

# Glycoprotein Ib $\alpha$ Polymorphism T145M, Elevated Lipoprotein-Associated Phospholipase A<sub>2</sub>, and Hypertriglyceridemia Predict Risk for Recurrent Coronary Events in Diabetic Postinfarction Patients

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To explore altered platelet function in recurrent coronary event risk among diabetic postinfarction patients, we investigated a function-altering genetic polymorphism (T145M) in the von Willebrand factor binding region of the platelet glycoprotein Ib $\alpha$  (GPIb $\alpha$ ) subunit. The study comprised diabetic and nondiabetic patients of the Thrombogenic Factors and Recurrent Coronary Events postinfarction study. Cox proportional hazards multivariable modeling, adjusted for significant clinical covariates, was performed using the polymorphism and metabolic, inflammatory, and thrombogenic blood markers. Nondiabetic patients demonstrated risk for elevated lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>). In contrast, diabetic patients demonstrated significant and independent risk for the M allele of the T145M polymorphism (MT plus MM versus TT, hazard ratio [HR] 3.73, 95% CI 1.90–7.33,  $P < 0.001$ ), hypertriglyceridemia (2.91, 1.52–5.56,  $P = 0.001$ ), and elevated Lp-PLA<sub>2</sub> (2.78, 1.45–5.35,  $P = 0.002$ ). Joint risk (one, two, or three risk factors) expressed as relative outcome rates (compared with no risk factors) were 2.4, 4.0, and 8.2, respectively. We conclude that the M allele of the T145M polymorphism of the GPIb $\alpha$  subunit predicts risk for recurrent coronary events in diabetic postinfarction patients, but not in nondiabetic postinfarction patients, supportive of an important role for platelet hyperactivation in diabetic coronary heart disease. *Diabetes* 56:1429–1435, 2007

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apo, apolipoprotein; CAD, coronary artery disease; CHD, coronary heart disease; GP, glycoprotein; Lp-PLA<sub>2</sub>, lipoprotein-associated phospholipase A<sub>2</sub>; MI, myocardial infarction; PAI-1, plasminogen activator inhibitor-1; SNP, single-nucleotide polymorphism; THROMBO, Thrombogenic Factors and Recurrent Coronary Events.

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The Adult Treatment Panel III of the National Cholesterol Education Program recognizes type 2 diabetes as a coronary heart disease (CHD) risk equivalent in view of the high 10-year rate of development of new CHD in these patients (1). Furthermore, for type 2 diabetic patients with prior myocardial infarction (MI), more intensive prevention measures are indicated because of increased death rates experienced by these patients (1). In addition, type 2 diabetic patients with prior MI have higher rates of recurrent MI than patients with prior MI without diabetes (2). Progression of CHD in type 2 diabetic patients is only partially dependent on conventional risk factors (3,4); additional factors include long-term regulation of blood vessel structure and function by the renin-angiotensin-aldosterone system, vascular matrix fibrosis and stiffness associated with abnormalities of extracellular matrix turnover, oxidative stress, chronic low-grade inflammation, endothelial dysfunction, hypercoagulation, decreased fibrinolysis, and platelet hyperactivation (5,6).

With specific regard to platelets and CHD progression, platelet hyperactivation plays a significant role in the development of vascular thrombosis. Hyperactivation is related to multiple steps in the pathway of platelet activation. One of the earliest and most crucial of these is tethering of platelets to collagen of damaged endothelium by simultaneous binding of von Willebrand factor to collagen and to platelet glycoprotein (GP) Ib-IX-V complex (7,8). The GpIb complex is composed of four subunits (GPIb $\alpha$ , GPIb $\beta$ , GPIX, and GPV) with the binding site for von Willebrand factor on the NH<sub>2</sub>-terminal domain of the GPIb $\alpha$  subunit (9).

In a recent report, von Willebrand factor was found to be increased in diabetic patients of the Thrombogenic Factors and Recurrent Coronary Events (THROMBO) postinfarction study, suggesting endothelial damage as a major cause for recurrent coronary event risk (10). In view of higher levels of von Willebrand factor and the importance of platelet hyperactivation in diabetic patients, we hypothesized that platelet interactions with high levels of von Willebrand factor might also play a role in establishment of risk for recurrent coronary events. We tested this hypothesis by using a genetic polymorphism of the von Willebrand factor platelet binding site (T145M polymorphism of the GPIb $\alpha$  subunit) that has differential func-

tional effects and associations with clinical outcomes (8,11). Risk was assessed in post hoc analyses using multivariable modeling adjusted for significant clinical covariates in diabetic and nondiabetic patients of the prospective THROMBO study as a function of the T145M polymorphism and a set of metabolic, inflammatory, and thrombogenic blood markers.

