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Genetic Polymorphisms of Platelet Glycoprotein Ia and the Risk for Premature Myocardial Infarction

Effects on the Release of sCD40L During the Acute Phase of Premature Myocardial Infarction

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OBJECTIVES The aim of this research was to evaluate the effect of genetic polymorphisms C807T and G1648A of platelet glycoprotein Ia (GPIa), on the risk for myocardial infarction (MI) and on the release of soluble CD40 ligand (sCD40L) during the acute phase of MI and one year after the event.

BACKGROUND C807T and G1648A polymorphisms affect the density of GPIa on platelet surface, but their effect on the risk for MI and the release of sCD40L is unknown.

METHODS The study population consisted of 219 patients with premature MI and 389 controls. One year after the event, 67 patients and 232 controls were recalled for the follow-up study.

RESULTS The risk for MI in 807TT was 2.296 (95% confidence interval [CI]: 1.187 to 4.440) $p < 0.05$ versus CC + CT, 2.269 (95% CI: 1.085 to 4.745) $p < 0.05$ versus CC, and 2.135 (95% CI: 1.080 to 4.219) $p < 0.05$ versus CT. During the acute phase of MI, sCD40L was higher in 807CT + TT compared with 807CC ($p < 0.01$), an effect persisting after one year ($p < 0.01$). The carriage of 807T allele was an independent predictor for sCD40L during the acute phase of MI ($\beta = 9.442$ [standard error (SE): 2.526], $p = 0.001$) and in the same patients one year later ($\beta = 8.282$ [SE: 2.044], $p = 0.001$). In healthy individuals, 807T allele was associated with higher sCD40L levels compared with 807CC ($p < 0.05$), only among those with von Willebrand factor greater than or equal to median.

CONCLUSIONS Genetic polymorphism C807T increases the risk for premature MI. 807T allele is an independent predictor for sCD40L levels during the acute phase of premature MI as well as one year after the event, while it is associated with elevated sCD40L levels in healthy subjects, only in the presence of high von Willebrand levels. (J Am Coll Cardiol 2006;47:1959–66)

Platelets play a major role in the pathophysiology of acute myocardial infarction (MI) because they adhere to the vessel wall at the site of a vulnerable coronary plaque, and initiate thrombotic occlusion of the coronary vessel leading to myocardial ischemia and infarction (1). At the initial stages of MI, circulating platelets exposed to subendothelial collagen are activated and secrete several thrombotic and proinflammatory molecules (1). Soluble CD40 ligand (sCD40L), an important proinflammatory molecule secreted by activated platelets (2), is also involved in plaque destabilization (3,4) and thrombus formation (5,6) during the acute phase of MI.

Platelet adhesion to subendothelial collagen during acute coronary syndromes is mediated by specific receptors (integrins) on their surface, such as glycoproteins Ia/IIa (GPIa/IIa) (1,7–9) and VI (GPVI) (9). Therefore, as a platelet receptor for collagen, GPIa/IIa plays an important role in platelet adhesion and activation during MI (10). Two genetic polymorphisms on GPIa, the C807T and A1648G, have been associated with increased cardiovascular risk in some populations (11,12), although their role is largely controversial (13,14). The presence of 807T allele has been associated with increased expression of GPIa on platelet surface (15) and increased thrombogenicity (16), despite the fact that it does not affect the molecular structure of GPIa. G1648A polymorphism on GPIa gene leads to a Glu/Lys^{505} substitution in GPIa molecule, and it is responsible for the human platelet alloantigen system Bra/Brb (17), but its effect on the functional status of GPIa is unclear.

In the present study, we examined the potential role of C807T and G1648A polymorphisms of platelet GPIa, as risk factors for the development of premature MI. We also examined whether the presence of these polymorphisms modifies platelet activation and affects the release of sCD40L during the acute phase of premature MI, as well as one year after the event in the same patients, and in healthy individuals. Moreover, we examined the ability of von Willebrand factor (vWF) (a surrogate marker of endothelial injury and activation [18] and a direct stimuli for the platelet surface translocation of CD40L [19]) to modify the effect of these two polymorphisms on the release of sCD40L.

