FIC Federazione Italiana di Cardiologia
Italian Federation of Cardiology

Lipoprotein-associated phospholipase A_2 single-nucleotide polymorphisms and cardiovascular events in patients with coronary artery disease

Giuseppe Maiolino[a], Livia Lenzini[a], Luigi Pedon[b], Maurizio Cesari[a], Teresa Maria Seccia[a], Anna Chiara Frigo[c], Giacomo Rossitto[a], Brasilina Caroccia[a] and Gian Paolo Rossi[a]

Aims **We tested the hypothesis that variations in the *PLA2G7* gene encoding the lipoprotein-associated phospholipase A_2 (Lp-PLA_2), an enzyme deemed to have proatherogenic activity, affect the Lp-PLA_2 levels and predicts cardiovascular events.**

Methods **Using a prospective cohort study design, we investigated incident cardiovascular events as a function of the *PLA2G7* gene for rs1805017, rs1805018, and rs1051931 single-nucleotide polymorphisms (SNPs) in 643 randomly selected white patients from the GENICA Study, who at baseline underwent coronary angiography, measurement of Lp-PLA_2 mass and activity. Cardiovascular event-free survival was compared across the genotypes by Cox regression, propensity score matching, and haplotype analysis.**

Results **The rs1805018 SNP did not follow the Hardy–Weinberg equilibrium and was not further explored. The rs1805017 GG genotype had a lower Lp-PLA_2 mass and a higher Lp-PLA_2 activity, thus suggesting that this SNP is functional. Long-term follow-up (median 7.8 years) was obtained in 75% of the cohort and allowed recording of incident cardiovascular events in 25.8% of the patients. On Cox regression analysis, the common rs1805017 GG genotype predicted acute myocardial infarction (AMI) [hazard ratio 1.75, 95% confidence interval (CI) 1.03–2.99, $P = 0.041$]; this finding was confirmed on propensity score matching (82.6% AMI-free survival in GG vs. 94.4% in GA + AA, $P = 0.003$). The rs1805017 and rs1051931 G/G haplotype was also associated with AMI (52.7 vs. 42.2%, $P = 0.026$) and cardiovascular event incidence (49.5 vs. 41.7%, $P = 0.025$).**

Conclusion **In high-risk coronary artery disease patients of European ancestry, the *PLA2G7* rs1805017 GG genotype is associated with increased Lp-PLA_2 plasma activity and AMI.**

J Cardiovasc Med 2015, **16**:29–36

Keywords: atherosclerosis, coronary artery disease, lipoprotein-associated phospholipase A_2, prognosis, single-nucleotide polymorphism

[a]Department of Medicine – DIMED - Internal Medicine 4, [b]Divisione di Cardiologia, Ospedale di Cittadella, Cittadella and [c]Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Padua, Italy

Correspondence to Gian Paolo Rossi, MD, FACC, FAHA, Department of Medicine -DIMED- Internal Medicine 4, University Hospital, via Giustiniani, 2, 35126 Padova, Italy
Tel: +39 049 821 7821; fax: +39 049 821 7873;
e-mail: gianpaolo.rossi@unipd.it

Received 8 July 2013 **Revised** 19 February 2014
Accepted 19 February 2014

Introduction

The *PLA2G7* gene encodes the lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a Ca^{2+}-independent enzyme mostly produced by monocytes/macrophages,[1] which hydrolyzes oxidized phospholipids on the surface of low-density lipoproteins (LDLs),[2] producing oxidized fatty acids and lysophosphatidylcholine. These substances are pro-atherogenic,[2,3] as they induce expression of adhesion molecules and cytokines, downregulate nitric oxide, increase oxidative stress, and induce endothelial cell apoptosis.[4,5]

By contrast, atherosclerosis,[6,7] stroke,[8] and dilated cardiomyopathy[9] are associated with a point mutation (rs76863441) near the active site of the enzyme,[10] which is found in 4% of the Japanese[11] and implies undetectable plasma Lp-PLA_2 activity. However, the opposite results were found in a Korean population for the same variant.[12] A proatherogenic role of Lp-PLA_2 is supported by results from meta-analysis and prospective studies,[13,14] but it remains uncertain whether variations in the *PLA2G7* gene are associated with altered Lp-PLA_2 activity or cardiovascular risk in non-Asian populations, as data on three nonsynonymous single-nucleotide polymorphisms (SNP)s of the *PLA2G7* gene (rs1805017, rs1805018, and rs1051931) are conflicting. The rs1805017 G and rs1051931 A alleles were found to be associated with coronary artery disease (CAD),[15] but only the latter SNP correlated with severity of CAD in the Taiwanese people,[16] and with myocardial infarction in case–control studies.[15,16] No such associations,[17,18] or even an opposite relationship,[19] were seen in white patients harbouring the

1558-2027 © 2015 Italian Federation of Cardiology DOI:10.2459/JCM.0000000000000057

rs1051931 A allele.[19–21] Moreover, two recent meta-analyses including more than 10 000 individuals of European ancestry also found no association of any of the *PLA2G7* SNPs with a risk of CAD.[22,23]

Therefore, using a prospective cohort study design, Cox regression, and propensity score matching, which are regarded as providing more solid findings,[24] we sought to test the hypothesis that the rs1805017, rs1805018, and rs1051931 SNPs determine Lp-PLA_2 mass and activity, and predict cardiovascular events.