## RESEARCH DESIGN AND METHODS

The study population comprised patients of the THROMBO prospective postinfarction study. There were a total of 1,045 nondiabetic and diabetic patients, with 940 having complete biomarker results and 903 with T145M genotyping results. For the diabetic study group, there were a total of 199 patients, with 173 having complete serum biomarker levels and 145 with complete biomarker levels as well as T145M genotyping. Reference populations comprised nondiabetic patients of the THROMBO study (12). Details of the entire THROMBO study population have been reported previously (13), and, as noted, the study was carried out with approval of and according to guidelines of the research subjects review boards. Recurrent coronary outcome events for this study were cardiac death, MI, or unstable angina (hospitalization during follow-up with an increase in either frequency or duration of angina symptoms or with development of new angina at rest with both requiring ischemic electrocardiogram changes without enzyme elevation), whichever occurred first. Average patient follow-up was 26 months. Diabetes was identified as use of oral hypoglycemic agents or insulin.

**Blood markers.** At 2 months after index MI, blood markers were determined on fasting sera. Levels of the following 17 markers were determined as described previously (13,14): apolipoprotein (apo) B, total cholesterol, apoAI, HDL cholesterol, triglyceride, LDL peak particle diameter, glucose, insulin, plasminogen activator inhibitor-1 (PAI-1), lipoprotein(a), C-reactive protein, von Willebrand factor antigen, fibrinogen, D-dimer, factor VII, factor VIIa, and lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>). LDL peak particle diameter was determined by gradient gel electrophoresis as described previously (15,16). A commercial colorimetric assay (Cayman Chemical, Ann Arbor, MI) was used to determine plasma Lp-PLA<sub>2</sub> activity as described previously (12), using 2-thio PAF (platelet-activating factor) as substrate and according to manufacturer's directions; 5,5'-dithio-bis-(2-nitrobenzoic acid) was used to detect enzymatic hydrolysis of the acetyl thioester bond with monitoring at 405 nm. Two control products were run with each assay. Between-assay coefficients of variation were 1.6% for a low control and 5.6% for a control near the sample mean; all but two samples gave rates greater than the low control. Samples were run in duplicate, and the average coefficient of variation was 2.5%. Enzyme activity ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$ ) was measured.

**Genotyping of T145M polymorphism of Gp-Ib $\alpha$ .** Blood samples were collected at study enrollment, and buffy coats were isolated and stored at  $-70^{\circ}\text{C}$  until extracted for DNA analysis. Genotyping was performed through a contractual agreement with Millennium Pharmaceuticals (Cambridge, MA) using the TaqMan method with differentially labeled probes complementary to either the T nucleotide (coding for methionine) or C nucleotide (coding for threonine) of the Gp-Ib $\alpha$  sequence (reference single-nucleotide polymorphism [SNP] no. rs6065). Genotype frequencies in the study subjects were consistent with population diversity data reported in the National Center for Biotechnology Information (NCBI) reference SNP database (TT 76.4%, TM 20.5%, and MM 0.3%).

**Statistical analyses.** Study design involved post hoc analyses of the prospective THROMBO study. All analyses were performed with Statistica 7.0 (StatSoft, Tulsa, OK). Variables were adjusted for age using linear regression. The Mann-Whitney *U* test was used to detect significant differences between groups. A  $\chi^2$  test was used to detect significant differences in proportions and polymorphism distributions. The significance level for all testing was at the  $P < 0.05$  level unless otherwise stated.

Significant variables associated with time to outcome event were determined using Kaplan-Meier analysis (log rank statistic,  $P < 0.05$ ) and Cox multivariable proportional hazards regression modeling. To assess risk of secondary coronary events as a function of blood marker variables, Cox regression was applied using the previously noted 17 laboratory markers as independent variables. Each marker was treated as a dichotomized independent variable in separate univariate models. To determine optimal cut points for dichotomization of marker levels, two dichotomization schemes were applied to each variable. These were with cut points at the 75th and 50th percentile levels for all blood markers except for apoAI and HDL cholesterol, which were at the 50th and 25th percentile levels in accordance with inverse association of marker level with risk for these two parameters. For markers with univariate results significant in both dichotomization schemes, the best-fit result was used in subsequent analyses. The T145M polymorphism of

TABLE 1

Clinical characterization and blood marker means and SDs for patients with diabetes

Characteristic	Diabetes
Recurrence rate	29.5
Male subjects	67.1
Race (% white)	61.8
Prior MI	24.3
Statins	34.1
$\beta$ -Blockers	71.1
Aspirin	79.2
Ca channel blockers	22.5
Nitrates	45.1
ACE inhibitors	50.9
Oral anticoagulants	17.3
Age (years)	60.3 $\pm$ 11.4
BMI ( $\text{kg}/\text{m}^2$ )	29.2 $\pm$ 5.4
Blood markers	
apoB (g/l)	1.24 $\pm$ 0.29
Cholesterol (mmol/l)	5.11 $\pm$ 1.11
apoAI (g/l)	1.19 $\pm$ 0.26
HDL (mmol/l)	1.03 $\pm$ 0.34
Triglyceride (mmol/l)	2.36 $\pm$ 1.39
LDL peak diameter (nm)	26.16 $\pm$ 0.79
Glucose (mmol/l)	8.69 $\pm$ 3.91
Insulin (pmol/l)	211 $\pm$ 272
PAI-1 ( $\mu\text{g}/\text{l}$ )	35.0 $\pm$ 36.3
Lipoprotein(a) (mmol/l)	0.62 $\pm$ 0.61
CRP (mg/l)	7.42 $\pm$ 10.55
Von Willebrand factor (%)	175 $\pm$ 74
Fibrinogen (g/l)	3.74 $\pm$ 1.01
D-dimer ( $\mu\text{g}/\text{l}$ )	569 $\pm$ 485
Factor VII (%)	106 $\pm$ 47
Factor VIIa (ng/ml)	2.61 $\pm$ 1.77
Lp-PLA <sub>2</sub> ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$ )	25.43 $\pm$ 5.44

