Glycoprotein IIIa $P_{1}^{A1/A2}$ Polymorphism and Sudden Cardiac Death

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OBJECTIVES
We studied the association of the $P_{1}^{A1/A2}$ polymorphism with coronary thrombosis, myocardial infarction (MI) and sudden cardiac death (SCD) in autopsied victims of sudden death. Sudden cardiac death is one of the leading symptoms of coronary heart disease in early middle age. Platelet glycoprotein (GP)IIb/IIIa fibrinogen receptors play a key role in coronary thrombosis and MI. $P_{1}^{A1/A2}$ polymorphism of the gene for GPIIIa has been previously studied in hospital MI patients. Significance of the $P_{1}^{A1/A2}$ polymorphism in victims of SCD is not known.

METHODS
The $P_{1}^{A1/A2}$ polymorphism was studied in the Helsinki Sudden Death Study comprising 700 autopsied middle-aged white Finnish men (33 to 70 years, mean 53 years) who suffered sudden or violent out-of-hospital death.

RESULTS
Prevalence of the A2 allele decreased with age in the series. This decrease was observed among victims of SCD ($n = 281$) but not in men who died violently ($n = 258$) or of other diseases ($n = 127$). Of SCD victims below 50 years, 39.7% were carriers of the A2 allele compared with 28.3% among men under 50 who died of other causes (odds ratio [OR] 2.5, $p = 0.01$). Men with acute fatal coronary thrombosis ($n = 39$) were more often (OR 3.4, $p < 0.01$) carriers of the A2 allele than were men ($n = 242$) with SCD in the absence of acute coronary thrombosis (48.7% vs. 24.4%, respectively). In addition, men with MI and recent or old thrombosis ($n = 67$) were more often (OR 3.6, $p = 0.005$) carriers of the A2 allele than were men ($n = 123$) with MI in the absence of thrombosis (44.8% vs. 20.3%, respectively). These associations were especially strong in men under 60.

CONCLUSIONS
Our results suggest that the A2 allele of the $P_{1}^{A1/A2}$ polymorphism of GPIIIa is a major risk factor of coronary thrombosis and may be one important predictor of SCD in early middle age. (J Am Coll Cardiol 2000;36:1317–23) © 2000 by the American College of Cardiology

Sudden death is one of the main complications of coronary heart disease (CHD). Approximately 50% of deaths caused by CHD are sudden and take place outside a hospital (1,2). Moreover, in 20% to 25% of all cases of CHD, sudden death is the first and only manifestation of the disease (2), being the leading first symptom of CHD in early middle age (3,4). Knowledge of factors predisposing to sudden cardiac death (SCD) instead of stable CHD is very limited. Factors that may increase the risk of SCD are cigarette smoking (5–8) and parental history of myocardial infarction (MI) (6)

Fissuring or rupture of an atheromatous plaque, subsequent platelet aggregation and thrombus formation are key events in the development of acute myocardial infarction (AMI) and sudden death in patients with CHD (10–14), even though an acute myocardial lesion can only be found in approximately half of the victims of SCD, the deaths in the other half being due to arrhythmias arising from old infarct scars (2).

Acute occluding thrombosis is usually associated with transmural MI, whereas subendocardial MI often results from slower platelet-dependent progression of preexisting coronary narrowings (15–19). These two phenotypes of MI are clinically often presented as Q-wave and non-Q-wave infarctions, respectively (20).

Platelet glycoprotein (GP)IIb/IIIa fibrinogen receptors are activated and begin binding fibrinogen after rupture or fissuring of coronary plaques, resulting in platelet aggregation and thrombus formation. In addition to platelets, GPIIIa is abundantly expressed in endothelium and in vascular smooth muscle cells (vSMC) (21,22). $P_{1}^{A1/A2}$ polymorphism of the gene for GPIIIa is responsible for a change in the protein conformation and the spatial orientation of the ligand-binding region (23). Studies on the effect of the $P_{1}^{A1/A2}$ polymorphism on platelet function in vitro (24–28) have suggested that the polymorphism is functional, whereas studies on the association of the $P_{1}^{A1/A2}$ polymorphism with MI have aroused remarkable controversy (29–47). In our previous study (29), we found that the $P_{1}^{A2}$ allele was associated with MI because of coronary thrombosis. In the present study, we extended our previous study population to include another large autopsy series in order to further study the significance and age-dependency of the $P_{1}^{A1/A2}$ polymorphism in victims of SCD.
METHODS

