RAPID COMMUNICATION

Association of the Platelet Glycoprotein Ia C<sub>807</sub>T Gene Polymorphism With Nonfatal Myocardial Infarction in Younger Patients

By S. Santoso, T.J. Kunicki, H. Kroll, W. Haberbosch, and A. Gardemann

Recently, we have shown that two alleles of the glycoprotein (GP) Ia gene, designated C<sub>807</sub> and T<sub>807</sub>, are associated with low or high platelet GPIa-IIa density and consequently with slower or faster rate of platelet adhesion to type I collagen, respectively. This polymorphism could therefore present a genetic predisposition for the development of thrombotic disease and hemostasis. We investigated the relationship of the GPIa C<sub>807</sub>T dimorphism to the risk of coronary artery disease (CAD) and myocardial infarction (MI). An allele-specific polymerase chain reaction (PCR) was developed for genotyping of C<sub>807</sub>T polymorphism. DNA samples from 2237 male patients who underwent coronary angiography on account of coronary heart disease as verified illness or presumptive diagnosis were genotyped. The odds ratio was calculated as an estimate of the relative risk by multiple logistic regression. We found a strong association between the T allele and nonfatal MI among individuals younger than the mean age of 62 years (n = 1,057; odds ratio, 1.57; P = .004). The odds ratio of MI increased for T<sub>807</sub> carriers with decreasing age. The highest odds ratio was detected within the youngest 10% of the study sample (<49 years; n = 223; odds ratio, 2.61; P = .009). In contrast, no evidence of an association between C<sub>807</sub>T dimorphism with CAD was found. Our findings suggest that inherited platelet GP variations might have an important impact on acute thrombotic disease.

© 1999 by The American Society of Hematology.

ADHESION RECEPTORS of the integrin gene superfamily play important roles in the development of the mammalian vasculature, in securing hemostasis after blood vessel injury, and in restoring the integrity of the vessel during wound healing. Integrins are heterodimers composed of noncovalently associated α and β transmembrane subunits. On human platelets, five integrins (α<sub>IIb</sub>β<sub>3</sub>), α<sub>IIa</sub>β<sub>1</sub>, α<sub>3</sub>β<sub>1</sub>, α<sub>5</sub>β<sub>1</sub>, and α<sub>6</sub>β<sub>4</sub>) are currently recognized.1

The α<sub>IIb</sub>β<sub>3</sub> integrin, also known as glycoprotein (GP) Ia-IIa complex, is a major collagen receptor on platelets.2 Consequently, congenital and acquired deficiency of platelet GPIa<sup>3</sup>-<sup>5</sup> as well as autoantibodies against GPIa are associated with clinical bleeding diatheses characterized by impaired platelet response to collagen.6

Several nucleotide sequence variations of GPIa (α<sub>IIa</sub> integrin) gene have been described. The single point missense mutations A<sub>164G</sub> (Lys<sup>54Glu</sup>) and C<sub>253T</sub> (Thr<sup>74Met</sup>) are responsible for the formation of the Br<sup>v</sup>/Br<sup>b</sup> (HPA-5) and S<sup>it</sup>/S<sup>it</sup> alloantigens (HPA-12bw), respectively.7,9

Recently, linked silent polymorphisms in the coding region of GPIa gene at nucleotides 807 (C or T) and 873 (G or A) could be identified. Although these polymorphisms do not change the amino acid sequence of GPIa, a significant correlation between these polymorphisms and expression levels of GPIa-IIa on the platelet surface was found.10 Carriers of the allele bearing T<sub>807</sub>C<sub>873</sub> express high levels of GPIa-IIa, whereas individuals who carry the C<sub>807</sub>G<sub>873</sub> allele exhibit lower expression of the platelet integrin. Furthermore, we could demonstrate that the density of GPIa-IIa on the platelet surface correlates with the rate of platelet attachment in whole blood to type I collagen, even under high shear rates (1,500 sec<sup>-1</sup>) typical of the arterial vasculature.11 Thus, a high density of platelet surface GPIa-IIa could represent a potential risk factor for thrombotic disease.

In this study, we evaluated the clinical significance of the GPIa C<sub>807</sub>T dimorphism to coronary artery disease (CAD) and myocardial infarction (MI) in a population of 2,237 male individuals whose coronary anatomy was exactly defined by coronary angiography.