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
CI = confidence interval
GPIa = glycoprotein Ia
GPVI = glycoprotein VI
HDL = high-density lipoprotein
MI = myocardial infarction
PCI = percutaneous coronary intervention
sCD40L = soluble CD40 ligand
STEMI = ST-segment elevated myocardial infarction
vWF = von Willebrand factor

METHODS

Study population. This study enrolled a total number of 606 subjects: 219 patients of both genders with a first event of premature MI, and 387 controls of similar age and gender (Table 1). Patients aged <49 years old, consecutively admitted to coronary care unit with a first event of ST-segment elevated myocardial infarction (STEMI) were recruited. The diagnosis of STEMI was defined according to the guidelines (20). All patients were thrombolized and received the same standard therapy including aspirin, low-molecular-weight heparin, statin, intravenous nitrates, and angiotensin-converting enzyme inhibitors as appropriate.

Controls had no clinical evidence of coronary heart disease, stroke, or any atherosclerotic disease (based on a detailed medical history, physical examination followed by electrocardiogram). All patients and controls were Greek Caucasians, habitants of Athens in Greece. Demographic characteristics are presented in Table 1.

Protocol. The study was approved by the institutional ethics committee, and an informed consent was given by all the participants. Blood samples were obtained at the 24th h after admission for genotyping and for evaluation of serum levels of sCD40L and plasma levels of vWF. All patients were followed up prospectively for one year, and a well-defined group of patients with a stable clinical condition for at least the previous six months was recalled for the follow-up study one year after the event, and new blood samples were obtained. Exclusion criteria from the follow-up study were the existence of any inflammatory or infective disease, liver or renal disease, malignancy, heart failure defined as ejection fraction <45%, history of deep vein thrombosis or pulmonary embolism, while patients receiving non-steroid or anti-inflammatory drugs or anticoagulants (other than aspirin) were also excluded. To avoid possible influences of percutaneous coronary intervention (PCI), catheter-related acute phase reactions, or coronary artery bypass grafting (CABG) operation on the measured parameters during the acute phase of MI or at the follow-up, patients who required urgent PCI, coronary angiographic investigation, or CABG within the first 24 h of the event or <6 months before the follow-up study were also excluded. None of the patients included in the follow-up study received GPIIb/IIIa antagonists during and after the

Table 1. Demographic Characteristics and Genotype Distribution

	MI Cases	Control Subjects	p Value
Number of subjects	219	387	
Age (yrs)	47.97 ± 0.66	46.85 ± 0.41	0.229
Gender (male/female)	205/14	354/33	0.218
Current smokers/ex-smokers	195/4	220/13	0.0001
Hypercholesterolemia, n (%)	116 (52.9)	121 (31.3)	0.0001
Hypertension, n (%)	90 (41.1)	127 (32.8)	0.043
Diabetes mellitus, n (%)	25 (11.4)	43 (11.1)	0.548
BMI (kg/m^2)	27.84 ± 0.258	26.87 ± 0.22	0.006
Cholesterol levels (mg/dl)	251.42 ± 52.75	227.34 ± 2.60	0.0001
HDL (mg/dl)	35.83 ± 0.63	47.61 ± 1.51	0.0001
Triglycerides (mg/dl)*	138 (99–193)	112 (75–146)	0.0001
Fasting glucose (mg/dl)	115.42 ± 52.05	91.88 ± 1.12	0.0001
Genotype distribution C807T			
807CC, n (%)	73 (33.3)	143 (36.9)	
807CT, n (%)	112 (51.2)	214 (55.3)	
807TT, n (%)	34 (15.5)	30 (7.8)	
Recessive model			0.014†
807TT, n (%)	34 (15.5)	30 (7.8)	
807CC + 807CT, n (%)	185 (84.5)	357 (92.2)	
A1648G			
1648GG, n (%)	161 (73.5)	300 (77.6)	
1648AG, n (%)	50 (22.8)	77 (19.9)	
1648AA, n (%)	8 (3.7)	10 (2.6)	
Recessive model			0.395†
1648AA, n (%)	8 (3.7)	10 (2.6)	
1648GG + 1648AG, n (%)	211 (96.3)	377 (97.4)	

Values expressed as means ± SEM. *Value expressed as median (25th–75th percentile values); †Indicates p by chi-square analysis after adjustment for age, gender, and classic risk factors.
BMI = body mass index; HDL = high-density lipoprotein; MI = myocardial infarction.

acute MI. Of the 219 MI patients in the first part of the study, 76 fulfilled the inclusion criteria for the follow-up study, and 67 of them agreed to participate. All patients were under standard medication as appropriate.