Prospsective autopsy series of middle-aged men. Helsinki Sudden Death Study (HSDS) was designed to study the risk factors of sudden out-of-hospital cardiac death and can be conceived as a complementary study to the national WHO MONICA Project (1,48). The HSDS study comprised two prospective consecutive series of a total of 700 white Finnish men, who were subjected to a medicolegal autopsy at the Department of Forensic Medicine, University of Helsinki, in 1981 to 1982, and 10 years later in 1991 to 1992. The mean age in both series was 53 years (range 33 to 70). The reason for the medicolegal autopsy was unexpected sudden death occurring outside a hospital, often unobserved. In Finland, all cases of sudden out-of-hospital cardiac death must be subjected to medicolegal autopsy; this includes more than 40% of the total deaths in the studied age group. Thus, all men aged 33 to 70 who suffered sudden cardiac death outside hospital during the study years are included in this study. Causes of deaths were as follows: cardiac causes in 41.1% (n = 288), of which 230 (79.9%) were due to CHD without valvular disease, significant cardiomyopathy or other cardiac diseases; other diseases in 20.0% (n = 140) and suicides or accidents in 38.9% (n = 272). Characteristics of the study population are shown in Table 1. The study was approved by the Ethics Committee of The Department of Forensic Medicine, University of Helsinki.

Characteristics and phenotypes of MI. The presence of MI in the series was confirmed by macroscopic and histologic examination of the myocardium. The presence/absence of neutrophil granulocytes was considered diagnostic of an acute MI and the presence/absence of fibrous scar tissue diagnostic of an old MI. Based on the autopsy findings and nitro-blue-tetrazolium (NBT) staining (49), the MI was classified as either transmural or non-transmural. The presence of recent or organizing macroscopic coronary thrombosis was recorded while the coronary arteries were opened.

In 700 men, a total of 184 were found to have MI. Eighty-five men died of AMI with or without an old MI. Of the AMI cases, 39 were associated with coronary thrombosis, of which 24 were acute. Old nonfatal MI scar without AMI was found in an additional 99 cases, of which

### Abbreviations and Acronyms

- AMI = acute myocardial infarction
- BMI = body mass index
- CHD = coronary heart disease
- GPIIIa = glycoprotein IIIa
- HSDS = Helsinki Sudden Death Study
- MI = myocardial infarction
- NBT = nitro-blue-tetrazolium
- PHS = Physicians' Health Study
- SCD = sudden cardiac death
- vSMC = vascular smooth muscle cells

### Table 1. Characteristics of the Deceased in Relation to Causes of Death

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>Under 60</th>
<th>Violent Death</th>
<th>Other Dis.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Under 60</td>
<td>Violent Death</td>
<td>Other Dis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 288)</td>
<td>(n = 234)</td>
<td>(n = 100)</td>
<td></td>
</tr>
<tr>
<td>Age (SD)</td>
<td>56.0 (8.6)</td>
<td>49.2 (9.4)</td>
<td>53.5 (8.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Body mass index (SD)</td>
<td>25.9 (5.1)</td>
<td>23.8 (4.0)</td>
<td>23.5 (5.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Proportion of smokers</td>
<td>139/177 (78.5%)</td>
<td>125/164 (75.2%)</td>
<td>87/100 (76.0%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Alcohol consumption (SD)</td>
<td>125.4 (6.2)</td>
<td>105 (6.2)</td>
<td>96 (6.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>64/110 (31.6%)</td>
<td>49/84 (37.5%)</td>
<td>15/26 (30.8%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>31/110 (27.9%)</td>
<td>24/57 (21.0%)</td>
<td>7/13 (53.8%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Acute MI</td>
<td>80/110 (72.7%)</td>
<td>55/84 (66.0%)</td>
<td>25/37 (70.0%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Old infarct scar</td>
<td>121/110 (110.0%)</td>
<td>75/84 (89.0%)</td>
<td>46/37 (70.0%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Genotype</td>
<td>A1/A1</td>
<td>203</td>
<td>120</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>A2/A1</td>
<td>68</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2/A2</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