MATERIALS AND METHODS

Study Sample

DNA samples were collected from 2,250 consecutive male patients who underwent coronary angiography for diagnostic purposes. About 80% of the participants underwent coronary angiography on account of coronary heart disease (CHD) as verified illness or presumptive diagnosis. The remaining part of the group consisted almost completely of patients who underwent coronary angiography for clarification of restricted left ventricular function. In 90% of these patients CAD was proven as the reason for this dysfunction. Only in 10% of this subpopulation (2% of the total sample) was restricted left ventricular function caused by dilated cardiomyopathy or longstanding arterial hypertension. All patients who agreed to participate in the study were evaluated with a detailed questionnaire that provided information about coronary risk factors such as smoking, diabetes mellitus, and hypertension.

Coronary angiography was performed using the Judkins method. Coronary vessels with at least 50% stenosis were defined as diseased. The severity of CHD was also estimated by calculating the Gensini score and designated as CHD-score.12

From the Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig University Giessen, Giessen, Germany; The Scripps Research Institute, La Jolla, CA; The Max Planck Institute for Experimental and Clinical Research, Kereckhoff Hospital, Bad Nauheim, Germany; and the Institute for Clinical Chemistry and Pathobiology, Justus Liebig University Giessen, Giessen, Germany.

Submitted October 30, 1998; accepted January 27, 1999.

Supported by grants from the German Research Foundation (DFG Sa 4802/1-1) and a grant (to T.J.K.) from the Gustavus and Louise Pfeiffer Research Foundation (Denville, NJ).

Address reprint requests to S. Santoso, PhD, Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig University Giessen, Langhansstr. 7, D-35392 Giessen, Germany; e-mail: Santos.Santoso@immunologie.med.uni-giessen.de.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1999 by The American Society of Hematology.

0006-4971/99/9308-0034$3.00/0
Angina pectoris and acute MI were diagnosed according to criteria established by the World Health Organization.

**Measurements of Serum Enzymes and Substrates**

Triglycerides, total cholesterol, apolipoprotein B (apoB), apolipoprotein AI (apoAI), lipoprotein (a) [Lp(a)], and fibrinogen were measured by conventional methods of clinical chemistry. Hypertension (binary variable in the present study) was defined by either treatment or a diastolic blood pressure greater than 95 mm Hg on two consecutive visits for those untreated. Cigarette consumption was expressed as pack years (1 pack year = 20 cigarettes per day for 1 year). With the exception of 18 patients, all individuals with diabetes mellitus (n = 424) were classified as non-insulin-dependent diabetes mellitus.

**Definition of Low- and High-Risk Subpopulations**

With respect to continuous variables of coronary risk factors, low- and high-risk populations were defined according to the mean values and to the 10th, 25th, 50th, 75th, and 90th percentiles of these parameters. Low- and high-risk groups of the coronary risk factors hypertension and diabetes were defined by the absence or presence of these diseases. Thus, low and high risk groups were chosen a priori; subgroup analysis was not performed post hoc.

**Genotyping of GPIa C 807 T Dimorphism**

Leukocyte DNA was isolated from whole blood using standard procedures. The GPIa-specific polymerase chain reaction (PCR) primers used in this study were constructed based on the published GPIa cDNA and GPIa gene sequences. Five microliters of genomic DNA was added to a 50 µL reaction mixture containing 10 mmol/L Tris (pH 8.0), 50 mmol/L KCl, 2.75 mmol/L MgCl₂, 0.125 mmol/L of each dNTP, 0.25 µmol/L of each of intron specific primer (5'-gagcatcagcttaaatgtctgccctcgc-3') and sequence-specific antisense primer (5'-cctttgcatatgaaattggtacg-807-3' or 5'-cctttgcatatgaaattggtacg-807-3'), 0.125 µmol/L each of HGH I (CAGTGCCCTCCCCACAACCTCCCTTA-3') and HGH II (ATTCACCTACGAGATTCTGTTGTTTAC-3') primers, and 2.5 U TaqGold (Perkin Elmer, Vaterstetten, Germany). One mismatch base (A instead C; letter in bold) was introduced in both sequence specific primers to increase the specificity of hybridization. After initial denaturation at 96°C for 10 minutes, amplification was performed in a DNA thermocycler (GeneAmp PCR System 9600; Perkin Elmer) for 35 cycles (denaturation at 93°C for 50 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 15 seconds). The PCR products were analyzed by electrophoresis on 1.8% agarose gels using Tris-borate/EDTA buffer and visualized by ethidium bromide staining. DNA molecular marker V was used as the standard (Boehringer Mannheim, Mannheim, Germany). One mismatch base (A instead C; letter in bold) was introduced in both sequence specific primers to increase the specificity of hybridization. After initial denaturation at 96°C for 10 minutes, amplification was performed in a DNA thermocycler (GeneAmp PCR System 9600; Perkin Elmer) for 35 cycles (denaturation at 93°C for 50 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 15 seconds). The PCR products were analyzed by electrophoresis on 1.8% agarose gels using Tris-borate/EDTA buffer and visualized by ethidium bromide staining. DNA molecular marker V was used as the standard (Boehringer Mannheim, Mannheim, Germany).

