Glycoprotein IIIa PlA 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 PlA1/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 PlA 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 PlA2-positive genotype and coronary artery stenosis. The frequency of possessing the PlA2 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 PlA polymorphism was not directly associated with MI, the PlA2 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 PlA2 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 PlA polymorphism is involved in the development of CAD and MI. Men with the PlA2 allele may harbor more thin-walled, vulnerable coronary plaques, plaques prone to rupture, leading to massive, fatal thrombosis. In contrast, men homozygous for the PlA1 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 (αIIbβ3 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 (β3 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 αβ3 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 PlA polymorphism of the GPIIIa gene is produced by a single point mutation in exon 2 of the GPIIIa gene, leading to substitution of leucine (PlA1) for proline (PlA2) and

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From the Medical School, University of Tampere and Tampere University Hospital, Tampere, Finland (J.M., P.J.K.); the Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland (M.P.); Biometry, University of Tampere, School of Public Health, University of Tampere and Tampere University Hospital, Tampere, Finland (P.L.); the Department of Forensic Medicine, University of Helsinki, Finland (V.S., J.P., K.L., A.P.); and the Department of Clinical Pathology and Forensic Medicine, University of Kuopio, Kuopio, Finland (P.J.K.).

Correspondence to Dr Jussi Mikkelsson, Medical School, University of Tampere, POB 607, FIN—33101 Tampere, Finland. E-mail jms56215@uta.fi

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consequent changes in the protein conformation and spatial orientation of the fibrinogen-binding region. The functional importance of the PI\(^4\) polymorphism as an important inherited risk factor for MI was first suggested by Weiss and colleagues. Subsequent studies on associations between the PI\(^{A2}\) allele and acute coronary events have, however, been controversial. More controversy was added by the recent findings that platelets from individuals possessing the PI\(^{A2}\) allele bind either less or more fibrinogen than do platelets from PI\(^{A1}\) homozygotes and also by the finding that the response to thrombin differs between the genotypes.

In this study, we evaluated the association of platelet PI\(^{A1/A2}\) 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 unwatched. 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 = 64), and violent deaths (suicides and accidents) 40.0% (n = 120). 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 its site and has been successfully used in the routine postmortem diagnosis of thrombotic and other complications after coronary artery bypass surgery. The proximal, middle, and distal stenoses of the main trunks of the left anterior descending coronary artery, left circumflex artery, and right coronary artery were measured from the cast rubber model with a mauser.

The percent stenosis was obtained by dividing the diameter (millimeters) of the artery with the greatest diameter by the diameter of the nearest proximal undamaged part of the cast model of the artery. These measurements were available for 272 cases that composed the final study population.

Measuring the Area of Atherosclerosis by Computer-Assisted Morphometry of Coronary Arteries
Coronary arteries were fixed in 10% buffered formalin and stained for fat by the Sudan IV staining method. The areas of the fatty streaks, raised fibrous lesions, and complicated lesions (with fissures, hematoma, or thrombosis) were measured by computer-assisted morphometry.

Confirmation of MI
At autopsy, the presence of MI in the series was confirmed by macroscopic and histological examination of the myocardium. Coronary thrombosis was recorded during opening of the coronary arteries after angiography. Diagnostic studies of MI were done independent of any measurements of the cast or the arteries. Of the series of 272 cases, 34 men had died of recent MI and an additional 2 had suffered an acute, fatal, occluding coronary thrombosis without histological features of acute MI, owing to their short survival time. These 36 cases were grouped together as “recent MI cases” for statistical analysis.

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 PI\(^4\) 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.

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). Ex-smokers (n = 16) were excluded from statistical analysis. Hypertension had been diagnosed before death in 50 men and diabetes in 22. The final subpopulation with interview data thus consisted of 131 men.

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 logarithmically transformed data but are also presented as crude percentages and their 95% confidence intervals were calculated with Confidence Interval Analysis (CIA) software on a personal computer.