Materials and methods

Study participants and design

The GENICA is a prospective cohort study that enrolled consecutive white patients referred for coronary angiography. The protocol will be only briefly described as it was previously detailed.[25,26] Patient recruitment and baseline evaluation took place between 1999 and 2001. Predetermined follow-up evaluations were carried out after 5 (2004) and 10 (2009) years from the start of the recruitment. The protocol was approved by the institutional Ethics Committee (Comitato Etico dell'Azienda Ospedaliera di Padova) and all patients signed a consent form to participate in this study. Refusal to participate was the only exclusion criterion. All investigations were conducted according to the principles of the Declaration of Helsinki. Only two of the 1273 eligible patients denied consent. Information on medical history, smoking habits, presence/absence of arterial hypertension, diabetes mellitus, dyslipidaemia and current medications was gathered with a staff-administered questionnaire.[25,26] Definitions for BMI, smoking status, diabetes mellitus, impaired glucose tolerance, hypercholesterolaemia, and hypertriglyceridaemia were already reported.[25,26] Blood pressure was measured by mercury sphygmomanometer using Korotkoff phase V for diastolic, according to the WHO guidelines. Hypertension was defined as SBP of at least 140 mmHg or DBP of at least 90 mmHg, or use of antihypertensive drugs.

Coronary angiography

The measurement of left ventricular ejection fraction (LVEF) and of the CAD atherosclerotic burden (with a modified Duke Prognostic Index score) was carried out as described.[27,28]

Laboratory measurements

Patients were studied between 8.30 a.m. and noon. Blood samples were taken immediately before coronary angiography, put on ice, and centrifuged at 3000 *g* (at 4°C for 10 min). Total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, glycaemia, sodium, potassium, blood urea nitrogen, and creatinine levels were measured using conventional methods.

Lp-PLA_2 mass and activity assays were centralized at diaDexus (San Francisco, USA) and performed blindly with respect to the clinical data as described in the Supplemental Data: http://links.lww.com/JCM/A43.[14]

PLA2G7 genotyping

Blood was collected in EDTA acid and stored at −20°C until DNA was extracted using standard procedures and quantified by spectrophotometer as already reported.[26] Genotyping at the *PLA2G7* gene (NM_005084) SNPs +495 (Arg92His; rs1805017), +813 (Thr198Ile; rs1805018), and +1356 (Ala379Val; rs1051931) positions of mRNA sequence was performed with real-time PCR and TaqMan probes using the Roche LightCycler 480 Instrument and LightCycler 480 Probes Master (Roche Diagnostics Corporation) according to the manufacturer's instructions. Primers and probes sequences are reported as supplemental data (Supplemental Table 1, http://links.lww.com/JCM/A43). In a random sample of the patients, genotype results were confirmed by sequencing.

Follow-up and endpoints definition

Information on long-term outcome of the patients was gathered blind to their biochemical profile and genotype with a predefined form. Predetermined primary endpoint [cardiovascular events, including acute coronary syndrome (ACS), stroke and cardiovascular death] were defined following guidelines as reported.[14] Death due to congestive heart failure, ACS, stroke, and sudden death represented cardiovascular death.[29–31] A detailed definition of the other endpoints [acute myocardial infarction (AMI), ACS, stroke, and congestive heart failure] is provided in the Supplemental Data, http://links.lww.com/JCM/A43. All events were validated by the adjudication committee (G.P.R. and G.M.) blinded to patient data.

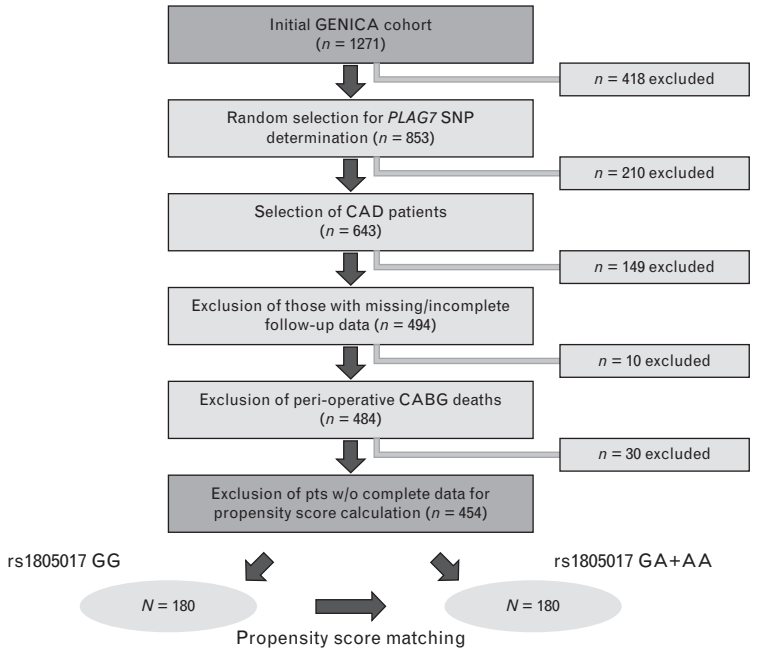
Statistical analysis

Utmost attention was put into minimizing the chances of selection, detection, and attrition bias.

The variables with a skewed distribution [serum triglycerides, high-density lipoprotein (HDL) and LDL-cholesterol, age, creatinine, modified Duke Prognostic Index score of coronary atherosclerotic burden, LVEF, Lp-PLA_2 mass] were examined after achievement of a Gaussian distribution with log or square root transformation. From the entire GENICA cohort, we selected 853 patients who were genotyped at the *PLA2G7* gene SNPs using a single random number generation. Of these, 643 had an angiographic demonstration of CAD and were further analysed (see Fig. 1).

Comparison of quantitative variables across SNPs was performed by analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. The frequencies of categorical CAD risk factors were compared with χ^2 analysis. Agreement with the Hardy–Weinberg equilibrium of the genotypes for the three SNPs was verified beforehand

Fig. 1



Data analysis flow chart. Flow chart of the study. CABG, coronary artery bypass graft; CAD, coronary artery disease.

using a χ^2 goodness-of-fit test, and SNPs that fulfilled this criterion were analysed further.