**Statistical Analysis**

Statistical analysis was performed using the SPSS software (Version 7.52; SPSS GmbH Software, Munich, Germany). Established risk factors of CAD and MI were identified by multiple regression analysis (extent of CAD and CHD score) or multiple logistic regression (absence/presence of CAD and MI). The χ² test was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium and to determine whether there was any significant difference in allele or genotype frequencies between cases and controls. The relationship between GPIa C 807 T gene dimorphism and the extent of CAD (CHD score) was determined by multiple regression analysis. Variables that showed significant association with CAD were introduced into the calculation. The relationship between the C 807 T dimorphism and the presence of CAD and MI was determined by multiple logistic regression with adjustment for other coronary risk factors. Odds ratios were calculated as an estimate of relative risk of CAD or of MI associated with the C 807 T genotype and adjusted for risk factors of CAD or of MI. For each odds ratio, we calculated two-tailed P values and 95% confidence intervals with adjustment for additional risk factors of CAD and MI by multiple logistic regression. All coronary risk factors such as age, apoAL and apoB remained to be included in all subgroup analyses; an exception was only made for binary variables such as diabetes or hypertension when high- or low-risk subpopulations were defined by the presence or absence of these parameters. A two-sided probability value of less than .05 was considered to indicate statistical significance.

**RESULTS**

**Genotyping Analysis of GPIa C807T Polymorphism**

We developed an allele-specific PCR approach to analyze the nucleotide C807T dimorphism. The common sense primer was set on the intron 6 bp downstream from the polymorphic exon (nucleotides 679-827). The allele-specific antisense primers (C or T) were located on the border of this exon. Genotyping analysis of three individuals by this sequence-specific PCR (PCR-SSP) technique is shown in Fig 1. Amplification of genomic DNA derived from donor 1 resulted in a 184-bp specific product with primer C, but not with primer T. In contrast, DNA from donor 3 could be amplified only with primer T. Both primers, C and T, amplified the 148-bp fragment from donor 2. In all reactions, the 500-bp internal control fragment of the HGH gene was present. These results indicate that donors 1, 2, and 3 represent CC homozygous, CT heterozygous, and TT homozygous genotypes, respectively. To validate these findings, nucleotide sequencing analysis was performed, and the results are in accordance (data not shown). In this study, 2,237 of 2,250 patients’ DNA could be genotyped. To insure the reproducibility of our results, 15% of the samples were rechecked, paying particular attention to heterozygosity. Reference DNA derived from CC, CT, and TT genotyped individuals (see above) were run as controls.

**Distribution of the GPIa C807T Genotypes**

In subjects without CAD, without MI, or without CAD and MI and in individuals without detectable angiographic signs of coronary arterial stenoses (CHD score = 0), the distributions of the GPIa C807T dimorphism were in Hardy-Weinberg equilibrium (data not shown). In the study sample, the genotype frequencies of CC, CT, and TT were 33.5% (n = 750), 52.5% (n = 1,174), and 14.0% (n = 313), respectively (Table 1). Age, total cholesterol, triglycerides, apoB, apoAL, Lp(a), fibrinogen, prevalence of arterial hypertension, diabetes mellitus, body mass index (BMI), and cigarette consumption were not different between the C807T genotypes of the total study population and of each subgroup (data not shown).

**Relation of Established Risk Factors and GPIa C807T Dimorphism to CHD**

Coronary risk factors. Established risk factors of CAD such as apoB (P < .0001), Lp(a) (P < .0001), hypercholesterolaemia (P < .001), hypertension (P < .002), diabetes (P < .01), smoking habit (P < .05), and age (P < .0001) could be demonstrated as risk factors for CAD. ApoAI (P < .005) and high apoAI/apoB ratios (P < .0001) were identified as protective factors against CAD (data not shown). Risk factors of MI, such as apoB (P < .0001), hypercholesterolaemia (P < .005),
fibrinogen levels \((P < .05)\), glucose \((P < .05)\), and smoking habit \((P < .001)\), could be detected; apoAI \((P < .005)\) and high apoAI/apoB ratios \((P < .005)\) were identified as protective factors against MI (data not shown).