Results
Prevalence of PI\(^{A1/A2}\) Alleles
In the population of 272 men, the frequency of PI\(^{A1}\) was 87% and that of PI\(^{A2}\) 13%. Respective frequencies for genotypes were 75.0% for PI\(^{A1/A1}\), 22.4% for PI\(^{A1/A2}\), and 2.6% for PI\(^{A2/A2}\). Frequencies of PI\(^{A1}\) and PI\(^{A2}\) 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%). The main characteristics of men with the PI\(^{A1/A1}\) genotype and men with the PI\(^{A2}\) 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 PlA1/A2 Polymorphism

For multivariate analysis, we chose the highest percent stenosis measured from silicone casts to represent the severity of coronary stenosis. Possession of the PlA2 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 PlA polymorphism in comparison of 1-vessel disease to multiple-vessel stenosis.

To discover the effect of the PlA 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 PlA2-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 PlA1/A2 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 PlA polymorphism (Table 2). However, when the results were adjusted for interview data in the subgroup of 131 men, those possessing the PlA2 allele...
had significantly ($P$<0.05) larger areas of complicated lesions in their coronary arteries compared with the PlA1 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 (i.e., the plaque area) to obtain the proportion of complicated lesions in the coronary plaques. In men with the PlA2 allele, the complicated area in the coronary plaques was also significantly ($P$<0.05) larger.

**MI and PlA1/A2 Polymorphism**

We could find no direct association between MI and the PlA polymorphism before ($P$=0.9) or after ($P$=0.5) adjusting for the interview data. However, the prevalence of the PlA2 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 PlA2 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 PlA2 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 PlA polymorphisms with coronary atherosclerosis, coronary narrowing, and MI. We found that possession of the PlA2 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 PlA 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.

Weiss et al.\(^\text{17}\) were the first to report an association between the PlA polymorphism and MI. They found that the PlA2 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,\(^\text{19,20,22}\) but conflicting results have also been reported\(^\text{18,21}\) in similar series comprising patients with MI as well as in a large, prospective series of >14,000 men.\(^\text{23}\)

Previous studies proposing an association between MI and the PlA2 allele have been criticized for a too small patient series for allelic association studies,\(^\text{17}\) for too few cases with thrombosis among patients with the PlA2 allele,\(^\text{25}\) or for an abnormally low percentage of the PlA2 allele among controls.\(^\text{17}\) 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.4,5,38–42 The frequency of thrombosis in our MI cases was similar to a recent large autopsy study on out-of-hospital ischemic heart disease.42

In their results, Ridker et al23 could confirm no connection between Pl polymorphism and cardiovascular events. Nor in our study was MI directly associated with the Pl polymorphism. However, 50% of our patients with MI associated with coronary thrombosis carried the PlA2 allele, whereas only 12.8% of the men with MI in the absence of thrombosis were PlA2-positive. We now believe that 1 explanation may be that patients with ruptured vulnerable plaques, subsequent acute thrombosis, and MI requiring thrombolysis become more or less preferentially selected for clinical hospital series. In contrast, men with MI caused by stable, stenosed plaques may die suddenly of arrhythmias and never reach the hospital. Thus, the results of Weiss and colleagues17 of a higher occurrence of PlA2 in patients with MI may have, at least in part, resulted from association of the PlA2 allele with more vulnerable coronary plaques. This might also be the possible explanation for the lack of any association in the study of Ridker et al,23 who obviously included among their MI cases both hospital infarcts and sudden out-of-hospital MI cases. On a population basis, our results are consistent with another recent study on Finnish MI survivors, which found the PlA2 allele to be associated with a higher risk for MI in a population-based sample.43 In further support of our results for coronary arteries, we also found the PlA2 allele to be associated with larger areas of complicated lesions in the abdominal aorta.44

The association between the PlA3 homozygotes and more stenotic, stable plaques may be the result of genotypic differences in the VSMC proliferation and fibrous tissue generation in the intima after endothelial injury, due to differences in the function of the endothelial/VSMC β, integrins, which mediate these responses.6,10,14,15 This hyperplastic response may be weaker in men with the PlA2 allele, and thus, the coronary plaques of men with the PlA2 allele may have thinner fibrous caps, and their plaques may be less stenotic and more prone to rupture. Genotypic differences in thrombosis may also be due to differences in platelet aggregability.17

Owing to the sudden and unexpected death, cholesterol levels of the deceased were not available because they had not been measured at any point during the lives of our cases. This is why we could not control for the possible confounding effect of serum cholesterol in the statistical analyses.

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 Pl 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 PlA3 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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