Data are % or means  $\pm$  SD. CRP, C-reactive protein.

Gp-Ib $\alpha$  was treated as a binary independent variable (0 for TT, 1 for TM or MM). The following clinical covariates were evaluated in separate univariate models: sex; race; smoking; prior MI (an MI sometime in the past before the MI of study enrollment); myocardial index, which is index infarct type by electrocardiogram (Q-wave versus non Q-wave); pulmonary congestion; ejection fraction during entrance MI-EF<sub>30</sub> ( $>0.30$  or  $\leq 0.30$ ); and claudication. Multivariable modeling with adjustment for resulting significant clinical covariates ( $P < 0.1$ ) was performed with simultaneous entry of the T145M polymorphism and all univariate significant ( $P < 0.05$ ) dichotomized blood markers as independent variables. Assessment of medication effects was performed by single entry into the resulting multivariable models of the following medications: statins,  $\beta$ -blockers, aspirin, calcium channel blockers, nitrates, ACE inhibitors, and oral anticoagulants ( $P < 0.05$ ). Joint risk for combinations of risk factors were analyzed by Kaplan-Meier analysis and evaluation of relative outcome event rates (compared with patients with no risk factors) for risk factor combinations.

## RESULTS

Clinical and laboratory characteristics of the diabetic study population ( $n = 173$ ) are given in Table 1. In comparison to nondiabetic patients ( $n = 767$ ) of the THROMBO study (17), the diabetic group had significantly higher proportions of female (32.9 vs. 22.9%) and black patients (38.2 vs. 21.5) as well as significantly higher proportions of patients with recurrent coronary outcome events (29.5 vs. 15.9), prior MI (an MI sometime in the past before the MI of study enrollment, 24.3 vs. 16.8), on nitrates (45.1 vs. 33.6), and on ACE inhibitors (50.9 vs. 33.4). In terms of means and SD, diabetic patients in comparison to nondiabetic patients had a significantly higher value of BMI (29.2  $\pm$  5.4 vs. 27.6  $\pm$  4.9  $\text{kg}/\text{m}^2$ ) and

**TABLE 2**  
Blood marker means and SDs for diabetic patients as a function of T145M polymorphism of GPIIb $\alpha$  subunit

Blood marker	TT	TM plus MM
apoB (g/l)	1.22 $\pm$ 0.28	1.27 $\pm$ 0.26
Cholesterol (mmol/l)	5.09 $\pm$ 1.09	4.9 $\pm$ 0.83
apoAI (g/l)	1.21 $\pm$ 0.28	1.12 $\pm$ 0.19
HDL (mmol/l)	1.07 $\pm$ 0.38	0.99 $\pm$ 0.26
Triglyceride (mmol/l)	2.44 $\pm$ 1.36	1.96 $\pm$ 0.67
LDL peak diameter (nm)	26.14 $\pm$ 0.84	26.11 $\pm$ 0.57
Glucose (mmol/l)	8.54 $\pm$ 3.72	8.97 $\pm$ 4.09
Insulin (pmol/l)	188 $\pm$ 176	220 $\pm$ 268
PAI-1 ( $\mu$ g/l)	35.1 $\pm$ 29.8	32.2 $\pm$ 30.8
Lipoprotein(a) (mmol/l)	0.58 $\pm$ 0.57	0.71 $\pm$ 0.71
CRP (mg/l)	7.86 $\pm$ 11.53	7.32 $\pm$ 9.82
von Willebrand factor (%)	180 $\pm$ 75	163 $\pm$ 59
Fibrinogen (g/l)	3.79 $\pm$ 1.06	3.65 $\pm$ 0.89
D-dimer ( $\mu$ g/l)	562 $\pm$ 483	503 $\pm$ 342
Factor VII (%)	104 $\pm$ 49	101 $\pm$ 39
Factor VIIa (ng/ml)	2.69 $\pm$ 1.84	2.53 $\pm$ 1.88
Lp-PLA <sub>2</sub> ( $\mu$ mol $\cdot$ min <sup>-1</sup> $\cdot$ ml <sup>-1</sup> )	25.84 $\pm$ 5.56	25.11 $\pm$ 4.76

