Glycoprotein IIIa PI A Polymorphism Associates With Progression of Coronary Artery Disease and With Myocardial Infarction in an Autopsy Series of Middle-Aged Men Who Died Suddenly

Jussi Mikkelsson, Markus Perola, Pekka Laippala, Vesa Savolainen, Jarkko Pajarinen, Kaisa Lalu, Antti Penttilä, Pekka J. Karhunen

Abstract—Glycoprotein IIIa (GPIIIa) has a key role in the aggregation of thrombocytes, and it also mediates intimal hyperplasia after endothelial injuries; the possible association of the PI A1/A2 polymorphism of the gene for GPIIIa with coronary thrombosis and with the progression of coronary artery disease (CAD) is still to be confirmed. Therefore, the association of the PI A polymorphism with the development of coronary atherosclerosis, coronary narrowing, and myocardial infarction (MI) was studied in a prospective, consecutive autopsy series of 300 middle-aged, white Finnish men (33 to 69 years) suffering sudden out-of-hospital or violent death. Coronary atherosclerosis was measured morphometrically and the coronary stenosis percentage determined from a cast rubber model of the coronary tree. We found a significant inverse relation ($P<0.01$) between the PI A2-positive genotype and coronary artery stenosis. The frequency of possessing the PI A2 allele was significantly (odds ratio [OR] 0.45, 95% confidence interval [CI] 0.22 to 0.98) lower among men with >50% coronary stenosis (18.3%) than among those with <25% stenosis (32.9%). Although the PI A polymorphism was not directly associated with MI, the PI A2 allele was present in 11 of the 22 men (50%) with MI and coronary thrombosis (OR 6.6, 95% CI 2.1 to 22.8) but in only 6 of the 47 (12.8%) with MI associated with severe stenosis in the absence of thrombosis. In line with this result, men possessing the PI A2 allele also had a larger area of fissured and ulcerated complicated lesions in their coronary arteries ($P<0.05$). The present results suggest that the PI A polymorphism is involved in the development of CAD and MI. Men with the PI A2 allele may harbor more thin-walled, vulnerable coronary plaques, plaques prone to rupture, leading to massive, fatal thrombosis. In contrast, men homozygous for the PI A1 allele may more often show stable plaques and present with infarction caused by progressive coronary stenosis. (Arterioscler Thromb Vasc Biol. 1999;19:2573-2578.)

Key Words: platelet glycoprotein GPIIb/IIIa complex ■ coronary thrombosis ■ myocardial infarction ■ polymorphisms ■ genetics

In addition to environmental and acquired risks, genetic factors most probably play a significant role in the development of coronary artery disease (CAD), thrombosis, and myocardial infarction (MI) and may modify the effect of other known risk factors. Platelet aggregation with subsequent thrombus formation is a key event in the development of acute coronary syndromes and the sudden death of patients with coronary heart disease. Fissuring or rupture of an atheromatous plaque is followed by the activation of platelet GPIIb/IIIa ($\alpha_{\text{IIb}}\beta_3$ integrin) fibrinogen receptors, resulting in fibrinogen binding, platelet aggregation, and thrombus formation.1-3 Slow progression of atherosclerosis is dependent on shear-induced platelet adhesion to the exposed subendothelial matrix and to an enlarging mural thrombus at sites of vessel stenosis, whereas acute occluding thrombosis is usually caused by rupture of a thin-walled, atheromatous plaque distant from the site of the culprit stenosis.4,5 GPIIIa ($\beta_3$-integrin) is also expressed in the endothelium and in vascular smooth muscle cells (VSMCs).6,7 Its function is related to VSMC responses to endothelial injuries caused by, eg, hemodynamic shear stress, smoking, diabetes, and hypertension.8-13 $\alpha_{\beta_3}$ integrin is a receptor that mediates different kinds of stimuli to VSMCs, causing their proliferation and subsequent fibrous tissue generation and intimal hyperplasia after injuries.6,10,14,15

Platelet PI A polymorphism of the GPIIIa gene is produced by a single point mutation in exon 2 of the GPIIIa gene, leading to substitution of leucine (PI A1) for proline (PI A2) and

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consequent changes in the protein conformation and spatial orientation of the fibrinogen-binding region. The functional importance of the PlA polymorphism as an important inherited risk factor for MI was first suggested by Weiss and colleagues. Subsequent studies on associations between the PlA allele and acute coronary events have, however, been controversial. More controversy was added by the recent findings that platelets from individuals possessing the PlA allele bind either less or more fibrinogen than do platelets from PlA homozygotes and also by the finding that the response to thrombin differs between the genotypes.

