Glycoprotein Ia gene C807T polymorphism and risk for major adverse cardiac events within the first 30 days after coronary artery stenting

Nicolas von Beckerath, Werner Koch, Julinda Mehilli, Corinna Böttiger, Albert Schömig, and Adnan Kastrati

The glycoprotein complex Ia/Ila (GP Ia/Ila) is a major collagen receptor on platelets and other cell types. Recently, linked polymorphisms within the coding region of the GP Ia gene (C807T and G873A) were identified that are related to GP Ia/Ila surface expression. The T807/A873 allele is associated with high expression, whereas the C807/G873 allele is associated with low surface expression of GP Ia/Ila. Subsequently, the T807 allele was found to be associated with coronary and cerebral infarction in younger patients. Platelet adhesion to the vessel wall plays a pivotal role in thrombosis after coronary artery stent placement. The goal of this study was to test whether C807T polymorphism is associated with a higher incidence of thrombotic events following coronary stenting. Consecutive patients treated with coronary stent placement (n = 1797) were genotyped for C807T polymorphism with polymerase chain reaction and allele-specific fluorogenic probes. The composite end point was defined as death, myocardial infarction, or urgent target vessel revascularization within 30 days of stent implantation. The genotype distribution of the study population was CC in 36.5%, CT in 46.7%, and TT in 16.8% of the patients. The incidence of the composite end point was 6.5% in T allele carriers and 5.3% in noncarriers (odds ratio for T allele carriage 1.23 [95% confidence interval, 0.81-1.86], P = .33). After adjusting for other baseline characteristics, the odds ratio for the composite end point was 1.15 (0.76-1.75). Therefore, C807T genotype has no significant influence on the major adverse events occurring after coronary artery stenting. (Blood. 2000;95:3297-3301)

Introduction

The integrin α2β1, (also known as glycoprotein Ia/Ila [GP Ia/Ila] or very late antigen 2 [VLA-2]) is a major platelet collagen receptor. GP Ia/Ila binding to subendothelial collagens plays an important role in platelet adhesion and activation following initial platelet vessel wall interaction mediated by von Willebrand factor. Deficiency of GP Ia 2-5 or the presence of inhibitory antibodies directed against the GP Ia/Ila complex results in impaired hemostasis and a bleeding disorder of variable extent. Kunicki et al found a 3- to 4-fold interindividual variation of GP Ia/Ila surface expression. A corresponding variation was observed for α2- but not for β3-subunit expression. In contrast, surface expression of other platelet membrane receptors like αIIbβ3, α3β1, and GP IV shows little interindividual variation. Recently, 2 silent, linked polymorphisms (C807T and G873A) within the coding region of the α gene have been identified that are associated with the number of GP Ia/Ila copies on the platelet membrane. Other polymorphisms of the α gene such as Br10 are also linked with C807T polymorphism. High GP Ia/Ila surface expression was found in platelets derived from T807 homozygotes, whereas low surface expression was found in platelets from C807 homozygotes. Because the number of GP Ia/Ila receptors on the platelet surface correlates with platelet adhesion to collagen, T807 may be associated with thrombosis. Several recent studies have investigated the possible association between C807T genotype and the incidence of thrombotic, ischemic events in a clinical setting. In a large consecutive series of patients including 1050 survivors of myocardial infarction and 1187 controls, an association between T allele and myocardial infarction was found in younger patients. Specifically, for patients less than 62 years old, the odds ratio (OR) for T807 carriers versus noncarriers was 1.57 (1.14-2.13) and for patients less than age 49 years, the respective OR was 2.61 (1.26-5.41). In a smaller study, a similar age-dependent association was also found for stroke patients, with an OR of 3.02 (1.20-7.61) for T allele carriers less than age 50 years. Moshfegh et al found a strong, age-independent association between T807 homozygosity and myocardial infarction. Two recent studies, however, found no association between C807T genotype and thrombotic diseases or myocardial infarction, showing that the clinical significance of this polymorphism is still not completely clarified. Therefore, further study of the role of C807T polymorphism in other thromboembolic disease states related to platelet-collagen interaction has recently been recommended.