The assumption that cases lost were similar to those available at follow up for the baseline variables and genotypes was verified beforehand using multiple regression analysis. The predictors of cardiovascular events were investigated by Cox stepwise (Wald) regression analysis, using inclusion and exclusion criteria of 0.10 and 0.05, respectively. The variables were entered in blocks and the covariates meeting the exclusion criteria were removed at each step before proceeding with the next block according to Tabachnick and Fidell.[32] All blocks were sized conservatively to include a number of variables not exceeding one-tenth of the total number of cardiovascular events analysed. The following blocks were used: block 1: age and BMI; block 2: LDL and HDL-cholesterol, estimated glomerular filtration rate; block 3: statin therapy; block 4: LVEF and Duke Prognostic Index of coronary atherosclerotic burden; block 5: *PLA2G7* genotypes (SNP).

Propensity score was calculated using logistic regression analysis by including all variables that could affect the outcomes which comprised sex, age, BMI, LDL and HDL-cholesterol, triglycerides, estimated glomerular filtration rate, homocysteine, arterial hypertension, smoking habit, LVEF, the CAD Duke index score, length of follow up, past medical history, and treatment variables.[33] To adjust for the imbalanced distribution of variables between rs1805017 G homozygotes patients and the A allele carriers, we performed a greedy 1:1 matching without replacement using a calliper of 0.2 standard deviations of the logit of the propensity score.[34] The even distribution of baseline variables between genotypes was then verified by comparing the standardized differences between the matched samples[35] (supplemental Table 2, http://links.lww.com/JCM/A43). We plot the event incidence, using the Kaplan–Meier method and compared the survival for the matched set with the test proposed by Klein and Moeschberger.[36]

Statistical significance was defined as a P value of less than 0.05. SPSS 20 for Mac (SPSS Italy Inc., Bologna, Italy) was used for all analyses.

Haplotype analysis

Linkage disequilibrium testing and haplotype analysis were performed with Haploview (Vers. 4.2 at http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview)[37] using an accelerated expectation-maximization algorithm. A global test of association between haplotypes and cardiovascular events was performed by means of a χ^2 test, followed by permutation of the significant association results in order to obtain a measure of significance corrected for multiple testing bias.

Results

Clinical characteristics at baseline

Tables 1 and 2 show the distribution of genotypes and the baseline clinical characteristics across the rs1805017 and rs1051931 polymorphisms, respectively (see also

Table 1 Demographic and clinical characteristics of the individuals according to rs1805017 genotype

	rs1805017 allele			
Variable	GG ($n=299$, 53.5%)	GA ($n=214$, 38.9%)	AA ($n=47$, 7.6%)	*P*
Age (years)	63.0 ± 10	63.0 ± 10	63.0 ± 12	0.73
Sex (male, %)	75.6	80.5	83.0	0.66
Nonsmokers/smokers/Ex (%)	40/14/46	33/13/54	23/15/62	0.15
BMI (kg/m^2)	26.9 ± 4	27.1 ± 4	26.7 ± 4	0.58
Serum creatinine, (μmol/l)	91 ± 26	94 ± 27	92 ± 26	0.69
Serum K^+ (mmol/l)	4.2 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	0.77
Serum Na^+ (mmol/l)	140 ± 3	140 ± 2	139 ± 3	0.74
Heart rate (beats/min)	65 ± 9	66 ± 11	68 ± 10	0.60
SBP (mmHg)	135 ± 18	134 ± 17	132 ± 14	0.34
DBP (mmHg)	78 ± 10	77 ± 9	79 ± 9	0.28
Serum glucose (mmol/l)	6.3 ± 1.9	6.4 ± 2.4	5.8 ± 1.3	0.48
Total cholesterol (mg/dl)	205 ± 39	208 ± 46	204 ± 46	0.46
HDL-cholesterol (mg/dl)	46 ± 11	46 ± 10	47 ± 11	0.57
LDL-cholesterol (mg/dl)	131 ± 31	135 ± 37	126 ± 31	0.40
Triglycerides (mg/dl)	140 ± 71	148 ± 97	160 ± 195	0.46
Homocysteine (μmol/l)	12.4 ± 7.4	12.1 ± 6.0	13.0 ± 6.2	0.51
Left ventricular EF (%)	64 ± 14*	61 ± 14	55 ± 15*	0.015
Lp-PLA$_2$ mass (ng/ml)	365.2 ± 103**	383.5 ± 81**	393.1 ± 111	0.047
Lp-PLA$_2$ activity (nmol/ml/min)	116.9 ± 28***	113.5 ± 26	105.1 ± 24***	0.020
Atherosclerotic burden (Duke score)	37 ± 21	39 ± 21	38 ± 24	0.82
Follow-up (years)	7.1 ± 2.4	6.9 ± 2.5	7.0 ± 3.4	0.33

Results are expressed as mean ± SD or percentages; comparisons across genotypes were made by ANOVA or χ^2. BP, blood Pressure; CAD, coronary artery disease; EF, ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Bonferroni post-hoc test comparisons: *left ventricular EF GG vs. AA, $P=0.024$; **Lp-PLA$_2$ mass GG vs. GA, $P=0.038$; ***Lp-PLA$_2$ activity GG vs. AA, $P=0.020$.

supplemental Tables 3–6, http://links.lww.com/JCM/A43). For rs1805017, the G and A allele frequency was 0.73 and 0.27, respectively. For rs1051931, the G and A allele frequency was 0.67 and 0.33, respectively. For the rs1805018 polymorphism, the genotype distribution was TT = 74.7%, TC = 20.4% and CC = 4.9%, with a T and C allele frequency of 0.85 and 0.15, respectively. The three SNPs were in low linkage disequilibrium: rs1051931 and rs1805018 $r^2=0$, rs1051931 and rs1805017 $r^2=0.061$, rs1805018 and rs1805017 $r^2=0.014$.

