct explanation points to the existence of (at least) two molecular ‘switches’ by which β3 integrin is involved in platelet adhesion and aggregation: one modification, involving amino acid position 1 at exons 2, is critical for activation directly by thromboxane A2 and indirectly by arachidonic acid, while the PlA1 polymorphism is not critical for activation by ADP and thrombin. This hypothesis is in agreement with the current knowledge indicating that different transduction pathways, proximal to the fibrinogen receptor, are utilized by different agonists (Du & Ginsberg, 1997). According to this model, the finding of increased sensitivity to acetylsalicylic acid may simply indicate that the drug unMASKS a latent defect by reducing the endogenous generation of thromboxane A2 and, thus, the β3 integrin modification by this pathway. However, because of the complexity of fibrinogen-receptor function, it cannot be excluded that the indicated molecular modification of β3 integrin may affect other signalling pathways involved in cellular activation. The involvement of the fibrinogen receptor in outside-in activation or amplification of signals has been already suggested (Shattil et al. 1997).

A possible explanation of the decrease of PlA2 platelet response to thromboxane A2 could be the result of a desensitization of the thromboxane A2 receptor following activation in vivo. According to this hypothesis, the PlA2 platelets could be activated in vivo and subsequently become hypo-responsive. However, from our data obtained by flow-cytometric analysis and on determination of β-thromboglobulin plasma levels, two investigations of possible platelet activation in vivo, we did not determine any up-regulation of PlA2 platelets with respect to PlA1 platelets.

From our data obtained by in vitro assay of platelet function and by ex vivo assessment of platelet activation markers, it seems that the suggested thrombogenic effect of the PlA2 polymorphism (Weiss et al. 1996; Carter et al. 1997; Walter et al. 1997; Garcia-Ribes et al. 1998; Pastinen et al. 1998; Zotz et al. 1998; Kastrati et al. 1999) is not related to platelet hyper-activity. This consideration is in accordance with others authors (Lasne et al. 1997). However, we did not observe a platelet hypo-reactivity to thrombin or ADP as described by Lasne et al (1997). This
discrepancy could be related to different experimental conditions (we studied washed platelets, Lasne and colleagues studied platelet-rich plasma). Basically, it cannot be excluded that the PlA2 genotype is linked to another yet unidentified genetic marker, which is the true risk determinant, or to other platelet disorders, where a defect of the thromboxane A2 receptor has been described (Higuchi et al., 1999). Further studies are necessary to clarify this point. It cannot be ruled out that other cellular sources than platelets are being affected, as the β3 subunit is part of the vitronectin receptor which is expressed in endothelial cells, smooth muscle cells and a number of other cells. As this integrin receptor (αvβ3) is highly involved in angiogenesis, polymorphism of glycoprotein β3 has also to be considered in relation to the pathophysiology of cardiac vasculature. It is known that the integrin αvβ3 could mediate platelet adhesion to the activated endothelial cells (Gawaz et al., 1997). More recently, in an animal model, an anti-thrombotic role for vitronectin was reported (Fay et al., 1999). This effect seems to be mediated by inhibiting platelet–platelet interactions and/or thrombin procoagulant activity. If these data are confirmed in humans, anomalous functioning of the anti-thrombotic role of the vitronectin receptor could be advanced to explain the role of PlA2 as a cardiovascular risk factor.

Because of the small sample size of the PlA2 subjects considered in this work (n = 14), the statistical power of the present study is low. This limitation of the study does not affect the finding of differences which are statistically significant (i.e. platelet responses to arachidonic acid and U46619). On the other hand, the negative findings (i.e. platelet responses in the resting condition or to ADP or thrombin) do not rule out the possibility that some small difference may actually exist. However, the differences between the non-significant values of the PlA2 and PlA1 groups are so small that they probably do not have a relevance at the clinical level.

The differences in dose–inhibition curves with acetylsalicylic acid are in accordance with a recent letter, Cooke et al. (1998), showing a greater sensitivity to acetylsalicylic acid in PlA1/PlA2 platelets than in PlA2/PlA1 platelets. These authors suggested that differences in exposure to aspirin at the time of onset of myocardial infarction might account for some of the discrepancies amongst studies on PlA1 as a risk factor for ischaemic coronary events.

In conclusion, our findings are not consistent with the hypothesis that the purposed increase of cardiovascular risk in these subjects may be as a result of fibrinogen receptor hyper-activation. On the contrary, the PlA2 allele is associated with a platelet functional deficiency, specifically linked to the activation of the fibrinogen receptor by thromboxane A2.

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Platelet Function and PlA2 Polymorphism