Stent placement in coronary arteries is now the most common interventional treatment for symptomatic coronary artery disease. Compared to plain angioplasty (percutaneous transluminal coronary angioplasty), stenting allows for a higher primary success rate and a reduced restenosis rate. Early thrombotic events remain, however, a serious problem of coronary stenting. The placement of coronary stents induces marked platelet activation. Moreover, animal studies have shown that, besides the interaction of the stent surface with the flowing blood, the extent of vessel wall injury has an influence on the formation of stent thrombosis. Because collagens are major components of the subendothelial matrix, differential expression of GP Ia/Ila may have an effect on platelet adhesion and activation after coronary stent placement.
This study was designed to test whether the C807T polymorphism, which is associated with elevated GPIa/IIa surface expression, results in an increased risk of adverse thrombotic events following coronary artery stenting.

**Patients and methods**

**Patients**

This study included all patients with stable or unstable angina pectoris who underwent a stent implantation procedure in the period from April 1996 through January 1998. The patients who underwent coronary stenting in the setting of acute myocardial infarction were excluded from the study.

Implantation of various slotted-tube stents was performed as previously described. More than 95% of the stents were hand-crimped on conventional angioplasty balloons and delivered under fluoroscopic guidance. Therapy after the procedure consisted of aspirin (100 mg twice daily, indefinitely) and ticlopidine (250 mg twice daily for 4 weeks; Tildyk, Sanofi-Winthrop, Munich, Germany). Patients with angiographic appearance of thrombus on the stented site received additional therapy with abciximab (Lilly Deutschland GmbH, Giessen, Germany) given as bolus injection during the stent insertion procedure and as a 12-hour continuous infusion thereafter. The decision to give abciximab was left to operator’s discretion. Patients with symptoms or signs of recurrent ischemia after the intervention underwent repeat angiography.

**Genotyping**

Genomic DNA was extracted from 200 µL peripheral blood leukocytes with the QIAamp Blood Kit (Qiagen, Hilden, Germany) or the High Pure PCR Template Preparation Kit (Boehringer Mannheim, Mannheim, Germany). Genotyping of the C807T polymorphism was performed with the ABI Prism Sequence Detection System (PE Applied Biosystems, Weiterstadt, Germany). The use of allele-specific fluorogenic probes in the 5′ nucleotide reaction combines DNA amplification and genotype determination into a single assay. The nucleotide sequences of primers and probes were as follows: forward primer 5′ CAT CCC AGA CAT CCC AAT ATG G 3′, reverse primer 5′ GCC CTA TTA GCA CCA AAA ATT ACC TT 3′, allele C807T probe 5′ TCT TGC AGG TCA ATT 3′, allele T807 probe 5′ ACC TCA AACA ATG TGG GAA CAA TTC AA 3′. Allele C807T probe was labeled with the fluorescent dye: 6-carboxy-fluorescein (FAM) and allele T807 probe was labeled with VIC (PE Applied Biosystems, patent pending) at their 3′ ends. The probes were labeled with the quencher 6-carboxy-tetramethyl-rhodamine (TAMRA) at their 3′ ends. The thermocycling protocol consisted of 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 62°C for 1 minute. The C807T Br genotypes were determined by allele-specific restriction enzyme analysis, as previously described. Primers 5′ GTG ACC TAA AGA AAG AGG 3′ and 5′ TCT CTA TGG AAA ATG GCA G 3′ were used to amplify a 274-bp sequence containing the Br polymorphic site. Polymerase chain reaction (PCR) products were digested with MnII. Restriction fragments were separated by electrophoresis in 8% polyacrylamide gels (Novex, Frankfurt/Main, Germany) and, after staining with ethidium bromide solution, identified by 312-nm UV transillumination.