The control group in the follow-up study consisted of 232 individuals selected from the initial study cohort to have similar age, gender, and the major risk factors as the patient group (Table 1). This design allows safer comparisons between the groups, because it diminishes any effect of the underlying risk factors on the expression of sCD40L and endothelial activation. All the control subjects had no evidence of cardiovascular disease (such as coronary artery disease or stroke), and they had normal electrocardiograms and exercise stress tests. The same general exclusion criteria used for patients were also applied to the control group. All the participants in the follow-up study were asked to abstain from tobacco, alcohol, and caffeine-containing beverages during the evening before blood sampling, and they discontinued their medication for 12 h before the study. Venous blood samples were centrifuged at 3,500 rpm at 4°C for 15 min, and plasma or serum was collected and stored at −80°C until assayed.

Biochemical measurements. Routine chemical methods were used to determine serum concentrations of total cholesterol, high-density lipoprotein (HDL), and triglycerides. Enzyme linked immunosorbent assays were used for the determination of plasma levels of vWF (Asserachrom, Diagnostica Stago, Asnières sur Seine, France), and sCD40L (Bender Medsystems, Vienna, Austria).

DNA extraction and genotyping. Genomic deoxyribonucleic acid was extracted from 2 to 5 ml of whole blood using standard methods (QIAamp DNA blood kit, Qiagen, Germantown, Maryland). The detection of C807T polymorphism on GPIa gene was performed by polymerase chain reaction as previously described (11,21), with the following primers: sense 5′-ACCTTGCATATTGAATTGCTT-3′ and antisense 5′-GTGTTTAACTTGAACACATAT-3′, and the polymerase chain reaction products were digested by the *Taq*I restriction enzyme (New England BioLabs, Ipswich, Massachusetts). Digested fragments were visualized on 3% agarose gel, after ethidium bromide staining under ultraviolet light. Similarly, G1648A polymorphism was detected using the following primers: sense 3′-GTTGATGTGGATAAA GACACC-5′ and antisense 3′-ATGATGAAATGTAA ACCATAC-5′, and the polymerase chain reaction products were digested by Mnl I restriction enzyme (New England Biolabs), electrophoresed with 2.5% agarose gel, and visualized under ultraviolet using ethidium bromide staining, as previously described (12).

Statistical analysis. We tested the allele frequencies conformed to Hardy-Weinberg equilibrium proportions by use of the chi-square test. According to the applied power analysis based on previous reports (22), a total sample size of 230 subjects (cases and controls) was adequate to detect at least a 15% difference in the frequency of the examined alleles between groups, achieving statistical power 90% at $p = 0.05$ probability level (p value). The number of both cases and controls was, however, increased ($n = 606$) to permit further analyses in subgroups. Qualitative variables are presented as absolute and relative frequencies. Genotype and allele frequencies were compared between groups by chi-square analysis, and conditional multiple logistic regression analysis was used to estimate odds ratios and 95% confidence intervals (CI), of the development of MI as a function of C807T or A1648G polymorphisms. All odds ratios were adjusted for age, gender, and atherosclerosis risk factors, such as hypertension, diabetes mellitus, hypercholesterolemia, obesity, and smoking. Continuous variables were tested for normal distribution by the Kolmokorov-Smirnov test. Normally distributed variables are presented as mean values ± SEM, while not normally distributed variables were log-transformed for analysis and are presented in the non-logarithmic format as median (25th to 75th percentiles values). The log-transformed variable (triglyceride levels) was confirmed to have normal distribution. Comparisons of continuous variables between patients and controls or between the genotypes were performed by the unpaired Student *t* test if normally distributed, or by the Mann-Whitney *U* test if non-normally distributed. The change of sCD40L from baseline (acute phase) to the one-year follow-up value and the comparison of this change between the different genotype groups was assessed by analysis of variance (ANOVA) for repeated measurements. Comparisons of the sCD40L level between the "control group" and the "group of patients during the acute phase" or between the "control group" and the "group of patients at follow-up" were performed by using ANOVA for multiple comparisons followed by Bonferroni correction.