In this study, we evaluated the association of platelet PlA polymorphism with the development of CAD, coronary narrowing, and MI in a prospective autopsy series of middle-aged, white Finnish men who had died suddenly or violently. This population is particularly suitable for genetic association studies of coronary heart disease because Finns have 1 of the world’s highest death rates due to CAD, and the population is genetically young and relatively isolated.

Methods

Prospective Autopsy Series of Middle-Aged Men

The original study population comprised a prospective series of 300 white men, aged 33 to 69 years, who were subjected to a medicolegal autopsy at the Department of Forensic Medicine, University of Helsinki, from 1991 to 1992. This autopsy series included 42% of all deaths of those <65 years of age during those years in the area of Helsinki and its surroundings. The reason for the medicolegal autopsy was unexpected sudden or violent death occurring outside a hospital, often unwitnessed. The cause of death was recent MI in 12.0% (n = 36), old MI in 11.0% (n = 33), sudden coronary death with no histological MI in 10.3% (n = 31), other cardiac causes 5.3% (n = 16), other diseases 21.3% (n = 33), sudden coronary death with extension at 72°C for 60 seconds. The final extension step was at 72°C for 4 minutes. The 266 bp-product was then incubated at 37°C for 1 hour with 10 U of MspI. The resulting fragments were then separated by size in a 2% agarose gel and visualized by ethidium bromide staining.

Risk Factors for CAD

A spouse, relative, or close friend of the deceased could be interviewed in 147 cases. Among questions pertaining to risk factors for sudden death, questions were included that delineated past and recent smoking habits as well as previous illnesses. On the basis of these interviews, men were classified as smokers (n = 99) and nonsmokers (n = 32). The study was approved by the Ethics Committee of the Department of Forensic Medicine, University of Helsinki.

Measuring the Percent Stenosis in Silicone Rubber Casts of the Coronary Arteries

At autopsy, coronary angiography was performed by use of vulcanizing liquid-silicone rubber mixed with lead oxide as the contrast medium. This procedure does not dislodge attached thrombus from the vessel or the arteries. Of these recent MI cases, 16 (44.4%) were associated with acute coronary thrombosis. Old, nonfatal MI was diagnosed in an additional 33 cases, of which a macroscopic organizing thrombus was observed in 6 cases (18.2%). Thus, among these 69 men with MI, 22 (31.9%) were associated with coronary thrombosis, whereas in the remaining 47 (68.1%) men with MI, a thrombus could not be found.

Determination of PlA Genotypes

The polymorphism of cytosine/thymine in exon 2 of the GPIIIA gene was detected by polymerase chain reaction and restriction digestion. Genomic DNA (10 to 30 ng) extracted from frozen cardiac muscle samples taken at autopsy was used in each amplification. DNA was amplified with a PTC 100 (Perkin-Elmer) for 37 cycles of denaturation at 94°C for 45 seconds, annealing at 53°C for 45 seconds, and extension at 72°C for 60 seconds. The final extension step was at 72°C for 4 minutes. The 266 bp-product was then incubated at 37°C for 1 hour with 10 U of MspI. The resulting fragments were then separated by size in a 2% agarose gel and visualized by ethidium bromide staining.

Statistical Analysis

The data analysis for stenosis and areas of atherosclerotic changes was based on ANCOVA, in which the possible confounding effects of age, body mass index, diabetes, hypertension, and smoking (if data were available) were taken into account by including them into the model as covariates. The results for stenosis are based on logaritically transformed data but are also presented as crude frequencies. Significance at the 0.05 level was accepted to be statistically significant. Analysis of variance was performed with SPSS Statistical Software (1993 version). The odds ratios and their 95% confidence intervals were calculated with Confidence Interval Analysis (CIA) software on a personal computer.

