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Lack of relationship between the Pl^{A1}/Pl^{A2} polymorphism of platelet glycoprotein IIIa and premature myocardial infarction

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Abstract

Background The Pl^{A1}/Pl^{A2} polymorphism of the platelet glycoprotein IIIa has been variably associated with an increased risk of coronary thrombosis.

Materials We investigated the linkage between the Pl^{A1}/Pl^{A2} polymorphism and the risk of myocardial infarction in 98 patients who suffered their first myocardial infarction at the age of 45 years or less and 98 well-matched control subjects without coronary artery disease. Lipid parameters were measured using conventional methods of clinical chemistry; Pl^{A} genotypes were determined by polymerase chain reaction and restriction enzyme digestion.

Results There was no significant difference in the prevalence of Pl^{A2}-positive genotypes (either Pl^{A1}/Pl^{A2} or Pl^{A2}/Pl^{A2}) between patients and control subjects ($\chi^2 = 0{\cdot}66$, d.f. $= 1$, $P = 0{\cdot}41$).

Conclusions These results suggest that the Pl^{A2} polymorphism of the platelet glycoprotein IIIa does not contribute to the genetic susceptibility to premature myocardial infarction.

Keywords Glycoprotein IIb-IIIa polymorphism, myocardial infarction, risk factors.
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Introduction

The role of endovascular thrombus formation in myocardial infarction (MI) and unstable angina is well established [1]. Platelets are a key component in the process of thrombus formation, and the inhibition of platelet aggregation is associated with a marked reduction in morbidity and mortality associated with unstable coronary syndromes [2,3]. Activation of the platelet membrane receptor glycoprotein IIb/IIIa to bind fibrinogen, von Willebrand factor or fibronectin represents the final common pathway for platelet aggregation, and a recent study has shown a protective effect of a non-peptide glycoprotein IIa/IIIb antagonist in unstable angina [4]. The gene for glycoprotein IIIa (GP IIIa) is highly polymorphic; the substitution of cytosine for thymidine at position 1565 in exon 2 of the glycoprotein IIIa gene results in either a leucine or proline at position 33 of the mature GP IIIa and accounts for the Pl^{A1}/Pl^{A2} polymorphism [5]. Recently, a linkage between the Pl^{A2} polymorphism of the platelet GP IIIa and the risk of MI has been reported [6]. However, conflicting results on the association of this polymorphism with the risk of coronary artery disease (CAD) have been reported [7–10], and data on an Italian population are still lacking. One possible reason for the discrepant results could be the elevation of the mean age of the patients. In fact, if this polymorphism represents an inherited risk factor for MI, its prevalence should be higher in subjects with premature coronary events. To test this hypothesis, the Pl^{A1}/Pl^{A2} polymorphism of the platelet GP IIIa was studied in a group of 98 consecutive Italian patients who suffered their first episode of MI at the age of 45 years or less.

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Patients and methods

Selection of patients and control subjects

Platelet GP IIIa genotyping was performed on 98 consecutive patients aged 45 years or less who were admitted to the coronary care unit (S. Giovanni Battista Hospital and

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Table 1 Baseline characteristics of case patients and control subjects.

Clinical characteristic	Case patients ($n = 98$)	Low-risk case patients ($n = 33$)*	Control subjects ($n = 98$)	*P*-value
Age (years)	40 ± 4	40 ± 5	41 ± 4	NS
Sex (M/F)	94/4	30/3	94/4	NS
Diabetes mellitus (%)	4	3	4	NS
Positive familiar history for CAD (%)	36	33	11	< 0·0002
Smokers (%)	78	67	57	< 0·006
Hypertension, *n* (%)	22	0	5	< 0·0003
BMI (kg m^{-2})	26 ± 3	24 ± 3	25 ± 3	NS
Total cholesterol (mmol L^{-1})	5·7 ± 1·2	5·0 ± 0·8	5·2 ± 1·0	< 0·0004
Triglyceride (mmol L^{-1})	1·8 ± 1·3	1·3 ± 0·5	1·2 ± 0·7	< 0·000001
LDL-cholesterol (mmol L^{-1})	3·6 ± 1·2	2·9 ± 0·7	3·2 ± 0·9	< 0·0005
HDL-cholesterol (mmol L^{-1})	1·3 ± 0·2	1·4 ± 0·2	1·5 ± 0·4	< 0·00002
Apolipoprotein B (mmol L^{-1})	3·0 ± 0·9	2·3 ± 0·5	2·4 ± 0·6	< 0·000001

Plus/minus values are means ± SD. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* Low-risk case patients had MI and only one of the following risk factor for CAD: dyslipidaemia (total cholesterol level > 6·20 mmol L^{-1} or LDL-cholesterol > 3·36 mmol L^{-1} or HDL-cholesterol < 1·03 mmol L^{-1} or triglycerides level > 2·25 mmol L^{-1}), body mass index > 27 kg m^{-2}, hypertension, current or former smoker and diabetes mellitus.