MI = myocardial infarction; SD = standard deviation.
a macroscopic organizing thrombus was observed in 14 cases and an acute thrombus without AMI in 5 cases. An additional 10 men had suffered an acute, fatal occluding coronary thrombosis without histologic features of MI, due to their short survival time. These 10 men were included among MI cases. Of a total of 194 men with MI, 68 men (35.1%) were associated with coronary thrombosis, whereas MI in the remaining 126 men (64.9%) was due to severe coronary stenosis without thrombosis; acute thrombus was found in 39 cases. The prevalence of men with SCD due to CHD is shown in Figure 1. Men with thrombosis and MI were, on average, younger (mean 56.6 years) compared to men with MI in the absence of thrombosis (mean 59.2 years). Acute MI was more often found and an old infarct scar less often found in men with thrombosis compared with men having MI without thrombosis (data not shown).

**DNA extraction and Pl A1/A2 genotyping.** In the 1981–1982 series DNA was extracted from paraffin-embedded samples of cardiac muscle using a method described by Isola et al. (50). In the 1991–1992 series DNA isolation was performed from frozen (–70°C) cardiac muscle samples by the standard phenol-chloroform method. The polymorphism of cytosine/thymidine in exon II of the glycoprotein IIIa gene was detected by PCR and restriction digestion. Primer sequences and PCR protocol have been previously described in detail (29). Genotyping was successful in 666 cases. In the 1991–1992 series 99.7% of the samples were successfully genotyped, whereas 91.8% of the samples from the 1981–1982 series could be genotyped.

**Risk factors of coronary artery disease and sudden death.** A spouse, relative or close friend of the deceased could be interviewed in 500 (71.4%) cases. Questions delineated past and recent smoking and drinking habits as well as previous illnesses. On the basis of these interviews, men were classified as smokers (n = 353) and nonsmokers (n = 88). Ex-smokers (n = 67) were included in the class of smokers for statistical analysis. Average daily alcohol consumption of the deceased was calculated from information given by the interviewed persons. One bottle of beer, glass of wine and shot of spirits were each considered to contain 12 g of pure alcohol and to equal one drink. On the basis of questions on previous illnesses, 107 men had suffered from hypertension and 113 men from diabetes.

**Statistical analysis.** Characteristic differences between causes of deaths were bivariately analyzed with Student t-tests (Table 1). Analyses of the effect of genotype on MI with/without thrombosis and comparisons between acute thrombosis cases and other SCD victims were based on logistic regression, where the confounding effects of age, body mass index, smoking, alcohol consumption (in grams), hypertension and diabetes were taken into account by including them in the model as covariates. We also analyzed the trend for the prevalence of genotypes in different age groups and groups of causes of deaths using chi-square tests and logistic regression (Tables 2–4). All data analyses were performed both with and without the interview data. The computation was carried out with STATISTICA/WIN (Version 5.0, Statsoft Inc., Tulsa, Oklahoma) and BMDP Statistical Software on a SUN/UNIX mainframe.

**RESULTS**

**Prevalence of Pl A1/A2 alleles.** Frequencies of PlA1 and PlA2 in the genotyped population of 666 men were 0.85 and 0.15.

**Table 2.** Distribution of Pl A1/A2 Genotypes in Relation to Causes of Sudden Death

<table>
<thead>
<tr>
<th></th>
<th>&lt;50 Years</th>
<th>50–60 Years</th>
<th>&gt;60 Years</th>
<th>p Value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1/A1</td>
<td>A2+</td>
<td>A1/A1</td>
<td>A2+</td>
</tr>
<tr>
<td>All causes</td>
<td>173</td>
<td>79 (31.3%)</td>
<td>169</td>
<td>64 (27.5%)</td>
</tr>
<tr>
<td>SCD</td>
<td>41</td>
<td>27 (39.7%)</td>
<td>79</td>
<td>28 (26.2%)</td>
</tr>
<tr>
<td>Violent</td>
<td>100</td>
<td>40 (28.6%)</td>
<td>57</td>
<td>28 (32.9%)</td>
</tr>
<tr>
<td>Other diseases</td>
<td>32</td>
<td>12 (27.3%)</td>
<td>33</td>
<td>8 (19.5%)</td>
</tr>
</tbody>
</table>

SCD = sudden cardiac death.
0.15, respectively. The allele frequencies were almost identical in both autopsy series. Genotype frequencies were 73.1% for PlA1/A1, 24.2% for PlA1/A2, and 2.7% for PlA2/A2. Frequencies of PlA1 and PlA2 were identical (0.85 vs. 0.15) in the subpopulation with interview data. On population basis the frequency in Finland of A2 allele is 0.14 and the frequency of A2-positive genotypes is 26.5% (51), which is almost identical to the frequencies in our series, 0.15% and 26.9%, respectively.