**Angiographic assessment**

Lesions were classified according to the modified American College of Cardiology/American Heart Association grading system. Type B2 and C lesions were considered complex lesions. The diagnosis of reduced left ventricular function was established in the presence of at least 2 hypokinetic segments in the contrast angiogram. The diagnosis of stent closure was established angiographically in the presence of a flow of grade 0 or 1 according to the Thrombolysis in Myocardial Infarction (TIMI) trial. The quantitative analysis was performed off-line for angiograms before and immediately after stent placement using the automated edge-detection system CMS (Medis Medical Imaging Systems, Nuenen, The Netherlands) and computing the following parameters: minimal lumen diameter, reference diameter, percent diameter stenosis, and diameter of the maximally inflated balloon.

**Definitions and end point of the study**

Procedural failure was defined as failure to place the stent at the desired position or to achieve a final diameter stenosis of less than 30% by visual inspection. The end point of adverse events within the first 30 days after the procedure was a combination of death from any cause, myocardial infarction, and severe myocardial ischemia requiring urgent revascularization by means of coronary bypass surgery or percutaneous intervention. Myocardial infarction was defined as new abnormal Q waves on the electrocardiogram (Q-wave myocardial infarction) or a value of creatine kinase or its MB isoenzyme at least 3 times the upper limit of normal (non-Q-wave infarction). Cardiac enzymes were systematically determined after the intervention.

Assuming that about 70% of patients are T807 carriers, the sample size in our study was estimated to provide 80% power for detecting an increase in the risk for adverse events from 6% in CC patients to 10% in those who carry the T allele.

**Statistical analysis**

Discrete variables are expressed as counts and compared with χ2 or Fisher exact test as appropriate. The Hardy-Weinberg equilibrium was tested according to Guo et al. Continuous variables are expressed as mean ± SD and compared by means of the unpaired, 2-sided t test. The possible association between the presence of the T807 allele and adverse events was also assessed after adjusting for other factors using multiple logistic regression analysis. Together with the genotype, all factors resulting with a P value less than .05 in the univariate analysis were entered into the multivariate model as potential confounding factors. The risk associated with each factor was assessed on the basis of the respective OR and the corresponding 95% confidence interval (CI). All statistical analyses were performed using S-Plus software (Mathsoft, Seattle, WA). Statistical significance was assumed for P values less than .05.

**Results**

The study included 1797 patients. C807T genotype distribution was CC in 36.5%, CT in 46.7%, and TT in 16.8% of the patients. Br genotype was a/a in 0.7%, a/b in 18.2%, and b/b in 81.1% of the patients. Table 1 shows the baseline characteristics of the patients according to their C807T genotype. TT patients were significantly older and more likely to have diabetes and reduced left ventricular function. Table 2 shows comparable angiographic and procedural characteristics for CC, CT, and TT patients. The proportion of patients with successful procedures was similar among the 3 genotypes. Table 4 displays the adverse events within 30 days of coronary artery stenting according to C807T genotype. No significant differences were found for any of the events and for the composite end point of the study. The incidence of adverse major events was 6.5% in T allele carriers and 5.3% in noncarriers (OR for T allele carriage, 1.23 [95% CI, 0.81-1.86], P = .33). The events occurred
Before stenting

Ostial lesions, % 7.9 6.9 7.9 .71

Target vessels .66

TT patients (incidence of adverse events: 4.7% in CC, 5.7% in CT, and 4.7% in overwhelmingly during the first 4 days after stenting (83%); even during this period there was no significant difference in the incidence of adverse events: 4.7% in CC, 5.7% in CT, and 4.7% in TT patients (P = .66). In addition, subacute stent thrombosis was observed in 10 (1.6%) CC, 16 (2.0%) CT, and 6 (2.0%) TT patients (P = .31). Table 5 displays the adverse events within 30 days of coronary artery stenting according to Br genotype. Here also there was no significant difference among the 3 genotypes.