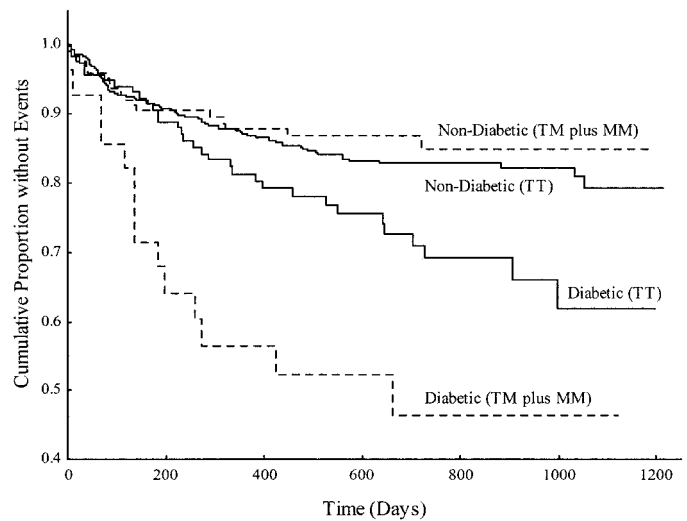
Data are means  $\pm$  SD. There were no statistically significant differences. CRP, C-reactive protein.

significantly higher levels of glucose (8.69  $\pm$  3.91 vs. 5.01  $\pm$  1.18 mmol/l), insulin (211  $\pm$  272 vs. 120  $\pm$  168 pmol/l), PAI-1 (35.0  $\pm$  36.3 vs. 26.6  $\pm$  24.7  $\mu$ g/l), C-reactive protein (7.42  $\pm$  10.55 vs. 4.36  $\pm$  6.87 mg/l), von Willebrand factor (175%  $\pm$  74 vs. 142%  $\pm$  62%), fibrinogen (3.74  $\pm$  1.01 vs. 3.47  $\pm$  0.82 g/l), and D-dimer (569  $\pm$  485 vs. 473  $\pm$  415  $\mu$ g/l). None of the lipoprotein-related traits, including levels of Lp-PLA<sub>2</sub>, were significantly different between diabetic and nondiabetic patients.

**Gp-Ib $\alpha$  polymorphism (T145M).** Genotype frequencies in the study population (80.7, 17.9, and 1.4% for TT, TM, and MM, respectively; *n* = 145) were in Hardy-Weinberg equilibrium. Distribution of frequencies as a function of race (81.6, 17.4, and 1.0% for whites and 78.7, 19.2, and 2.1% for blacks, respectively) were not different from each other (*P* = 0.83). For subsequent analyses, the polymorphism was dichotomized as TM plus MM versus TT. Based on this dichotomization, there were no significant differences as a function of variant in the clinical markers of Table 1 except for sex (TM plus MM; 10.0% in women, 24.2% in men, *P* = 0.041) and no differences in proportions of patients on medications. Additionally, there were no significant differences in blood marker levels as a function of the dichotomized polymorphism as shown in Table 2, and there was no difference in variant distribution between diabetic and nondiabetic patients (*n* = 663).

**TABLE 3**  
Significant variables of Gp-Ib $\alpha$  polymorphism (T145M), blood markers, and clinical covariates as predictors of recurrent coronary outcome events in univariate Cox proportional hazards analysis in diabetic postinfarction patients

Variable	Dichotomization cut point	Hazard ratio (95% CI)	<i>P</i>
T145M	TT/TM plus MM	2.31 (1.23–4.33)	0.009
Glucose	10.55 mmol/l (190 mg/dl)	1.99 (1.13–3.52)	0.017
HDL	0.98 mmol/l (38 mg/dl)	0.52 (0.30–0.93)	0.026
Triglycerides	2.07 mmol/l (183 mg/dl)	2.03 (1.14–3.64)	0.017
Factor VIIa (ng/ml)	2.22	0.54 (0.31–0.97)	0.038
Lp-PLA <sub>2</sub> ( $\mu$ mol $\cdot$ min <sup>-1</sup> $\cdot$ ml <sup>-1</sup> )	24.92	1.86 (1.05–3.31)	0.034
Prior MI	No/yes	2.23 (1.26–3.94)	0.006
Claudication	No/yes	2.57 (1.39–4.76)	0.003



**FIG. 1.** Kaplan-Meier curves of proportion of patients without recurrent coronary events as a function of Gp-Ib variant for diabetic patients (log rank *P* = 0.013) and for nondiabetic patients (log rank *P* = 0.49) as labeled.

**Gp-Ib $\alpha$  polymorphism (T145M) and risk of recurrent coronary events.** Diabetic postinfarction patients with the M allele demonstrated a recurrent coronary event rate of 50%, versus 27.4% (*P* = 0.02) for patients homozygous for the T allele, whereas there was no difference in corresponding rates for nondiabetic patients (16.4 vs. 13.7%, *P* = 0.45). To confirm that the effect of the T145M polymorphism on outcome events was specific to diabetic patients, interaction between diabetes and the T145M polymorphism was tested in the Cox regression for the combined nondiabetic and diabetic patient groups. Results demonstrated the interaction term to be of statistical significance (*P* = 0.013), underscoring the specific effect of the polymorphism on diabetic patients. The effect of the polymorphism is illustrated by Kaplan-Meier curves (Fig. 1) of the proportion of diabetic postinfarction patients without recurrent coronary events as a function of dichotomized T145M polymorphism, showing significantly poorer outcome over time for patients with the M allele (*P* = 0.013) in contrast to nondiabetic postinfarction patients, who demonstrated no difference (*P* = 0.49).