The rs1805017 polymorphism affected the Lp-PLA$_2$ mass (Table 1), while both the rs1805017 (Table 1) and the rs1805018 SNPs influenced the Lp-PLA$_2$ activity (Supplemental Table 7, http://links.lww.com/JCM/A43). Apart from the Lp-PLA$_2$ mass and activity, the LVEF also differed ($P=0.015$) across the rs1805017, but not the rs1051931, genotypes. Moreover, there was no association between the CAD burden and the rs1805017 genotype. These findings suggest that the rs1805017 SNP is functional, as it affects the synthesis and activity of Lp-PLA$_2$.

Table 2 Demographic and clinical characteristics of the individuals according to rs1051931 genotype

	rs1051931 allele			
Variable	GG ($n=289$, 46.5%)	GA ($n=256$, 41.3%)	AA ($n=76$, 12.2%)	*P*
Age (years)	63.8 ± 10	64.0 ± 9	62.4 ± 10	0.43
Sex (male, %)	81.0	77.8	82.9	0.52
Nonsmokers/smokers/Ex (%)	31/18/51	38/12/50	36/17/47	0.20
BMI (kg/m^2)	26.7 ± 4	27.3 ± 4	27.4 ± 4	0.10
Serum creatinine (μmol/l)	96 ± 42	97 ± 80	93 ± 20	0.79
Serum K^+ (mmol/l)	4.2 ± 0.4	4.3 ± 0.4	4.2 ± 0.3	0.33
Serum Na^+ (mmol/l)	139 ± 3	140 ± 2	140 ± 3	0.29
Heart rate (beats/min)	66 ± 10	65 ± 10	68 ± 12	0.28
SBP (mmHg)	133 ± 18	134 ± 18	136 ± 21	0.47
DBP (mmHg)	78 ± 9	78 ± 10	79 ± 11	0.68
Serum glucose (mmol/l)	6.4 ± 2.4	6.3 ± 2.0	6.4 ± 2.2	0.75
Total cholesterol (mg/dl)	205 ± 44	205 ± 46	209 ± 38	0.78
HDL-cholesterol (mg/dl)	45 ± 10	46 ± 13	45 ± 12	0.89
LDL-cholesterol (mg/dl)	132 ± 35	131 ± 34	134 ± 29	0.83
Triglycerides (mg/dl)	143 ± 89	145 ± 81	144 ± 87	0.96
Homocysteine (μmol/l)	11.8 ± 6.3	12.6 ± 7.5	10.7 ± 4.6	0.10
Left ventricular EF (%)	60 ± 15	62 ± 14	62 ± 14	0.21
Lp-PLA$_2$ mass (ng/ml)	382.4 ± 98	368.3 ± 96	365.8 ± 92	0.22
Lp-PLA$_2$ activity (nmol/ml/min)	112.8 ± 26	116.2 ± 28	117.9 ± 26	0.26
Atherosclerotic burden (Duke score)	41 ± 20	38 ± 20	38 ± 19	0.25
Follow-up (years)	6.6 ± 3	6.9 ± 2	6.8 ± 2	0.59

Results are expressed as mean ± SD or percentages; comparisons across genotypes were made by ANOVA or χ^2. Using the Bonferroni post-hoc test there was a significant difference for Lp-PLA$_2$ mass between groups GG vs. GA and GG vs. AA and for Lp-PLA$_2$ activity and left ventricular EF between groups GG vs. AA. BP, blood pressure; CAD, coronary artery disease; EF, ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

On the basis of the different Lp-PLA_2 mass and activity values between the patients carrying the A allele and those homozygous for the G allele, further analyses were performed assuming the A allele to play a dominant effect, for example by comparing AA + AG vs. GG genotypes. These genotype groups were compared using the propensity score matching. Interestingly, the A allele was associated with a higher Lp-PLA_2 mass and a lower Lp-PLA_2 activity. The survival analysis was undertaken only for the rs1805017 ($\chi^2 = 0.15$, $P = $ NS) and the rs1051931 ($\chi^2 = 2.54$, $P = $ NS) SNPs, as they agreed with the Hardy–Weinberg equilibrium.