Stepwise multivariate analysis was undertaken with sCD40L as dependent variable and clinical factors (age, gender, hypertension, diabetes, smoking, hypercholesterolemia, and obesity, cholesterol levels, triglyceride levels, HDL levels) and C807T or A1648G polymorphisms as independent variables. We included as independent variables in each multivariate model the respective polymorphism (CC/CT + TT for C807T or GG/AG + AA for A1648G) as well as those that showed a significant association with the dependent variable in univariate analysis, at 15% significance level. A backward elimination procedure was applied in all multivariate models (using $p < 0.05$ as the threshold for removing a variable from the model).

All reported p values are based on two-sided tests and compared with a significance level of 5%. SPSS version 12.0 (SPSS Inc., Cary, North Carolina) software was used for all the statistical calculations.

RESULTS

Clinical characteristics and genotype distribution. The study population consisted of 606 subjects. The population characteristics, serum lipid and glucose levels at baseline, and genotype frequencies are presented in Table 1. There

Table 2. Odds Ratios as Estimates of Relative Risk for Premature Myocardial Infarction in Carriers of 807T and 1648A Alleles

Genotypes	Crude Odds Ratios (95% CI)	p Value	Odds Ratios (95% CI)*	p Value
C807T				
TT vs. CC + CT	2.187 (1.298–3.686)	0.003	2.296 (1.187–4.440)	0.014
TT vs. CC	2.220 (1.260–3.911)	0.006	2.269 (1.085–4.745)	0.030
TT vs. CT	2.165 (1.260–3.722)	0.005	2.135 (1.080–4.219)	0.029
CT vs. CC	1.025 (0.713–1.474)	0.893	0.991 (0.626–1.568)	0.968
A1648G				
AA vs. GG + AG	1.429 (0.556–3.677)	0.459	1.820 (0.459–7.219)	0.395
AA vs. GG	1.491 (0.577–3.851)	0.410	1.888 (0.460–7.750)	0.378
AA vs. AG	1.232 (0.455–3.334)	0.681	1.630 (0.415–6.404)	0.484
AG vs. GG	1.210 (0.808–1.813)	0.355	1.018 (0.603–1.718)	0.946

p values by chi-square analysis. *Adjusted for age, gender, and classic risk factors.
CI = confidence interval.

was a significant difference in the prevalence of 807TT genotype in cases compared with controls ($p < 0.05$) (Table 1). The risk for MI in TT was significantly increased compared with all the other genotypes (Table 2), and remained significantly elevated after adjustment for age, gender, and classic risk factors (Table 2). On the other hand, although the prevalence of 1648AA genotype in cases was slightly higher compared with controls, this difference did not reach statistical significant, even after adjustment for age, gender, and classic risk factors for atherosclerosis (Tables 1 and 2).

The angiographic extent of coronary artery disease in patients as well as demographic characteristics, genotype distribution, lipid levels, and medication of patients and controls in the follow-up study are presented in Table 3. In the patient group, 14 had CABG operation and 29 had PCI at least six months before the follow-up study, while there was no difference in genotype distribution among those patients.