**Multivariable risk as a function of Gp-Ib $\alpha$  polymorphism (T145M) and blood markers.** Table 3 gives significant results for predictors of recurrent coronary outcome events from univariate Cox proportional hazards regression of the T145M polymorphism, clinical covariates (*P* < 0.1), and the 17 blood markers of Table 1 dichoto-

TABLE 4

Results of Cox multivariable modeling adjusted for significant clinical covariates and medication effects in diabetic postinfarction patients

Variable	Dichotomization cut point	Hazard ratio (95% CI)	<i>P</i>
T145M	TT/TM plus MM	3.73 (1.90–7.33)	<0.001
Triglyceride	2.07 mmol/l (183 mg/dl)	2.91 (1.52–5.56)	0.001
Lp-PLA <sub>2</sub> (μmol · min <sup>-1</sup> · ml <sup>-1</sup> )	24.92	2.78 (1.45–5.35)	0.002
Prior MI	No/yes	1.73 (0.91–3.28)	0.095
Claudication	No/yes	2.25 (1.12–4.52)	0.023
Calcium channel blockers	No/yes	2.68 (1.39–5.18)	0.003

mized as described above (75th percentile for glucose and 50th percentile for HDL cholesterol, triglycerides, factor VIIa, and Lp-PLA<sub>2</sub>; variables with significant associations with recurrent coronary outcome were only significant in one of the two dichotomization schemes). Results of multivariate modeling using simultaneous entry of all variables of Table 3 demonstrated continued significance of all variables except glucose and factor VIIa. Furthermore, to test medication effects on the model, multivariable models using all the variables of Table 3 except glucose and factor VIIa were performed with single entry of each of the seven medications of Table 1. Only calcium channel blockers were found to be significant in this series of models. Furthermore, HDL cholesterol lost significance in this model. Thus, the final multivariable model adjusted for significant clinical covariates (prior MI and claudication) and medication effects (calcium channel blockers) revealed as significant: T145M polymorphism of Gp-Ibα, triglycerides, and Lp-PLA<sub>2</sub>. Hazard ratios, 95% CIs, and *P* values are given in Table 4, along with Kaplan-Meier curves for triglycerides and Lp-PLA<sub>2</sub> in Figs. 2A and B, respectively.

A series of additional analyses were performed to address several issues. First, to rule out potentially confounding effects on results of the sex difference in the proportion of patients with the M allele as noted above (10.0% in women, 24.2% in men), the complete model was run additionally adjusted for sex. Results were essentially unchanged. Lack of a sex effect was also supported by similar ratios of secondary recurrent event rates for patients with the M allele to patients homozygous for the T allele (2.08 and 1.81 for female and male subjects, respectively) as well as similar values for hazard ratios in patients with the M allele versus patients homozygous for the T allele in sex-specific Cox analyses adjusted for significant clinical covariates (3.25, *P* = 0.16 and 2.91, *P* = 0.008 for female and male subjects, respectively). Close approach but failure to reach statistical significance in female subjects likely resulted from low patient numbers. Second, effects of race on results were also assessed. As noted above, distributions of T145M variants were not different as a function of race. This was consistent with results of multivariable analyses with race forced into the model (either alone or with sex forced in as well), again, giving essentially identical results. Third, to assess effects of inclusion of all diabetic patients regarding the T145M polymorphism, univariate Cox analysis was performed using mean substitution for the value of the T145M variable for diabetic patients without genotyping. Results for the total population of diabetic patients (*n* = 199) were essentially unchanged.

Further analyses were performed to evaluate two-factor interactions among the three significant marker variables

(T145M polymorphism, triglycerides, and Lp-PLA<sub>2</sub>); no significant interactions were found. To explore joint risk associated with combinations of the three risk markers, Kaplan-Meier curves (Fig. 3) were generated for patients having zero, one, two, and three risk markers. Log rank *P* values for comparison of curves relative to patients with no risk markers were 0.15, 0.018, and <0.00001 for one,