Follow-up data

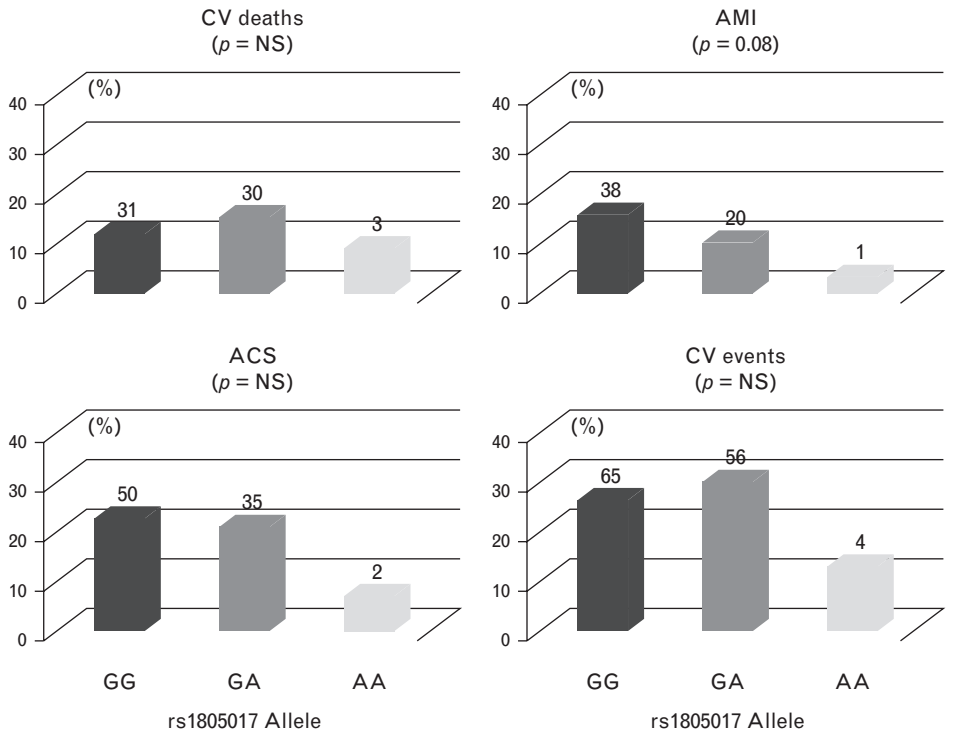
Seventy-five percent ($n = 484$ patients) of the total cohort was available for the survival analysis after exclusion of those with missing follow-up data ($n = 149$) and patients ($n = 10$) who did not meet the end-point definition for cardiovascular death as they died perioperatively due to the coronary artery bypass surgery or its complications. Multiple regression analysis showed that the cases lost and those available at follow up were similar for all baseline variables potentially affecting the outcomes, including risk factors, past medical history, therapy and genotype distribution.

The median length of follow-up was 7.8 years (interquartile range 7.4–8.3 years). On χ^2 and Kaplan–Meier analysis, we found no differences in cardiovascular death and event rates across rs1051931 genotypes (see Supplementary Figure 1 and 2, http://links.lww.com/JCM/A43). Of the 484 patients with available rs1805017 genotype data, 64 (13.2%) had a cardiovascular death, 87 (18.0%) an ACS, 59 (12.2%) an AMI and 125 (25.8%) a cardiovascular event. Figure 2 shows the cardiovascular death and events according to rs1805017 genotype.

Cox regression analysis showed that although the rs1805017 showed no association with cardiovascular death and events, the rs1805017 GG genotype predicted AMI [hazard ratio 1.75, 95% confidence interval (CI) 1.03–2.99, $P = 0.041$] along with LVEF (hazard ratio 0.97, 95% CI 0.95–0.98, $P < 0.0001$) and Duke prognostic index of coronary atherosclerotic burden (hazard ratio 1.01, 95% CI 1.00–1.03, $P = 0.045$).

To verify the robustness of this association, we performed propensity score matching analysis in the 454 patients who had complete data for all the variables necessary to compute the score. Among these patients, 180 (83%) of the 217 patients carrying the A allele, either homozygous or heterozygous, could be matched with an equal number of GG homozygous patients by propensity score. Overall, these patients had 51 (14.2%) cardiovascular deaths, 73 (20.3%) ACSs, 43 (11.9%) AMIs and 102 (28.3%) cardiovascular events. Patients carrying the GG genotype

Fig. 2



Cardiovascular events by rs1805017 single-nucleotide polymorphism. The bar graphs show cardiovascular death and events rate by GG, GA, AA rs1805017 genotype (the absolute number of events is shown above each column). ACS, acute coronary syndrome; AMI, acute myocardial infarction.

showed a similar cardiovascular death rate (87.3 vs. 92.0%, respectively, $P = NS$), a borderline significantly worse cardiovascular event-free survival (73.3 vs. 80.8%, respectively, $P = 0.066$) and a worse AMI-free survival (82.6 vs. 94.4%, respectively, $P = 0.003$) than the patients carrying the GA or AA genotypes (Fig. 3).

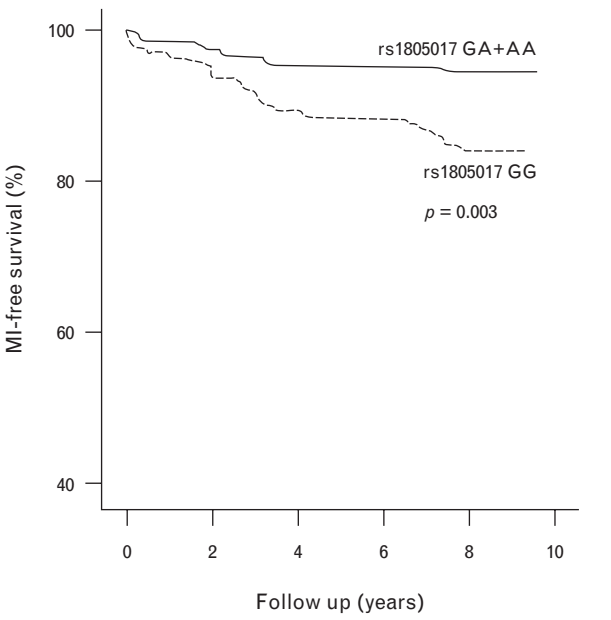
Haplotype analysis

The rs1805017 and rs1051931 genotypes for the *PLA2G7* gene generated four possible haplotypes (Table 3). The G/G haplotype was more common in patients with AMI (52.7 vs. 42.2%, $P = 0.026$) and cardiovascular events (49.5 vs. 41.7%, $P = 0.025$) than in those experiencing no events. This finding was confirmed after correction with permutation test ($P = 0.040$ and $P = 0.020$, respectively). Notably, in spite of this association with AMI, there was no association between any haplotype and cardiovascular death.