Table 6 shows the results of the different subgroup analyses. The lack of association between C807T genotype and early outcome after stenting was independent of the procedural success, administration of abciximab, and age. Of the 38 patients with procedural failure, 13 (34.2%) incurred an early major adverse event without significant differences among the 3 genotypes: 29.4% in CC, 41.2% in CT, and 25.0% in TT patients (P = .65). Six (16.2%) patients died during the first 4 days after stenting (83%); even during this period there was no significant difference in the incidence of adverse events: 4.7% in CC, 5.7% in CT, and 4.7% in TT patients (P = .66). In addition, subacute stent thrombosis was observed in 10 (1.6%) CC, 16 (2.0%) CT, and 6 (2.0%) TT patients (P = .31). Table 5 displays the adverse events within 30 days of coronary artery stenting according to Br genotype. Here also there was no significant difference among the 3 genotypes.

A multivariate model for the composite end point was constructed and age, diabetes, unstable angina, reduced left ventricular function, a history of bypass surgery, multivessel disease, and number of stents implanted (all factors with a P < .2 in the univariate analysis) as well as C807T genotype were entered as potential independent predictors. The adjusted OR for T allele homozygosity versus C allele homozygosity was 1.04 (0.60-1.82) and for T allele carriage 1.15 (0.76-1.75). The multivariate analysis revealed no interaction between the C807T genotype and other factors with respect to the occurrence of adverse events.

Discussion

The main result of this study is that the T807 allele of the GP Ia gene is not associated with an increased risk of major adverse events following coronary artery stenting. This result was obtained in a large consecutive series of patients who were carefully monitored for the occurrence of adverse thrombotic events. Most thrombotic events occur during the first days following stent implantation. Serial determination of creatine kinase and its myocardial isoenzyme allowed registration of postprocedural thrombotic events causing non–Q-wave infarction. Such events are often caused by embolization of platelet aggregates into more distal vessels or side branch occlusions as opposed to the less frequent complete thrombotic occlusion of the stented artery. Therefore, the composite end point of this study allowed an extensive evaluation of postprocedural thrombosis as a prerequisite to investigate a prothrombotic genetic factor in this specific setting. In our study group, the T allele frequency was 0.40, which is identical to the value reported for the group of patients studied by Santoso et al and slightly higher than the T allele frequency found in healthy blood donors. We also genotyped the patient population for the GP Ia Br polymorphism and found no correlation with early outcome after stenting. So far, as in the case of our study, Br* has only been found in homozygous or heterozygous C allele carriers. Moreover, Br* is rarely encountered in whites with an allele frequency of around 0.1, which corresponds to the frequency

Table 5. Major adverse events during the first 30 days after stent placement according to Br genotype

<table>
<thead>
<tr>
<th>Event</th>
<th>a/a (n = 13)</th>
<th>a/b (n = 327)</th>
<th>b/b (n = 1457)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
<td>9 (0.6)</td>
<td>.35</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0</td>
<td>17 (5.2)</td>
<td>64 (4.4)</td>
<td>.60</td>
</tr>
<tr>
<td>Q wave</td>
<td>0</td>
<td>2 (0.6)</td>
<td>22 (1.5)</td>
<td>.40</td>
</tr>
<tr>
<td>Non-Q wave</td>
<td>0</td>
<td>15 (4.6)</td>
<td>42 (2.9)</td>
<td>.23</td>
</tr>
<tr>
<td>Urgent revascularization</td>
<td>0</td>
<td>8 (2.5)</td>
<td>40 (2.8)</td>
<td>.80</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.
Table 6. Major adverse events during the first 30 days after stent placement in different subsets of patients according to the C807T genotype