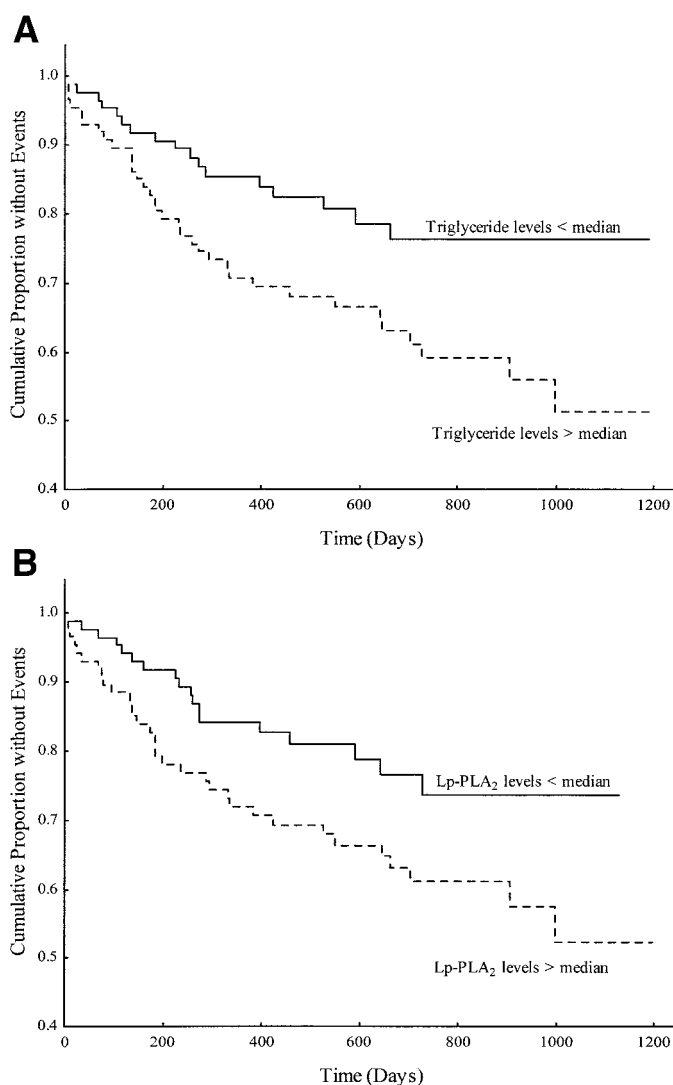
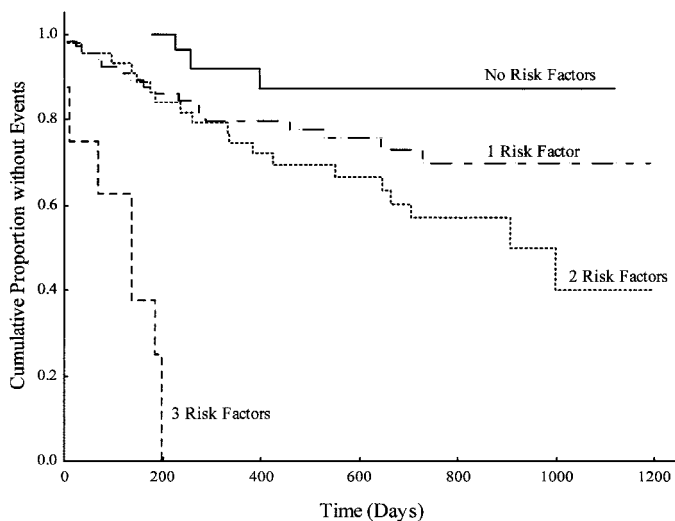


FIG. 2. Kaplan-Meier curves of diabetic patients showing proportion without recurrent coronary events. *A*: Curves as a function of triglycerides dichotomized according to the 50th percentile (log rank *P* = 0.015). *B*: Curves as a function of Lp-PLA<sub>2</sub> dichotomized according to the 50th percentile (log rank *P* = 0.031). Solid line, <50th percentile in concentration; dashed line, >50th percentile in concentration.



**FIG. 3.** Kaplan-Meier curves of diabetic patients showing proportion without recurrent coronary events as a function of number of risk factors (Gp-Ib variant, triglyceride, and Lp-PLA<sub>2</sub>). Log rank *P* values for comparison of curves relative to patients with no risk factors are as follows: one risk factor, *P* = 0.15; two risk factors, *P* = 0.018; and three risk factors, *P* < 0.00001. Solid line, patients with no risk factors; line with long and short dashes, patients with one risk factor; dotted line, patients with two risk factors; line with short dashes, patients with three risk factors.

two, and three risk markers, respectively. Recurrent coronary event rates as a function of number of risk markers and *P* values relative to patients with zero higher risk markers were: 10.7% and 26.2% (*P* = 0.099), 43.2% (*P* = 0.005), and 87.5% (*P* = 0.0002) for zero, one, two, and three markers, respectively. Thus, relative outcome rates (compared with patients with no risk factors) for risk factor combinations of one, two, or three risk factors were: 2.4, 4.0, and 8.2, respectively. This represents an approximately multiplicative relationship of recurrent risk with risk factor number.

## DISCUSSION

Results of the current work on diabetic patients of the THROMBO prospective postinfarction study, using multivariable modeling adjusted for clinical risk factors, demonstrated significant and independent association with risk of recurrent coronary events for the M allele of the T145M polymorphism of the platelet GPIIb/IIIa subunit, hypertriglyceridemia, and elevated Lp-PLA<sub>2</sub>. Additionally, joint risk increased approximately multiplicatively with number of risk factors. In contrast, for nondiabetic postinfarction patients, the T145M polymorphism was not a predictor of risk, nor was hypertriglyceridemia.