Discussion

We previously found that high Lp-PLA_2 activity predicted cardiovascular events in this prospectively studied cohort of white patients with angiographically detected CAD.[14] We extended this finding by showing that variation at the *PLA2G7* gene involving three common nonsynonymous SNPs, the rs1805017, rs1805018, and rs1051931, affected the Lp-PLA_2 mass and activity, and predicted cardiovascular events.

Fig. 3



Myocardial infarction free survival. Kaplan–Meier curve shows myocardial infarction (MI)-free survival in the propensity score matched high-risk patients divided according to the rs1805017 genotype into homozygous wild-type allele carriers (GG) and variant allele carriers (GA and AA). Patients carrying the variant allele had a significantly lower MI-free survival.

Table 3 Estimated haplotype frequencies

Haplotype rs1805017/rs1051931	Frequencies
G/G	0.440
G/A	0.289
A/G	0.235
A/A	0.037

PLA2G7 genotypes, Lp-PLA_2 mass and activity, and coronary artery disease burden

We found that the rs1805017 A allele exerted opposite effects on Lp-PLA_2 activity and mass levels, which were reduced and increased, respectively, which agrees with available data,[18,22,23] and suggests that the A allele may be associated with the production of a higher amount of a less active form of the enzyme. The underlying mechanisms are worth further specific research.

In spite of this functional effect of the rs1805017 SNP, we found no association of any PLA2G7 SNPs with the angiographically determined CAD burden, a finding that at first sight might seem contradictory given the effect of Lp-PLA_2 activity and SNPs (see later) on cardiovascular events. However, it has to be remembered that plaque growth and plaque destabilization, the latter occurring mostly at sites determining stenoses less than 50%,[38,39] were repeatedly shown to have different determinants.[14]

rs1805017 and rs1051931 effects on cardiovascular events

Of the three SNPs, only the rs1805017 and the rs1051931 followed the Hardy–Weinberg equilibrium in our cohort and, therefore, were further analysed, which disclosed a novel finding: in CAD patients, the rs1805017 GG genotype implied an increased incidence of AMI, a finding that was confirmed after correction for baseline coronary atherosclerotic burden and LVEF (Fig. 3). This result is partly at variance with previous negative findings,[15,18,20,22,40] and with two recent meta-analyses.[22,23]

For the rs1051931 A allele, in keeping with most reports,[17,18,22,40,41] but at variance with the detrimental effect found in cohorts comprising Asian or African-American individuals, and also with the protective effect observed in patients of European ancestry,[15,16,42] we found no association of the SNP with cardiovascular events.[19,20] These conflicting results could be explained by several reasons, including serendipity, differences of ethnicity, inclusion criteria,[15–20] study design and endpoints. Of note, a prospective cohort study design was used in only two cases;[18,20] adjustment for potential confounders by a multivariate analysis was not consistently performed, and propensity score matching had never been used prior to the present study. As a result of all these reasons, even the results of meta-analyses on SNPs performed on thousands of patients should be taken cautiously. It has to be acknowledged that our

study differs from most previous reports in that: (a) it exploited use of a prospective cohort design; (b) it included patients with angiographically documented CAD, thus allowing to incorporate the baseline coronary atherosclerotic burden as a covariate; (c) it used propensity score matching to balance the uneven distribution of a large number of cardiovascular risk factors at baseline between genotype groups.

Haplotype effects on cardiovascular events

Given the observed trend towards differences in cardiovascular events distribution across alleles (see supplemental Figure 1, http://links.lww.com/JCM/A43), we undertook a haplotype analysis, which showed that the presumably functional SNP rs1805017 and the rs1051931 G/G was the only haplotype (Table 3) associated with AMI and cardiovascular events, albeit not with cardiovascular death. This finding may reflect the higher Lp-PLA_2 activity of carriers of the rs1805017 G allele. It differs from the protective effect of the G/A haplotype on cardiovascular events reported in the only previous study demonstrating an effect of the *PLA2G7* haplotypes on cardiovascular events.[20]

Limitations and strengths of the study

As in many other prospective long-term follow up studies,[43–45] the patients lost at follow up and those with uncertain outcomes collectively comprised a nontrivial part of our cohort (25%). It might therefore be argued that a selection bias affected our results. However, comparison for genotype distribution and all relevant baseline covariates showed no significant differences between the cohort available and those lost to follow up. This finding indicates that they were similar in terms of overall cardiovascular risk profile, *PLA2G7* genetic profile, and CAD burden.

As we explored only the three most thoroughly investigated SNPs that were known to be common in Europe up to 2009, when our second follow-up assessment started we cannot exclude the possibility that other SNPs demonstrated subsequently as affecting Lp-PLA_2 activity[22,23,46] could improve the precision of *PLA2G7* genotyping in predicting cardiovascular events.

This study has, however, several strong points, including the prospective design and the comprehensive information on the patients' features at baseline. The latter allowed calculation of the propensity score, which overcomes the limitation in number of covariates to be considered in classical multivariate Cox's regression. We could therefore consider some major determinants of cardiovascular events at follow up in CAD patients that were never examined before, such as the atherosclerotic burden and the LVEF. Thus, even though the study might seem small in size, it was highly powered as a result of the very high incidence (25.8%) of cardiovascular events observed. Moreover, matching such a number of patients ($n = 180$) for all the potentially relevant variables affecting cardiovascular outcomes with a propensity score is an important accomplishment that supports the strength of our findings.