**Association of PlA1/A2 genotypes with the causes of sudden death.** The frequency of A2 allele was similar among cases of SCD, violent deaths and deaths due to other diseases (p > 0.1). However, the frequency of the A2 allele decreased with age in the series (p < 0.05). Frequency of the A2 allele in men dying suddenly under the age of 50 (n = 252) was 31.3%, and it decreased to 20.0% in men over 60 (n = 181) (Tables 1 and 2). This decrease was observed only among cases of SCD, in whom the A2 allele was found in 39.7% of the 68 men under 50, in 26.2% of the 107 men between 50 and 60 and in 21.7% of the 106 men over 60 (p < 0.05). In cases of violent deaths and deaths due to other diseases, the frequency of men with A2 allele did not change significantly with age (p > 0.1) (Table 2). When we compared the groups with different causes of death in men under 50, the A2 allele was associated with an increased risk of SCD compared to death due to violent causes or other diseases (OR 2.5, 95% CI 1.2 to 5.3; p = 0.01).

**Coronary thrombosis and PlA1/A2 polymorphism.** In cases of fatal acute thrombosis (n = 39), 19 (48.7%) were carriers of A2 allele compared with 59 (24.4%) of the 242 men with SCD without acute thrombosis (OR 3.4, 95% CI 1.5 to 6.3; p < 0.01). In men under 60, 17 (60.7%) of the 28 men with acute fatal thrombosis had the A2 allele and it was found in 38 (25.9%) of the 147 men with SCD without acute thrombosis (OR 4.6, 95% CI 2.0 to 11.2; p < 0.0005) (Table 3). Thirty (44.8%) of the 67 men with MI and acute or old coronary thrombosis carried the A2 allele, whereas it was found in 25 (20.3%) of the 123 men with MI in the absence of thrombosis (OR 3.6, 95% CI 1.4 to 9.2; p = 0.005). In men under 60, 23 (60.5%) of the 38 men with MI and coronary thrombosis possessed the A2 allele compared with 11 (17.5%) of the 63 men with MI in the absence of thrombosis (OR 8.0, 95% CI 2.3 to 28.2; p = 0.001) (Table 4). The associations of the A2 allele with thrombosis weakened in men over 60 (p = 0.1).

Even though the number of A2 homozygotes was too small for conclusions, coronary thrombosis was present in three of the four A2 homozygotes with MI, whereas it was found in 37 (27.4%) of the 135 A1 homozygotes with MI (p = 0.07; OR 7.95, 95% CI 0.8 to 80.5). The above associations of the A2 allele with thrombosis were similar both in men with interview data on smoking, alcohol consumption, diabetes and hypertension and in the excluded men without interview data (data not shown).

The results were similar and independently significant in both the 1981–1982 and the 1991–1992 series. The A2 allele was found in 19 (42.2%) of the 45 men with MI due to thrombosis in the 1981–1982 series and in 11 (50%) of the 22 men with MI due to thrombosis in the 1991–1992 series (29), whereas the A2 allele was present in 19 (25.0%) of the 76 men with MI in the absence of thrombosis in the 1981–1982 series and in 6 (12.8%) of the 47 men with MI in the absence of thrombosis in the 1991–1992 series (29).

**DISCUSSION**

**Major findings of the study.** We found that the A2 allele of the PlA1/A2 polymorphism of the gene for GPIIIa was associated with acute fatal coronary thrombosis. The A2 allele was also overrepresented in individuals with MI and acute or old thrombosis. These associations were especially strong in men under the age of 60. In addition, the A2 carriers under the age of 50 were at an increased risk of SCD. This suggests that the A2 allele may be a possible predictor of SCD due to coronary thrombosis in early middle age or is in linkage disequilibrium with another nearby gene.