Polymorphisms C807T and A1648G and sCD40L variations during MI. Serum levels of sCD40L were significantly increased during the acute phase of MI in both carriers of 807T (807TT + 807CT) allele (by 4.12 μg/l [95% CI: 3.00 to 5.24] $p < 0.0001$ vs. follow-up) and in 807CC homozygotes (by 2.36 μg/l [95% CI: 1.0.81 to 3.638] $p < 0.001$ vs. follow-up). The change in sCD40L from rest to the value during the acute phase of MI was greater among the carriers of the T allele compared with 807CC homozygotes ($p < 0.05$). Furthermore, sCD40L level was significantly higher in carriers of the 807T allele compared with 807CC homozygotes, both during the acute phase of MI and at follow-up (Fig. 1). Carriers of the 807T allele had higher levels of sCD40L both during the acute phase of MI and one year after the event, compared with healthy controls ($p < 0.01$ for both vs. control, after Bonferroni correction) (Fig. 1). However, patients with 807CC genotype had higher levels of sCD40L compared with healthy individuals only during the acute phase of MI, but not one year after the event ($p < 0.05$ for acute phase vs. control subjects, after Bonferroni correction) (Fig. 1). Among healthy individuals, there was no significant difference in sCD40L levels between carriers of 807T and 807CC homozygotes (Fig. 1). Healthy individuals were divided according to median vWF levels (median [25th to 75th percentile] for healthy controls: 72.84% [52.35 to 90.96]), to examine whether the effect of C807T polymorphism on the release of sCD40L was dependent on the degree of endothelial damage. Indeed, it was found that although there was no significant difference in sCD40L between genotypes among subjects with vWF < median (mean vWF: 50.5 ± 1.2%), in the presence of high vWF levels (vWF ≥ median, mean vWF: 97.7 ± 2.1%) carriers of 807T allele had significantly higher levels of sCD40L compared with 807CC homozygotes ($p < 0.05$) (Fig. 2a). Among patients at follow-up, carriers of the 807T allele had higher levels of sCD40L compared with 807CC independently from vWF levels (median [25th to 75th percentile] of vWF at follow-up: 87.1% [72.84 to 96.60]), as presented in Figure 2b. Similarly, carriers of the 807T allele also had higher levels of sCD40L during the acute phase of MI compared with 807CC, independently from vWF levels (median [25th to 75th percentile] of vWF levels during the acute phase: 92.12% [71.1 to 112.0]).

Serum levels of sCD40L during the acute phase of MI were similar between carriers of the 1648A allele (1648AA + 1648AG) (10.80 ± 2.57 μg/l) and 1648GG homozygotes (11.17 ± 1.09 μg/l, p = NS), while one year after the event there was still no difference in sCD40L levels between the genotypes (7.94 ± 1.80 μg/l in 1648A carriers vs. 7.26 ± 0.86 μg/l in 1648GG, p = NS). Similarly, among healthy individuals, carriers of 1648A allele had similar levels of sCD40L (4.87 ± 0.65 μg/l) compared with 1648GG homozygotes (5.19 ± 0.28 μg/l, p = NS), although significantly lower than patients during the acute phase ($p < 0.01$ for all genotypes) or at follow-up ($p < 0.05$ for all genotypes). Plasma levels of vWF did not modify the potential effect of A1648G polymorphism on the variations of sCD40L in any of the study groups.

Multivariate analysis. In multivariate analysis, it was found that during the acute phase of premature MI, the presence of 807T allele was an independent predictor for sCD40L levels ($\beta = 9.442$ [SE: 2.526], $p = 0.001$). Other

Table 3. Demographic Characteristics, Medication, and Genotype Distribution Among the Participants in the Follow-Up Study

	MI Cases	Control Subjects	p Value
Number of subjects	67	232	
Distribution of C807T			
CC/CT/TT	20/33/14	84/127/21	
Distribution of A1648G			
GG/AG/AA	51/13/3	182/40/10	
Demographic characteristics			
Age (yrs)	48.51 ± 0.76	50.60 ± 0.81	0.253
Gender (male/female)	63/4	213/19	0.795
Hypercholesterolemia, n (%)	32 (47.7)	74 (31.9)	0.042
Active smokers, n (%)	25 (37.3)	128 (55.2)	0.001
Hypertension, n (%)	31 (46.2)	94 (40.5)	0.575
Diabetes mellitus, n (%)	9 (13.4)	36 (15.5)	0.702
BMI (kg/m^2)	27.15 ± 0.43	26.53 ± 0.26	0.186
Cholesterol levels (mg/dl)	185.29 ± 6.00	178.25 ± 2.17	0.237
HDL (mg/dl)	42.51 ± 1.71	47.46 ± 1.89	0.293
Triglycerides (mg/dl)	135.0 (103.5–166.0)	112.0 (80.0–154.0)	0.013
Fasting glucose (mg/dl)	109.61 ± 6.13	91.21 ± 1.58	0.0001
Medication			
Nitrates, n (%)	2 (3.0)	0 (0)	0.050
Angiotensin receptor antagonists, n (%)	15 (22.4)	19 (8.2)	0.003
Angiotensin-converting enzyme inhibitors, n (%)	42 (62.7)	70 (30.2)	0.001
Diuretics, n (%)	24 (35.8)	53 (23.3)	0.039
Calcium-channels inhibitors, n (%)	9 (11.9)	11 (4.7)	0.045
Beta-blockers, n (%)	46 (68.7)	14 (6.0)	0.001
Statins, n (%)	47 (70.1)	76 (32.8)	0.001
Anti-diabetic medication, n (%)	9 (13.4)	33 (14.2)	0.870
Aspirin, n (%)	66 (98.5)	33 (14.2)	0.001
Amiodarone, n (%)	2 (3.0)	0 (0)	
Extend of CAD at baseline (vessels with >50% stenosis)			
1 vessel	35 (52.2)	—	
2 vessels	19 (28.3)	—	
3 vessels	13 (19.4)	—	
CABG operation after the event, n (%)	14 (25.3)	—	
PCI after the event, n (%)	29 (43.2)	—	