Conclusion

We found an association between variation at the *PLA2G7* gene and Lp-PLA_2 mass and activity, and also with cardiovascular events in white patients with CAD. Specifically, we showed that the rs1805017 SNP not only influenced Lp-PLA_2 mass and activity but also predicted AMI, independently of all the major cardiovascular risk factors including the coronary atherosclerotic burden. This finding suggests a role of Lp-PLA_2 in atherosclerotic plaque destabilization, thus pointing to Lp-PLA_2 activity as therapeutic target for preventing cardiovascular events in high-risk patients. Given its design, our study cannot provide mechanistic insight; therefore, the causal role of Lp-PLA_2 could be conclusively proven or challenged by the results of ongoing clinical trials involving the use of Lp-PLA_2 inhibitors: the STABILITY[47] and the SOLID-TIMI 52.[48]

Acknowledgements

We are thankful to Dr Robert L. Wolfert for the Lp-PLA_2 mass and activity measurement. This study was supported by grants from the Italian Cabinet of University and Scientific Research (MURST) to A.C.P. (9906193152_001/06) and by research grants from Regione Veneto (863/01/98), Unindustria of Treviso and F.O.R.I.C.A. to Prof. G.P. Rossi.

There are no conflicts of interest.

References

1 Asano K, Okamoto S, Fukunaga K, *et al.* Cellular source(s) of platelet-activating-factor acetylhydrolase activity in plasma. *Biochem Biophys Res Commun* 1999; **261**:511–514.
2 MacPhee CH, Moores KE, Boyd HF, *et al.* Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. *Biochem J* 1999; **338**:479–487.
3 Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest* 1992; **90**:1138–1144.
4 Inoue N, Takeshita S, Gao D, *et al.* Lysophosphatidylcholine increases the secretion of matrix metalloproteinase 2 through the activation of NADH/NADPH oxidase in cultured aortic endothelial cells. *Atherosclerosis* 2001; **155**:45–52.
5 Takahashi M, Okazaki H, Ogata Y, Takeuchi K, Ikeda U, Shimada K. Lysophosphatidylcholine induces apoptosis in human endothelial cells through a p38-mitogen-activated protein kinase-dependent mechanism. *Atherosclerosis* 2002; **161**:387–394.
6 Yamada Y, Yoshida H, Ichihara S, Imaizumi T, Satoh K, Yokota M. Correlations between plasma platelet-activating factor acetylhydrolase (PAF-AH) activity and PAF-AH genotype, age, and atherosclerosis in a Japanese population. *Atherosclerosis* 2000; **150**:209–216.
7 Yamada Y, Ichihara S, Fujimura T, Yokota M. Identification of the G994–>T missense in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. *Metabolism* 1998; **47**:177–181.
8 Hiramoto M, Yoshida H, Imaizumi T, Yoshimizu N, Satoh K. A mutation in plasma platelet-activating factor acetylhydrolase (Val279–>Phe) is a genetic risk factor for stroke. *Stroke* 1997; **28**:2417–2420.

9 Ichihara S, Yamada Y, Yokota M. Association of a G994−>T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with genetic susceptibility to nonfamilial dilated cardiomyopathy in Japanese. *Circulation* 1998; **98**:1881–1885.

10 Stafforini DM, Satoh K, Atkinson DL, *et al.* Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. *J Clin Invest* 1996; **97**:2784–2791.

11 Miwa M, Miyake T, Yamanaka T, *et al.* Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. *J Clin Invest* 1988; **82**:1983–1991.

12 Jang Y, Kim OY, Koh SJ, *et al.* The Val279Phe variant of the lipoprotein-associated phospholipase A2 gene is associated with catalytic activities and cardiovascular disease in Korean men. *J Clin Endocrinol Metab* 2006; **91**:3521–3527.

13 Thompson A, Gao P, Orfei L, *et al.* Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. *Lancet* 2010; **375**:1536–1544.

14 Maiolino G, Pedon L, Cesari M, *et al.* Lipoprotein-associated phospholipase A2 activity predicts cardiovascular events in high risk coronary artery disease patients. *PLoS One* 2012; **7**:e48171.

15 Sutton BS, Crosslin DR, Shah SH, *et al.* Comprehensive genetic analysis of the platelet activating factor acetylhydrolase (PLA2G7) gene and cardiovascular disease in case-control and family datasets. *Hum Mol Genet* 2008; **17**:1318–1328.

16 Liu PY, Li YH, Wu HL, *et al.* Platelet-activating factor-acetylhydrolase A379 V (exon 11) gene polymorphism is an independent and functional risk factor for premature myocardial infarction. *J Thromb Haemost* 2006; **4**:1023–1028.

17 De Caterina R, Talmud PJ, Merlini PA, *et al.* Gruppo Italiano Aterosclerosi. Strong association of the APOA5-1131T > C gene variant and early-onset acute myocardial infarction. *Atherosclerosis* 2011; **214**:397–403.

18 Hoffmann MM, Winkler K, Renner W, *et al.* Genetic variants and haplotypes of lipoprotein associated phospholipase A2 and their influence on cardiovascular disease (The Ludwigshafen Risk and Cardiovascular Health Study). *J Thromb Haemost* 2009; **7**:41–48.

19 Abuzeid AM, Hawe E, Humphries SE, Talmud PJ, HIFMECH Study Group. Association between the Ala379Val variant of the lipoprotein associated phospholipase A2 and risk of myocardial infarction in the north and south of Europe. *Atherosclerosis* 2003; **168**:283–288.

20 Ninio E, Tregouet D, Carrier JL, *et al.* Platelet-activating factor-acetylhydrolase and PAF-receptor gene haplotypes in relation to future cardiovascular event in patients with coronary artery disease. *Hum Mol Genet* 2004; **13**:1341–1351.

21 Wootton PT, Stephens JW, Hurel SJ, *et al.* Lp-PLA2 activity and PLA2G7 A379 V genotype in patients with diabetes mellitus. *Atherosclerosis* 2006; **189**:149–156.

22 Grallert H, Dupuis J, Bis JC, *et al.* Eight genetic loci associated with variation in lipoprotein-associated phospholipase A2 mass and activity and coronary heart disease: meta-analysis of genome-wide association studies from five community-based studies. *Eur Heart J* 2012; **33**:238–251.