Results of population studies assessing the T145M polymorphism and atherosclerosis-related disease risk have been mixed (18–30). Multiple population studies failed to show differences in T145M variant frequencies between case and control subjects (18–23), including one in diabetic patients (19). However, other studies have shown associations of the polymorphism and coronary artery disease (CAD), all with M allele-associated risk (24–27). These include associations of the M allele with severity of CAD in MI and angina patients <60 years old (24); incidence of MI or unstable angina in postinfarction patients (25); coronary thrombosis, fatal MI, and sudden cardiac death for early middle-aged patients (26); and postoperative myocardial ischemia in vascular surgery

patients (27). For cerebrovascular disease, one study demonstrated no association (28), whereas others demonstrated associations of the M allele with various aspects of cerebrovascular disease (25,29,30). Results of the current work are in agreement with most studies described above in demonstrating M allele-associated risk. However, our finding of M allele-associated risk only in diabetic postinfarction patients is a new finding that was likely facilitated by performing separate studies in nondiabetic and diabetic patient groups.

As in population studies, results of platelet function studies for T145M variants are mixed (31–38). No differences for variants were reported for ADP-induced platelet aggregation and von Willebrand factor binding (31), von Willebrand factor binding to truncated GPIIb/IIIa T and M variant proteins (32), and collagen-epinephrine closure time (33). However, other studies do report differences, including greater inhibition of von Willebrand factor-mediated platelet agglutination by aurintricarboxylic acid (a von Willebrand factor antagonist) for M allele carriers, but no difference in prolongation of collagen-ADP closure time (34); increased ristocetin-induced and shear stress-induced agglutination and shortened collagen-epinephrine closure time for homozygous T subjects (35); greater binding of von Willebrand factor to T variant NH<sub>2</sub>-terminal fragments of GPIIb/IIIa-transfected Chinese hamster ovary (CHO) cells (36); mildly shortened collagen-ADP closure time for CAD patients homozygous for T (37); and, in CHO cell transfectants of T145M and a second GPIIb/IIIa polymorphism (variable number tandem repeats) variants, stronger interaction of von Willebrand factor with the M variant of T145M (38). Thus, there is ample evidence demonstrating functional differences in T145M variants.

Results of the current study show a striking difference between diabetic and nondiabetic patients regarding M allele-associated risk. We speculate that endothelial dysfunction is a significant process underlying this difference. This notion is supported by a recent report of greater association in diabetic patients of endothelial dysfunction with cardiovascular mortality than in nondiabetic patients (39). Indeed, endothelial dysfunction is more pronounced in diabetic patients of the current study, as demonstrated by higher levels of two common serum markers of endothelial dysfunction (von Willebrand factor and PAI-1). Thus, in diabetic patients, it may be that greater levels of endothelial dysfunction accentuate effects of functional differences between T145M variants through increased platelet binding to endothelium resulting from the endothelial damage and high von Willebrand factor levels associated with endothelial dysfunction. In addition to T145M, results of the current study for diabetic patients show significant and independent risk associated with hypertriglyceridemia and elevated Lp-PLA<sub>2</sub>. Hypertriglyceridemia is a well-known feature of diabetic dyslipidemia and associated risk (40). Lp-PLA<sub>2</sub>, an emerging biomarker of atherosclerotic disease (41–43), is a circulating enzyme that hydrolyzes altered phospholipids of oxidized lipoprotein particles to generate atherogenic species (44,45). It has special affinity for highly oxidized small dense LDL particles characteristic of diabetic dyslipidemia (46). Risk factor interactions are likely responsible for the extremely poor outcome in diabetic patients having all three risk factors. Thus, hypertriglyceridemia may drive formation of easily oxidizable small dense LDL particles (40) with subsequent hydrolysis by Lp-PLA<sub>2</sub> to generate proinflam-

matory species highly injurious to vascular endothelium, resulting in intensified endothelial dysfunction.

Study limitations included analysis of a single SNP of GPIb $\alpha$  rather than more extensive haplotype analysis. Although haplotype analysis is often useful in narrowing the localization of the functional genetic variant, the validity of our study conclusions linking genetic variation in GPIb $\alpha$  to coronary risk stands; in fact, the T145M SNP is a coding variant that demonstrates functional differences in GPIb $\alpha$  subunit action. Study results were possibly limited by underrepresentation of diabetes through use of diabetic medication history for identification, in that although the outcome event rate was substantial (29.5%), the number of diabetic patients ( $n = 173$ ) may have been insufficient to demonstrate significance of additional clinical risk factors and blood markers. However, this may also indicate robustness in risk factor study findings. Further limitations included the small proportion of M allele patients (19.3%) precluding subgroup analysis and blood marker levels at only one time point. Also, further patient information on exercise, diet, social support, depression, and ethanol use might have proven useful. Strengths of the work include a prospective study with long-term follow-up and availability of a set of thrombogenic, lipid, inflammatory, and metabolic blood markers in postinfarction patients, an important group with limited studies available (especially diabetic postinfarction patients).