Maria Vittoria Hospital, Turin, and the Hospital of Savigliano) with a diagnosis of MI according to the criteria of the World Health Organization [11]. None of the patients had a previous MI. All patient underwent coronary angiography.

Platelet GP IIIa genotyping was also performed on 98 control subjects matched with the case patients for sex, diabetes status and age, but without history or electrocardiographic signs of CAD. The control subjects were selected from the medical records of the patients admitted to the hospitals. Demographic data were obtained from each subject and included age, sex, smoking history, blood pressure, diabetes status, personal and family history of CAD. A positive family history was considered if the case patients or the control subjects had a first-degree relative with coronary disease at the age of 55 years or less for men or at the age of 65 years or less for women. Hypertension was defined as a systolic or diastolic blood pressure higher than 140/90 mmHg or the use of antihypertensive drugs, according to the JNC-V criteria [12].

Among the case patients, a low-risk subgroup for CAD was defined by the presence of no more than one of the following risk factors: dyslipidaemia [total cholesterol level > 6·20 mmol L^{-1} or low-density lipoprotein (LDL)-cholesterol > 3·36 mmol L^{-1} or high-density lipoprotein (HDL)-cholesterol < 1·03 mmol L^{-1} or triglyceride level > 2·25 mmol L^{-1}], body mass index greater than 27 kg m^{-2}, hypertension, current or former smoker and diabetes mellitus.

Laboratory analyses

Serum glucose, total cholesterol, HDL-cholesterol, apoB and triglycerides were measured using conventional methods of clinical chemistry. LDL-cholesterol was calculated using the Friedewald equation.

Genomic DNA was amplified by polymerase chain reaction (PCR) using primers flanking the part of the genomic DNA that contains the C→T substitution at position 1565 in exon 2 of the glycoprotein IIIa gene; the C→T substitution creates a *Msp*I restriction enzyme cleavage site that allows the Pl^{A1} allele to be distinguished from the Pl^{A2} allele [13]. The PCR products were digested with *Msp*I (MspI, MBI Fermentas, Lithuania), generating two fragments of 279 bp and 197 bp in the presence of the Pl^{A1} allele, whereas three fragments of 197 bp, 173 bp, and 106 bp were observed when the Pl^{A2} allele was present. The product of digestion was electrophoresed through a 10% non-denaturing polyacrylamide gel and visualized using silver stain.

Statistical analysis

The sample size was established after a pilot study indicating that the frequency of the Pl^{A2} allele in the Turin metropolitan area was not higher than 15%; in the case patients we expected a frequency of Pl^{A2} alleles twice that of control subjects, according to the study of Weiss *et al.* [6]; we set the size of the sample at 98 subjects to limit the beta-error to 0·2, with a one-sided error of 0·05.

Data are expressed as means ± SD. For normally distributed variables, the between-group differences were analysed using analysis of variance. Triglycerides values were logarithmically transformed when a parametric test was used, in view of their skewed distribution. Allele frequencies were estimated by the gene counting method, and the Hardy–Weimberg equilibrium was tested using the chi-square test; the chi-square test was also used to compare the distribution of Pl^{A} genotypes in the case patients and the control group and for comparison among groups of non-continuous variables.

Table 2 Distribution of Pl^{A1}/Pl^{A2} genotypes and allele frequencies in case patients and control subjects.