Values expressed as mean ± SEM or median (25th–75th percentile values) unless indicated otherwise.
BMI = body mass index; CABG = coronary artery bypass grafting operation; CAD = coronary artery disease; HDL = high-density lipoprotein; MI = myocardial infarction; PCI = percutaneous coronary intervention.

predictors of sCD40L levels during the acute phase of MI were smoking (β = 8.206 [SE: 3.204], p = 0.014), diabetes mellitus (β = 10.207 [SE: 4.359], p = 0.024), and obesity (β = 4.758 [SE: 2.114], p = 0.030). In the same subjects one year after the event, the presence of the 807T allele was still an independent predictor for sCD40L levels (β = 8.282 [SE: 2.044], p = 0.001), as well as diabetes mellitus (β = 7.843 [SE: 3.523], p = 0.031) and obesity (β = 4.463 [SE: 1.714], p = 0.013). Among healthy controls, the presence of T allele was not an independent predictor for sCD40L levels.

DISCUSSION

In the present study, we examined the effect of genetic polymorphisms C807T and A1648G of platelet GPIa, on the risk for premature MI. We have shown that only C807T polymorphism is independently associated with increased risk for MI. We have also shown that the presence of 807T allele is an independent predictor for the release of sCD40L during the acute phase of MI, an effect that persists in the same patients one year after the event. In healthy subjects, the presence of 807T allele was associated with increased levels of sCD40L only in the presence of high vWF levels.

Genetic polymorphisms C807T and G1648A on platelet GPIa, and the risk for premature MI. Evidence suggests that platelet GPIa/IIa (integrin $\alpha 2\beta 1$) and GPVI (9) are major platelet receptors for collagen (7,8). Glycoprotein Ia/IIa also modifies the GPIb-IX, vWF axis (8) and participates in platelet adhesion to subendothelial collagen and activation. Therefore, any variation in platelet GPIa density could become a potential risk factor for hemostatic abnormalities (8). Kunicki et al. (23) first described that genetic polymorphisms within the GPIa gene are associated with variations in platelet GPIa/IIa expression levels. Silent polymorphism C807T (codon Phe^{224}) in GPIa gene affects the density of this integrin on platelets surface, in a way that 807T allele expresses higher levels of GPIa than 807C allele (15,24). On the other hand, G1648A polymorphism leads

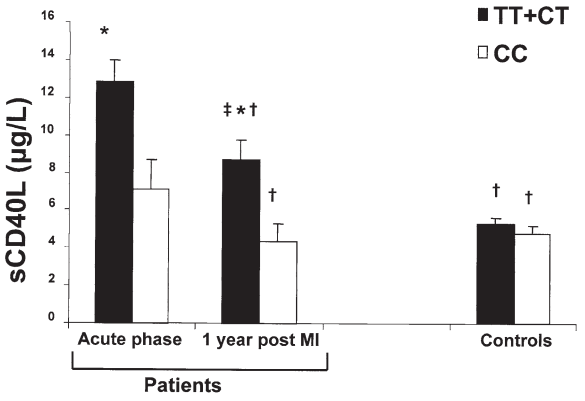

Figure 1. Serum levels of soluble CD40 ligand (sCD40L) were significantly higher in carriers of the 807T allele both during the acute phase of myocardial infarction (MI) and one year after the event in the same subjects, compared with 807CC homozygotes. Although sCD40L level was increased in all genotypes during the acute phase of MI compared with the values one year after the event, the increase in 807T carriers was greater than the increase in 807CC homozygotes ($p < 0.05$). Levels of sCD40L were similar between the genotypes in the control group. **Solid bars** = carriers of the 807T allele (807TT + 807CT); **open bars** = 807CC homozygotes. *$p < 0.01$ vs. CC; †$p < 0.01$ vs. acute phase (after Bonferroni correction); ‡$p < 0.01$ vs. controls (after Bonferroni correction).