Results

Prevalence of PlA Alleles

In the population of 272 men, the frequency of PlA was 87% and that of PlA was 13%. Respective frequencies for genotypes were 75.0% for PlA, 22.4% for PlA, and 2.6% for PlA. Frequencies of PlA and PlA were identical (87% and 13%) in the subpopulation of 131 men for whom interview data were available. The genotypes for the 272 men were in Hardy-Weinberg equilibrium. The frequencies of alleles in this series were similar to those reported on a population basis in Finland (A2 14%, A1 86%)33. The main characteristics of men with the PlA genotype and men with the PlA allele in the population of 272 men and in the subpopulation of 131 are presented in Table 1. No significant differences existed in these parameters between the 2 populations or between genotypes.
Coronary Narrowing and the \( \text{PlA}^{1/2} \) Polymorphism

For multivariate analysis, we chose the highest percent stenosis measured from silicone casts to represent the severity of coronary stenosis. Possession of the \( \text{PlA}^{2} \) allele was significantly \((P<0.01)\) associated with less-severe coronary stenosis. This association remained significant \((P<0.05)\) when interview data were brought into the model (Table 2), and the association was also significant in the group of men with no interview data. No further association was found for the \( \text{PlA}^{1} \) polymorphism in comparison of 1-vessel disease to multiple-vessel stenosis.

To discover the effect of the \( \text{PlA}^{1} \) genotype on the progression of stenosis, we divided the men into 3 groups on the basis of measurements of the coronary silicone rubber casts: men with smooth, healthy coronary arteries or narrowings <25%, men with moderate coronary narrowing between 25% and 50%, and men with severe stenosis of >50% in any of the main coronary artery trunks. There was a significant, gradual decrease in the \( \text{PlA}^{2} \)-positive genotype (Figure 1) across the range from the first group of men with stenosis <25% to the third group with >50% stenosis (OR 0.47, 95% CI 0.23 to 0.99), and also when comparing the second group with stenosis between 25% and 50% to the third group (OR 0.68, 95% CI 0.35 to 1.4). This association remained significant and similarly gradual after we adjusted the interview data and also in men with no interview data.

Coronary Atherosclerosis and \( \text{PlA}^{1/2} \) Polymorphism

The highest percentage for each morphological change in total vessel area in the 3 vessels was chosen to represent the severity of that individual variable for atherosclerosis. The mean percent areas of fatty streaks \((P=0.6)\), raised fibrous lesions \((P=0.2)\), and complicated lesions \((P=0.2)\) showed no association with \( \text{PlA}^{1} \) polymorphism (Table 2). However, when the results were adjusted for interview data in the subgroup of 131 men, those possessing the \( \text{PlA}^{2} \) allele

### TABLE 1. Characteristics by \( \text{PlA}^{1/2} \) Genotype in the Study Population of 272 Men and in the Subpopulation of 131 Men With Interview Data

<table>
<thead>
<tr>
<th></th>
<th>All A1/A1</th>
<th>A2/A1 + A2/A2</th>
<th>All A1/A1</th>
<th>A2/A1 + A2/A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>204</td>
<td>68</td>
<td>95</td>
<td>36</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>52.4 (SD 9.5)</td>
<td>51.2 (SD 9.7)</td>
<td>53.1 (SD 10.0)</td>
<td>50.6 (SD 10.5)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.0 (SD 4.8)</td>
<td>25.4 (SD 5.3)</td>
<td>25.2 (SD 4.9)</td>
<td>25.8 (SD 5.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td>76 (80%)</td>
<td>23 (64%)</td>
<td>76 (80%)</td>
<td>23 (64%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>27 (28.9%)</td>
<td>13 (36.1%)</td>
<td>10 (10.5%)</td>
<td>3 (8.3%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10 (10.5%)</td>
<td>3 (8.3%)</td>
<td>10 (10.5%)</td>
<td>3 (8.3%)</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac diseases</td>
<td>80</td>
<td>28</td>
<td>37</td>
<td>15</td>
</tr>
<tr>
<td>Other diseases</td>
<td>40</td>
<td>16</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Violent death</td>
<td>84</td>
<td>24</td>
<td>38</td>
<td>11</td>
</tr>
</tbody>
</table>

BMI indicates body mass index.

### TABLE 2. Association of \( \text{PlA}^{1} \) Genotype With Morphometrically Measured Lesions of Corona...
had significantly (P<0.05) larger areas of complicated lesions in their coronary arteries compared with the PI_A1 homozygotes (Table 2). To further analyze this association, we divided the crude area of complicated lesions by the crude summed areas of fatty and fibrous lesions (ie, the plaque area) to obtain the proportion of complicated lesions in the coronary plaques. In men with the PI_A2 allele, the complicated area in the coronary plaques was also significantly (P<0.05) larger.