**Relation of the GPIa C 807 T dimorphism to CAD.** In the total sample, the frequencies of the C and T alleles did not differ between subgroups of patients without and with single, double, or triple vessel disease (Table 1). An association of the gene polymorphism with CAD was also not detected when CAD was defined as CHD score according to Gensini.12 These observations also apply to analyses of low- and high-risk populations (data not shown).

**Relation of the GPIa C 807 T dimorphism to MI.** In the total sample, no association was found between the presence of the GPIa T 807 allele and the risk of MI (Table 1). However, a strong association of the T 807 allele with the risk of MI was detected in individuals who were younger than the mean age of 62 years (odds ratio, 1.57; \(P = .004\); Table 2). When the upper limit of the participant’s age was further reduced, an even stronger association of the T allele with the risk of MI was observed. For example, for T allele carriers younger than 49 years of age (10th percentile), an odds ratio of 2.61 was calculated \((n = 223; P = .009\); Table 2).

In addition, we analyzed the association of the T allele in low- and high-risk patients. In high-risk patients with BMI greater than the mean value of 26.9 kg/m², the odds ratio in T allele carriers younger than 62 and 49 years of age suffering from MI was 1.94 \((n = 503; P = .003)\) and 4.92 \((n = 117; P = .003)\), respectively (Table 2). Inclusion as well as exclusion of other coronary risk factors had no influence on the association of the T allele with MI (data not shown).

**DISCUSSION**

Human platelet GPs play a major part in platelet adhesion and aggregation, key events in the development of thrombosis and hemostasis. Thus, any variation in platelet GP density could

---

**Table 1. Distribution of the GPIa C 807 T Genotypes in Patients With or Without CAD and With or Without MI**

<table>
<thead>
<tr>
<th>Controls/Cases</th>
<th>Mean Age (± SD)</th>
<th>n</th>
<th>n(CC)</th>
<th>n(CT)</th>
<th>n(TT)</th>
<th>C/T Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>± CAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHD score = 0</td>
<td>54.1 ± 11.0</td>
<td>167</td>
<td>57</td>
<td>87</td>
<td>23</td>
<td>0.60 (0.55-0.65)</td>
</tr>
<tr>
<td>No vessel disease*</td>
<td>58.5 ± 10.7</td>
<td>502</td>
<td>176</td>
<td>257</td>
<td>69</td>
<td>0.61 (0.58-0.64)</td>
</tr>
<tr>
<td>Single vessel disease</td>
<td>61.2 ± 9.7</td>
<td>450</td>
<td>160</td>
<td>226</td>
<td>64</td>
<td>0.61 (0.58-0.64)</td>
</tr>
<tr>
<td>Double vessel disease</td>
<td>62.5 ± 9.7</td>
<td>484</td>
<td>172</td>
<td>236</td>
<td>76</td>
<td>0.60 (0.57-0.63)</td>
</tr>
<tr>
<td>Triple vessel disease</td>
<td>63.7 ± 8.6</td>
<td>801</td>
<td>242</td>
<td>455</td>
<td>104</td>
<td>0.59 (0.56-0.61)</td>
</tr>
<tr>
<td>Single, double, or triple vessel disease</td>
<td>62.7 ± 9.3</td>
<td>1,735</td>
<td>574</td>
<td>917</td>
<td>244</td>
<td>0.60 (0.58-0.61)</td>
</tr>
<tr>
<td>± MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MI</td>
<td>61.4 ± 9.9</td>
<td>1,187</td>
<td>416</td>
<td>605</td>
<td>166</td>
<td>0.60 (0.59-0.62)</td>
</tr>
<tr>
<td>At least 1 MI</td>
<td>62.2 ± 9.5</td>
<td>1,050</td>
<td>334</td>
<td>569</td>
<td>147</td>
<td>0.59 (0.57-0.61)</td>
</tr>
</tbody>
</table>

*Persons without any detectable stenosis of coronary arteries (CHD score = 0) or patients with less than 50% stenosis of coronary arteries.*