to a Glu/Lys^{505} substitution in the GPIa molecule, and it is responsible for the human platelet antigen system Bra/Brb (17). Although these polymorphisms do not affect the functional status of GPIa, they seem to influence its density on the platelet surface (15,25). It has been proposed that C807T polymorphism may affect the stability of mRNA or it may be linked to genetic alteration(s) located in regulatory regions of the GPIa gene, while G1648A could affect the stability of the protein or the formation of the GPIa/IIa complex, thus determining the number of GPIa/IIa complexes (14).

Genetic polymorphism C807T has been associated with the development of stroke (26) or MI in younger individuals (11,27,28) and especially among young smokers (11). Recent evidence suggests that 807T allele may also predict recurrent acute coronary syndromes (29), possibly as a result of the increased thrombogenicity accompanying the presence of 807T allele. However, other studies failed to demonstrate such an effect on the risk for MI (13,22,30,31), or restenosis (32) and events (33) after angioplasty. Similarly, the effect of G1648A polymorphism on the risk for MI is also controversial. The first reports suggested that the presence of 1648A allele may be a risk factor for MI (12) although this relationship is also questioned (14). Based on these controversial reports, it can be hypothesized that other parameters such as the specific population characteristics may modify the effect of this polymorphism on the risk for MI.

In the present study, we have shown that only C807T polymorphism was an independent risk factor for the development of premature MI. Homozygosity for the 807T allele was associated with an increased risk for MI (odds ratio: 2.2 compared with 807C homozygotes). These results were compatible with previous reports suggesting an increase of the risk for MI by 2.6 to 3.3 (11,27) in 807T homozygotes.

Genetic polymorphisms C807T and G1648A on platelet GPIa, and the release of sCD40L during the acute phase of MI. Platelet activation is a key feature in the pathogenesis of acute coronary syndromes (1,34,35). Exposure of circulating platelets to subendothelial collagen leads to their activation and the secretion of several thrombotic and proinflammatory molecules during the acute phase of MI (1). Soluble CD40 ligand, an important proinflammatory molecule secreted by activated platelets (2), is involved in plaque destabilization (3,4) and thrombus formation (3,5,6) during the acute phase of MI (3,6). In addition, ligation of CD40 mediates an array of proinflammatory effects in subjects with risk factors for atherosclerosis such as smokers (36), patients with diabetes mellitus (37) or hypercholesterolemia (38), including the expression of cytokines, chemokines, adhesion molecules, matrix metalloproteinases, and growth factors (2,39), and it is a strong predictor for clinical outcome in patients with advanced atherosclerosis (3). It has