<table>
<thead>
<tr>
<th>Patients with successful procedure, n</th>
<th>807 CC</th>
<th>807 CT</th>
<th>807 TT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>1 (0.2)</td>
<td>5 (0.6)</td>
<td>1 (0.3)</td>
<td>.39</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>28 (4.4)</td>
<td>34 (4.1)</td>
<td>13 (4.3)</td>
<td>.97</td>
</tr>
<tr>
<td>Any major adverse event</td>
<td>30 (4.7)</td>
<td>50 (6.1)</td>
<td>16 (5.4)</td>
<td>.51</td>
</tr>
</tbody>
</table>

Patients without abciximab therapy, n

| Death                               | 0 (0)   | 0 (0)   | 1 (0.2)  | .74 |
| Myocardial infarction               | 13 (6.9) | 13 (4.6) | 5 (5.8)  | .77 |
| Any major adverse event             | 15 (6.8) | 16 (5.6) | 6 (7.0)  | .82 |

Patients <60 y, n

| Death                               | 0 (0)   | 0 (0)   | 1 (0.5)  | .88 |
| Myocardial infarction               | 6 (8.1) | 4 (6.8) | 1 (0.5)  | .88 |

Patients >60 y, n

| Death                               | 0 (0)   | 0 (0)   | 0 (0)    |   |
| Myocardial infarction               | 0 (0)   | 0 (0)   | 0 (0)    | .77 |
| Any major adverse event             | 0 (0)   | 0 (0)   | 0 (0)    | .77 |

Numbers in parentheses are percentages.

found also in the present study, and platelet adhesiveness to collagens was not dependent on Br polymorphism.10

The number of GP Ia/Ila receptors exposed on the surface of a single platelet, as assessed by radioactive ligand binding, has been reported to be in the range of 1000 to 3000.7 With flow cytometry, surface expression of GP Ia/Ila receptors was found to be twice as high in T807 homozygotes as compared with C807 homozygotes.8,9 In heterozygotes, mean fluorescence of the fluorogenic antibody directed either against GP Ia or the GP Ia/Ila complex was in between the values for C807 and T807 homozygotes.8,9 In a family, the number of GP Ia/Ila receptors was quantified with radioactive ligand binding assay and the mean values obtained were 2325 for the TT father, 933 for the CC mother, and 1612 and 1606 for the CT sons.9 Moreover, direct evidence indicates that C807T-related variation of GP Ia/Ila surface expression affects platelet-collagen interaction. First, in a flow chamber, C807T genotype was associated with the rate of platelet attachment to collagen I at high shear.11 Second, in patients with a moderate deficiency of von Willebrand factor, the T807 allele was associated with longer closure times in a high shear stress system that simulates platelet-based hemostasis in vitro.55

Coronary artery stent placement causes marked platelet activation as has been shown experimentally and in clinical trials.22,23,36-38 Following stent implantation, surface expression of the activated fibrinogen receptor and P-selectin increase and, concomitantly, platelet count and plasma fibrinogen concentration decrease.22,39 Differences between stent-induced thrombosis and spontaneous coronary thrombosis may underlie the lack of an association between C807T genotype and the incidence of early adverse events following coronary artery stenting. Spontaneous coronary thrombosis is a result of platelet adhesion, activation, and aggregation following rupture or erosion of an atherosclerotic plaque.40,41 which often causes only a mild or moderate coronary stenosis.42 Tissue factor is particularly abundant in the lipid core of these plaques and is exposed to the flowing blood after plaque disruption.43 Therefore, tissue factor–induced thrombin generation may play an important role in platelet activation on the plaque surface. Accordingly, in perfused human arterial segments, addition of tissue factor pathway inhibitor resulted in a decrease of platelet and fibrinogen deposition on disrupted atherosclerotic plaques.44 In contrast, high-grade coronary lesions that are treated with stent placement are usually caused by collagen-rich fibrotic plaques that contain less tissue factor.45 Exposure of the thrombogenic device itself may play a significant role in marked platelet activation that follows coronary stent placement.7,32 Thus, the mode of platelet activation may differ in both types of arterial thrombosis. This may influence the function of GP Ia/Ila and the relative importance of this adhesion molecule.

References