---

![Fig 1. Schematic illustration of the localization of the primers for genotyping of C 807 T dimorphism (top). Allele-specific amplification (PCR-SSP) was performed with intronic common sense primer (92V) and antisense primers C807 or T 807 (96C or 96T). Representative results of GPIa genotypes determined by PCR-SSP of three individuals: homozygous CC (lanes 2), heterozygous CT (lanes 3), and homozygous TT (lanes 4). Genomic DNA were amplified using specific primer for C807 (left panel) or for T807 allele (right panel) and were analyzed on 1.8% agarose gel electrophoresis. The GPIa bands represent the allele-specific PCR product and the HGH bands the internal control. DNA size standards V (Boehringer Mannheim) were used as standard (lanes 1). Negative controls are shown in lanes 5.](image-url)
become a potential risk factor for hemostatic abnormalities. GPIa-IIα–mediated adhesion of platelets to collagens appears to have a significant physiological importance for normal hemostasis. Kunicki et al.10 could identify that polymorphisms within the GPIa gene are associated with variations in platelet GPIa-IIα expression levels. Platelets from individuals bearing the T 807 allele express high levels of GPIa-IIα, whereas individuals who carry the C 807 allele exhibit a lower density of the platelet integrin. Interestingly, high GPIa-IIα expression levels only depend on the presence of the T 807 allele. Heterozygous individuals express almost similar number of GPIa copies as individuals homozygous for T 807. More recently, we could demonstrate under whole blood arterial conditions that the rate of platelet attachment to type I collagen increases with increasing density of GPIa-IIα. Platelets derived from T 807 donors adhere significantly faster than platelets from C 807 donors.11

The fact that fibrillar collagens as major components of the subendothelial matrix are potent inducers of thrombus formation has led us to the hypothesis that increased expression of GPIa-IIα on the platelet can increase the risk of thrombotic disease, whereas decreased expression can impair hemostasis.

To prove this hypothesis, we analyzed the relationship between the C 807/T dimorphism and CHD in a study sample of 2,237 male individuals whose coronary anatomy was defined by means of coronary angiography. We found a strong association between the T 807 allele with MI among younger individuals. The odds ratio for the risk of MI increased for T 807 carriers with decreasing age; the highest odds ratio was found within patients younger than 49 years of age (10th percentile of age in our study sample). This observation might be explained by the fact that the inheritance of T 807 allele is associated with MI, whereas most other risk factors of MI develop in the course of life. Consequently (and in line with our observations) a higher risk for T allele carriers to suffer an acute MI should be predicted in younger individuals. Nevertheless, it has to be considered that only survivors of MI have been analyzed in the present study. Because of the retrospective design of our study, it cannot be entirely excluded that the T allele, although associated with a higher rate of MI, may be protective against death by MI. In addition, it should be noted that the present study sample represents a selected population of patients based on referral for coronary angiography. Thus, it is possible that there is a referral bias in which patients with MI who underwent coronary angiography may differ from the population of patients with MI in general. Further investigations, especially prospective studies, are clearly needed to clarify these questions.

Although no association between BMI and MI could be identified (data not shown), our results allow the assumption that obesity and the T 807 allele might interact on the risk of MI. It has been shown that obesity is associated with an increased risk for MI due to high plasminogen activator inhibitor (PAI) levels, which consequently impair fibrinolysis. Although we did not measure PAI levels in our study participants, it can be speculated that in obese subjects increased PAI levels might interact with the T allele on the risk of MI.

In the consequence of our observation, Carlsson et al.18 analyzed the association between C 807/T polymorphism and stroke. Only patients with focal transient or complete neurological symptoms due to cerebral ischemia were included. Analogous to our investigations, an impact of the T807 allele for the risk of stroke was observed in younger individuals. Thus, the positive association between the T 807 allele with MI and stroke indicates that the GPIa T 807 genotype might have a functional role in acute thrombotic complications.

If the inheritance of the T 807 allele is associated with thrombosis, then the converse would be true, namely, that inheritance of the C 807 allele would be associated with the risk for bleeding in individuals otherwise predisposed towards abnormal hemostasis. Recently, Di Paola et al.19 reported a significant correlation between the presence of the C 807 allele and increased bleeding symptoms among individuals with type I von Willebrand’s disease.

These findings emphasize the potential importance of inherited differences in GPIa-IIα on the health and maintenance of the cardiovascular system.

ACKNOWLEDGMENT

The authors thank Monika Kämmel and Heike Wagner for their excellent technical assistance.

REFERENCES

3. Niewenhuis HK, Akkerman JWN, Houdijk WPM, Sixma JJ:
Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia. Nature 318:470, 1985


18. Carlsson LE, Santoso S, Spitzer S, Kessler C, Greinacher A: The α2 gene coding sequence T807/A873 of the platelet collagen receptor integrin α2β1 (GPIa-IIa) is a genetic risk factor for the development of stroke in younger patients. Blood (in press)

Association of the Platelet Glycoprotein Ia C<sup>807</sup>T Gene Polymorphism With Nonfatal Myocardial Infarction in Younger Patients

S. Santoso, T.J. Kunicki, H. Kroll, W. Haberbosch and A. Gardemann