**Genetics of MI and SCD.** The role of genetic factors is considered to be very important in sudden cardiac death and first MI; positive family history is an independent risk factor for primary cardiac arrest (52) and sudden death (6,9). The importance of genetic factors is also supported by the fact that the incidence of first MIs has not decreased, even though effective primary prevention has resulted in decreased CHD morbidity and mortality (53).

**Previous studies on association of the PlA1 polymorphism with MI.** Possible explanations for differences in previous studies on MI include the fact that in studies in which an association was found, the risk is increased in younger men (30–32,34,37), cases of Q-wave infarction (33,37) and smokers (37). Smoking has been suggested to interact with the PlA polymorphism (37,54). Almost all of our MI cases were smokers. Zotz et al. (34) also found that although the A2 allele is strongly associated with MI in young men in the
acute phase, no association was found when they analyzed the survivors of MI one year after the event. This is the most likely explanation for the lack of effect in the studies (38,39,44,45) in which MI cases were survivors analyzed from six months up to several years after the event.

Anderson et al. (36) also found a modest association of the PlA1 polymorphism with MI, and they had both acute cases and survivors as their case patients. In the study by Marian et al. (46) there was a trend towards an association, but no subgroup analysis was performed for their MI patients under 60. The explanation for the lack of effect in the studies by Osborn et al. (40) and Scaglione et al. (41) remains unclear, although one-third of the cases in the latter study had non-Q-wave infarction. Sperr et al. (43) studied only CHD patients. Control patients differed significantly from the normal population in the study by Samani et al. (47). The study by Ridker et al. (42) has previously been criticized for the low prevalence of traditional risk factors and low incidence of MI cases (55). The PHS study population is also ethnically mixed (56), which is a major confounder in genetic association studies (57). In addition, half of the men were randomized to aspirin, which is a confounder as it has been shown that platelets of men with the A2 allele are more responsive to the effect of aspirin than are platelets of A1 homozygotes (25,58).

Basic pathology of MI in relation to association studies of prothrombotic factors. We believe that some of the differences in the previous results may also be due to heterogeneity in the basic pathology of MI. Transmural (often Q-wave) infarction is nearly always caused by an occluding fibrin-rich (red) coronary thrombus (17,19,20,59), whereas in most (75% to 80%) patients with unstable angina pectoris who develop non-transmural (usually non-Q-wave) infarction, occluding fibrin-rich thrombi are absent (17,19,20,60,61) and when thrombotic material is present (in approximately 50% of cases), it is often composed of platelets (mural white thrombus) adhered to preexisting stenotic lesions (16,18,19,60). Q-wave infarction is a markedly more frequent MI type in early middle age (62,63) often because of the lack of hemodynamically significant collateral vessels (18,64). This and the limitations and strengths of the autopsy study are discussed in our previous study (29). The absence of thrombosis in many of our MI cases may be due to this difference, but it is also possible that in some cases of old MI there has been a thrombus that has been totally recanalized. However, in cases of acute MI this is highly unlikely, as complete spontaneous reperfusion usually takes weeks to occur (65,66).

Possible use of the A2 allele as a risk marker in primary prevention of MI/SCD. In the light of the two findings of the A2 allele being a risk factor for MI and possibly SCD, especially in early middle age, and platelets of men with the A2 allele responding to aspirin up to 10 times as effectively as those of men with A1 homozygotes (25,58), it seems possible that young asymptomatic men (especially smokers) with the A2 allele might benefit from aspirin as primary prevention of MI/SCD, as the A2 allele seems to represent an otherwise uncontrolled risk factor for coronary events (67,68). However, the A2 allele as a risk factor is likely to be most detrimental in a cluster of other risk factors such as smoking and dyslipidemia. The PlA1/A2 polymorphism may represent one factor in the future screening protocol of prothrombotic genetic markers used to evaluate risks in middle-aged men with family history of MI/SCD as well as those with clustering of conventional risk factors.

Conclusions. We conclude that the PlA2 allele of GPIIIa is strongly associated with coronary thrombosis and may predict the risk of SCD in early middle age. This association of A2 allele with coronary thrombosis may explain why the A2 allele is overrepresented in hospital series of young patients with impending Q-wave infarctions.

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REFERENCES