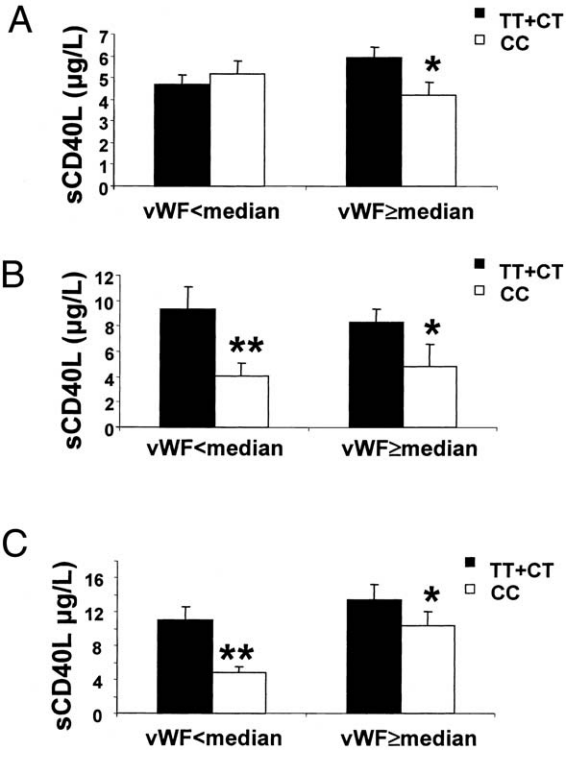

Figure 2. **(A)** In the control group, carriers of the 807T allele (807TT + 807CT) had significantly higher levels of soluble CD40 ligand (sCD40L) compared with 807CC homozygotes in the presence of high levels of von Willebrand factor (vWF ≥ median), while there was no difference in sCD40L between genotypes among healthy individuals with vWF < median (vWF median in controls: 72.84%). **(B)** Carriers of the 807T allele had significantly higher levels of sCD40L compared with 807CC one year after myocardial infarction (MI) independently from plasma vWF levels (vWF median for follow-up group: 87.1%). **(C)** Similarly, the presence of the 807T allele was associated with higher levels of sCD40L during the acute phase of MI, independently from vWF levels (median for vWF during the acute phase of MI: 92.12%). **Solid bars** = Carriers of the 807T allele (807TT + 807CT); **open bars** = 807CC homozygotes; *$p < 0.05$ and **$p < 0.01$ vs. carriers of T allele.

been recently reported by Schafer et al. (40) that a decrease of nitric oxide production in healthy volunteers increases CD40L expression on the platelet surface, suggesting that vascular endothelium is a key regulator of platelet CD40L expression.

Platelet activation is partly mediated by the adhesion to subendothelial collagen, an effect mediated by GPIa (7,8). In vitro evidence suggests that 807T allele of GPIa gene is associated with increased platelet reactivity (41) possibly due to its effect on the density of GPIa on platelets surface, an effect confirmed at a clinical level, in platelets from patients after CABG (42). However, the effect of GPIa and its genetic polymorphisms on the release of sCD40L has not been investigated until now.

In the present study, we hypothesized that GPIa may influence the release of sCD40L during the acute phase of MI, due to its role in the platelet-collagen interactions leading to platelet activation, and we examined whether C807T polymorphism (which modifies the density of GPIa on platelet surface) could affect sCD40L levels during the acute phase of premature MI. Moreover, in order to examine the ability of endothelial integrity to mask the effect of C807T on the release of sCD40L, we divided the control group according to plasma levels of vWF (a marker of endothelial cell integrity) (18). We found that the presence of 807T allele was an independent predictor of sCD40L during the acute phase of MI, an effect persisting (although in a lower degree) in the same subjects one year after the event. Interestingly, we also found that even in the control group, carriage of the 807T allele was associated with higher levels of sCD40L compared with 807C homozygotes only among those with vWF greater than or equal to the median, an effect not observed among those with vWF less than the median. This finding suggests that the presence of the 807T allele may participate in the pathogenesis of atherosclerosis and acute coronary syndromes only in the presence of endothelial damage, where platelet-collagen interactions have a significant contribution to platelet activation. A possible explanation for this effect is that endothelial injury and exposure of subendothelial collagen to circulating platelets seems to be the necessary dynamic precondition for any effect of C807T polymorphism or platelet GPIa density on the variability of sCD40L. As described by Tamura et al. (19), vWF can also induce CD40L expression on platelet surface especially under increased sympathetic stimulation that is present during MI. It is also likely that vWF-induced CD40L expression occurs in platelets adhering to exposed subendothelial matrix substances, such as collagen, and that the vWF-GPIb-alpha interaction induces thrombus formation on the collagen surface. Although existing evidence suggests that vWF induces CD40L expression by binding to GPIb-alpha (43), it is also likely that the elevation of vWF observed during MI (44) could induce higher platelet reactivity/activation and release of sCD40L in the presence of 807T allele in GPIa gene, because this glycoprotein modifies the GPIb-IX-vWF axis (8), although the underlying mechanisms require further investigation.

Conclusions. In the present study, we have shown that C807T but not G1648A polymorphism of platelet GPIa gene is an independent risk factor for premature MI. We have shown that the presence of 807T allele is an independent predictor for the variations of sCD40L both during the acute phase of MI as well as one year after the event in the same population. Furthermore, the presence of 807T allele affects the release of sCD40L in healthy subjects only in the presence of higher vWF levels.

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