23 Casas JP, Ninio E, Panayiotou A, *et al.* PLA2G7 genotype, lipoprotein-associated phospholipase A2 activity, and coronary heart disease risk in 10 494 cases and 15 624 controls of European Ancestry. *Circulation* 2010; **121**:2284–2293.

24 Rosenbaum PR, RDB. Reducing bias in observational studies using subclassification on the propensity score. *J Am Stat Assoc* 1984; **79**:516–524.

25 Rossi GP, Cesari M, De Toni R, *et al.* Antibodies to oxidized low-density lipoproteins and angiographically assessed coronary artery disease in white patients. *Circulation* 2003; **108**:2467–2472.

26 Rossi GP, Cesari M, Zanchetta M, *et al.* The T-786C endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA study. *J Am Coll Cardiol* 2003; **41**:930–937.

27 Cesari M, Pessina AC, Zanchetta M, *et al.* Low plasma adiponectin is associated with coronary artery disease but not with hypertension in high-risk nondiabetic patients. *J Intern Med* 2006; **260**:474–483.

28 Califf RM, Armstrong PW, Carver JR, D'Agostino RB, Strauss WE. 27th Bethesda Conference: matching the intensity of risk factor management with the hazard for coronary disease events. Task Force 5. Stratification of patients into high, medium and low risk subgroups for purposes of risk factor management. *J Am Coll Cardiol* 1996; **27**:1007–1019.

29 Maiolino G, Cesari M, Sticchi D, *et al.* Plasma adiponectin for prediction of cardiovascular events and mortality in high-risk patients. *J Clin Endocrinol Metab* 2008; **93**:3333–3340.

30 Rossi GP, Maiolino G, Seccia TM, *et al.* Hyperhomocysteinemia predicts total and cardiovascular mortality in high-risk women. *J Hypertens* 2006; **24**:851–859.

31 Rossi GP, Maiolino G, Zanchetta M, *et al.* The T(−786)C endothelial nitric oxide synthase genotype predicts cardiovascular mortality in high-risk patients. *J Am Coll Cardiol* 2006; **48**:1166–1174.

32 Tabachnick BG, Fidell LS, editors. Using multivariate statistics. 4th ed. Boston, MA: Allyn & Bacon; 2001.

33 Maiolino G, Pedon L, Cesari M, *et al.* Antibodies to malondialdehyde oxidized low-density lipoproteins predict long term cardiovascular mortality in high risk patients. *Int J Cardiol* 2013; **168**:484–489.

34 Austin PC. Some methods of propensity-score matching had superior performance to others: results of an empirical investigation and Monte Carlo simulations. *Biom J* 2009; **51**:171–184.

35 Heinze G, Juni P. An overview of the objectives of and the approaches to propensity score analyses. *Eur Heart J* 2011; **32**:1704–1708.

36 Klein JP, Moeschberger ML. *Survival analysis: techniques for censored and truncated data*. New York: Springer Verlag; 1997.

37 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**:263–265.

38 Ambrose JA, Tannenbaum MA, Alexopoulos D, *et al.* Angiographic progression of coronary artery disease and the development of myocardial infarction. *J Am Coll Cardiol* 1988; **12**:56–62.

39 Little WC, Constantinescu M, Applegate RJ, *et al.* Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? *Circulation* 1988; **78**:1157–1166.

40 Hou L, Chen S, Yu H, *et al.* Associations of PLA2G7 gene polymorphisms with plasma lipoprotein-associated phospholipase A2 activity and coronary heart disease in a Chinese Han population: the Beijing atherosclerosis study. *Hum Genet* 2009; **125**:11–20.

41 Jang Y, Waterworth D, Lee JE, *et al.* Carriage of the V279F null allele within the gene encoding Lp-PLA(2) is protective from coronary artery disease in South Korean males. *PLoS One* 2011; **6**:e18208.

42 Li L, Qi L, Lv N, *et al.* Association between lipoprotein-associated phospholipase A2 gene polymorphism and coronary artery disease in the Chinese Han population. *Ann Hum Genet* 2011; **75**:605–611.

43 Koh JS, Park JH, Shin DH, *et al.* Risk factors and effects on long-term outcomes of cardiac troponin I elevation after drug-eluting stent implantation in patients with stable coronary artery disease. *Am J Cardiol* 2012; **109**:461–465.

44 Vlek AL, van der Graaf Y, Sluman MA, Moll FL, Visseren FL, SMART Study Group. Metabolic syndrome and vascular risk in patients with peripheral arterial occlusive disease. *J Vasc Surg* 2009; **50**:61–69.

45 Panagiotakos DB, Pitsavos C, Chrysohoou C, *et al.* The effect of clinical characteristics and dietary habits on the relationship between education status and 5-year incidence of cardiovascular disease: the ATTICA study. *Eur J Nutr* 2008; **47**:258–265.

46 Suchindran S, Rivedal D, Guyton JR, *et al.* Genome-wide association study of Lp-PLA(2) activity and mass in the Framingham Heart Study. *PLoS Genet* 2010; **6**:e1000928.

47 White H, Held C, Stewart R, *et al.* Study design and rationale for the clinical outcomes of the STABILITY Trial (STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY) comparing darapladib versus placebo in patients with coronary heart disease. *Am Heart J* 2010; **160**:655–661.

48 O'Donoghue ML, Braunwald E, White HD, *et al.* Study design and rationale for the Stabilization of pLaques usIng Darapladib-Thrombolysis in Myocardial Infarction (SOLID-TIMI 52) trial in patients after an acute coronary syndrome. *Am Heart J* 2011; **162**:613–619; e1.