Variable genotype	All case patients No. (%)	Low-risk case patients No. (%)	Control subjects No. (%)
Pl^{A1}/Pl^{A1}	75 (76)	26 (79)	70 (71)
$Pl^{A1}Pl^{A2}$	19 (19)	7 (21)	27 (26)
Pl^{A2}/Pl^{A2}	4 (4)	0	1 (1)
$Pl^{A1}/Pl^{A2} + Pl^{A2}/Pl^{A2}$	23 (23)	7 (21)	28 (27)
Allelic frequency			
Pl^{A1}	0·86	0·89	0·85
Pl^{A2}	0·14	0·11	0·15

Results

Study participants

The 98 case patients had a Q-wave infarction or a non-Q-wave infarction (67·3% and 32·7% respectively); the myocardial infarction was anterior (50·5%), lateral (41·7%) or inferior (7·8%). The characteristics of the study population are shown in Table 1. The risk factor profile of the case patients differed significantly from that of control subjects; the risk profile of the case patients with a low risk of CAD was similar to that of the control group and significantly different from the risk profile of the whole case patients group (data not shown). The low-risk group of CAD consisted of 33 patients: five without risk factors and 28 with one risk factor (22 smokers, five patients with dyslipidaemia, one diabetic patient).

Frequencies of Pl^A genotypes

The Pl^A genotype distributions in case patients and control subjects were in Hardy–Weinberg equilibrium. The results of platelet GP IIIa Pl^A typing of case patients and control subjects are shown in Table 2 (genotype and allele frequencies). There was no significant difference in the prevalence of Pl^{A2}-positive genotypes (either Pl^{A1}/Pl^{A2} or Pl^{A2}/Pl^{A2}) between case patients and control subjects ($\chi^2 = 0{\cdot}66$, d.f. = 1, $P = 0{\cdot}41$) or between the low-risk patients and control subjects ($\chi^2 = 0{\cdot}68$, d.f. = 1, $P = 0{\cdot}40$). The odds ratios (ORs) were calculated as measures of the association of the Pl^{A2}-positive genotypes with MI. The ORs indicated no significant increase in risk of MI when either the case patients (OR 0·8, 95% CI 0·4–1·4) or the low-risk group (OR 0·7, 95% CI 0·3–1·7) were compared with control subjects. The prevalence of Pl^{A2}-positive genotypes between case patients with and without family history for CAD was not significantly different ($\chi^2 = 0{\cdot}15$, d.f. = 1, $P = 0{\cdot}69$).

Discussion

In a group of patients from the Baltimore area, Weiss and colleagues [6] found that the frequency of the Pl^{A2} allele was 2·1 times higher in patients with MI or unstable angina and more predictive for coronary thrombosis than established risk factors such as hypertension, smoking, hypercholesterolaemia or diabetes. The association of platelet GP IIIa Pl^{A2} gene polymorphism with MI was strongest in patients younger than 60 years, and this finding has been confirmed in another study by Carter *et al.* [9]. These results, however, have been challenged by other studies on European [7] and US patients [8,10], in which GP IIIa Pl^{A2} gene polymorphism was found not to be significantly related to MI.

If the Pl^{A1}/Pl^{A2} polymorphism represents a genetic coronary risk factor, one should expect a greater prevalence of this polymorphism among subjects in whom coronary events occur at a young age and/or in absence of other risk factors. However, our case–control study of Italian patients revealed no difference in Pl^{A1}/Pl^{A2} polymorphism in the GP IIIa gene between patients who suffered from MI at the age of 45 years or less and matched control subjects. Interestingly, our case patient group has a mean age almost 15 years younger than that studied by Weiss *et al.* [6]. A relatively high mean age of appearance of the first episode of MI may be a selection bias when a putative risk factor is inheritable; the Swedish Twin Study showed that inherited risk factors for CAD are relevant only for individuals with premature heart disease and that the genetic effect decreases at older ages [14]. Furthermore, we did not find an excess of polymorphism Pl^{A2} even in a subgroup of case patients at a lower risk of CAD or in patients with a positive family history for CAD. These results of subgroup analysis should, however, be considered with caution because the failure to find a positive result may be due to a type II error. Some differences between the study by Weiss *et al.* [6] and ours may account for the discrepancy in the results. The prevalence of diabetes is considerably lower among our subjects than among those studied by Weiss *et al.* (4% vs. 30%), suggesting a possible association of the Pl^{A2} polymorphism with diabetes. In addition, the difference in allelic distribution in Italian and other populations could be another possible explanation for the conflicting results. However, the frequency of the Pl^A genotypes in our control group is similar to those found in a north European population-based study [13] and to those of the control group of other studies on Pl^{A1}/Pl^{A2} polymorphism [6–10].

The present study does not provide evidence that the Pl^{A1}/Pl^{A2} polymorphism confers any significant increase in the risk of premature MI. This conclusion is strengthened by the choice of case patients who suffered from their first MI at a very young age and does not support the belief that the platelet GP IIIa Pl^{A2} gene polymorphism can be used as a predictor of MI.

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