MI and PI_A1/A2 Polymorphism
We could find no direct association between MI and the PI_A polymorphism before (P=0.9) or after (P=0.5) adjusting for the interview data. However, the prevalence of the PI_A2 allele was significantly higher (P<0.001) in men with an MI caused by coronary thrombosis than in men with an MI without thrombosis. Of the 22 men with an MI and coronary thrombosis, 11 had the PI_A2 allele, whereas in the 47 men with MI without thrombosis, it was present in only 6 (OR 6.6, 95% CI 2.1 to 22.8; Figure 2). When interview data was included in the analysis (data available for 40 of the cases with MI), the association of the PI_A2 allele still showed a significant association (P<0.005) with MI and thrombosis (Table 3). The association was also significant in the group of men with no interview data.

Discussion
Here we present the results of the first autopsy study on the association of PI_A polymorphisms with coronary atherosclerosis, coronary narrowing, and MI. We found that possession of the PI_A2 allele was associated with less-narrowed coronary arteries and a larger area of complicated lesions, both in coronary arteries and coronary plaques, as well as MI due to coronary thrombosis. This suggests an association for the PI_A polymorphism with the development of CAD. Because our measurements were made directly from the coronary arteries, the stage of CAD and the percent stenosis, as well as the presence of MI and coronary thrombosis, could be determined reliably. Our approach thus circumvents many of the problems encountered in phenotyping clinical patients with CAD or MI. The postmortem measurements of stenosis in our study may correlate well angiographically measured stenosis because we measured stenosis by using a vulcanized rubber model of the arteries, not by histological examination.34 Weiss et al17 were the first to report an association between the PI_A polymorphism and MI. They found that the PI_A2 allele was positively associated with MI and that this association was even stronger in their group of patients 60 years old. This finding has been supported by other studies,19,20,22 but conflicting results have also been reported18,21 in similar series comprising patients with MI as well as in a large, prospective series of >14 000 men.23

Previous studies proposing an association between MI and the PI_A2 allele have been criticized for a too small patient series for allelic association studies,17 for too few cases with thrombosis among patients with the PI_A2 allele,25 or for an abnormally low percentage of the PI_A2 allele among controls.17 Controversies involving these studies have also been...
ascribed to differences in the populations studied, to selection of controls, to ethnicity, and to definition of phenotype,17,19 as well as to the lack of any genetic effect in patients >60,17,24 and recently to the effect of use of aspirin.35 Another confounding factor between study populations has been the inclusion or exclusion of women. Possible sex differences in GPIIb/IIIa activation have been reported.36

Discrepancies in previous studies on the association of the PlA2 allele with MI may stem from the fact that MI does not always have a similar pathogenesis. In acute coronary thrombosis, the rupture of a vulnerable plaque with an extensive lipid core and a thin, fibrous cap accounts for two thirds of the cases, whereas the remaining one third are caused by erosion of fibrous plaques.4,37 In addition, in one quarter to three quarter of cases involving sudden cardiac death, no thrombosis can be found, and the recent MI is often due to progressive arterial narrowing, or death is caused by arrhythmias arising from old, myocardial infarct scars in the absence of a recent MI.4,37,42 The frequency of thrombosis in our MI cases was similar to a recent large autopsy study on out-of-hospital MI cases.4,37

In conclusion, we found a significant relation between possession of the PlA2 allele and slower progression of coronary artery stenosis. Our results thus support the concept of the functional importance of the PlA polymorphism. The higher prevalence of the PlA2 allele among men with MI associated with coronary thrombosis is likely due to the thinner fibrous caps of their atheromatous plaques and/or more reactive platelets. These thin-walled, vulnerable plaques are more prone to rupture and cause acute coronary thrombosis. On the other hand, in PlA1 homozygotes, intimal hyperplasia may be more extensive, resulting in progressive coronary stenosis with stable plaques and “silent” occlusion of the vessel lumen.

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References

TABLE 3. Association of PlA Genotypes With MI and the Presence or Absence of Coronary Thrombosis With P Values From Multivariate Analysis

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Men With Interview Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1/A1 A2/A1 A2/A1 + A2/A2 P</td>
<td>A1/A1 A2/A1 A2/A1 + A2/A2 P</td>
</tr>
<tr>
<td>MI and thrombosis</td>
<td>11 11 &lt;0.001</td>
<td>6 8 &lt;0.005</td>
</tr>
<tr>
<td>MI without thrombosis</td>
<td>41 6</td>
<td>24 2</td>
</tr>
</tbody>
</table>


34. Mann JM, Davies MJ. Assessment of the severity of coronary artery disease at postmortem examination: are the measurements clinically valid? Br Heart J. 1995;74:528–530.


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