In conclusion, results of the current study show that the M allele of the platelet GPI $\alpha$  subunit polymorphism (T145M), along with hypertriglyceridemia and elevated Lp-PLA $_2$ , predict risk for recurrent coronary events in diabetic postinfarction patients. In contrast, for nondiabetic postinfarction patients, only elevated Lp-PLA $_2$  predicts risk. These findings underscore differences in pathophysiological mechanisms associated with cardiovascular disease in diabetic patients and support an important role for endothelial dysfunction in diabetes. Further studies are needed to confirm the association of the M allele with risk in diabetic and other patient groups and to characterize at the molecular level the pathophysiological basis for this association.

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#### REFERENCES

- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 285:101-112, 2001
- Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229-234, 1998
- Laakso M: Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes* 48:937-942, 1999
- American Diabetes Association: Role of cardiovascular risk factors in prevention and treatment of macrovascular disease in diabetes. *Diabetes Care* 12:573-579, 1989
- Varughese GI, Tomson J, Lip GYH: Type 2 diabetes mellitus: a cardiovascular perspective. *Int J Clin Pract* 59:798-816, 2005
- Beckman JA, Creager MA, Libby P: Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 287:2570-2581, 2002
- Kunicki TJ: The influence of platelet collagen receptor polymorphisms in hemostasis and thrombotic disease. *Arterioscler Thromb Vasc Biol* 22:14-20, 2002
- Meisel C, Lopez JA, Stangl K: Role of platelet glycoprotein polymorphisms in cardiovascular diseases. *Naunyn-Schmiedeberg Arch Pharmacol* 369:38-54, 2004
- Ware J: Molecular analyses of the platelet glycoprotein Ib-IX-V receptor. *Thromb Haemost* 79:466-464, 1998
- Zareba W, Pancio G, Moss AJ, Kalaria VG, Marder VJ, Weiss HJ, Watelet LFM, Sparks CE: Increased level of von Willebrand factor is significantly and independently associated with diabetes in postinfarction patients. *Thromb Haemost* 86:791-799, 2001
- Yee DL, Bray PF: Clinical and functional consequences of platelet membrane glycoprotein polymorphisms. *Semin Thromb Hemost* 30:591-600, 2004
- Corsetti JC, Rainwater DL, Moss AJ, Zareba W, Sparks CE: High lipoprotein-associated phospholipase A $_2$  is a risk factor for recurrent coronary events in postinfarction patients. *Clin Chem* 252:1331-1338, 2006
- Moss AJ, Goldstein RE, Marder VJ, Sparks CE, Oakes D, Greenberg H, Weiss HJ, Zareba W, Brown MW, Liang CS, Lichstein E, Little WC, Gillespie JA, Van Voorhees L, Krone RJ, Bodenheimer MM, Hochman J, Dwyer EM, Arora R, Marcus FI, Watelet LFM, Case RB: Thrombogenic factors and recurrent coronary events. *Circulation* 99:2517-2522, 1999
- Corsetti JP, Zareba W, Moss AJ, Ridker PM, Marder VJ, Rainwater DL, Sparks CE: Metabolic syndrome best defines the multivariate distribution of blood variables in postinfarction patients. *Atherosclerosis* 171:351-358, 2003
- Rainwater DL, Moore PH, Shelledy WR, Dyer TD: Characterization of a composite gradient gel for the electrophoretic separation of lipoproteins. *J Lipid Res* 38:1261-1266, 1997
- Rainwater DL: Electrophoretic separation of LDL and HDL subclasses. *Methods Mol Biol* 110:137-151, 1998
- Corsetti JP, Zareba W, Moss AJ, Sparks CE: Serum glucose and triglyceride determine high-risk subgroups in non-diabetic postinfarction patients. *Atherosclerosis* 183:293-300, 2005
- Hato T, Minamoto Y, Fukuyama T, Fujita S: Polymorphisms of HPA-1 through 6 on platelet membrane glycoprotein receptors are not a genetic risk factor for myocardial infarction in the Japanese population. *Am J Cardiol* 80:1222-1224, 1997
- Sperr WR, Huber K, Roden M, Janisw M, Lang T, Graf S, Maurer G, Mayr WR, Panzer S: Inherited platelet glycoprotein polymorphisms and a risk for coronary heart disease in young central Europeans. *Thromb Res* 90:117-123, 1998
- Ito T, Ishida F, Shimodaira S, Kitano K: Polymorphisms of platelet membrane glycoprotein Ib alpha and plasma von Willebrand factor antigen in coronary artery disease. *Int J Hematol* 70:47-51, 1999
- Ardissino D, Mannucci PA, Merlini F, Duca F, Fèveau R, Tagliabue L, Tubaro M, Galvani M, Ottani F, Ferrario M, Corral J, Margaglione M: Prothrombotic genetic risk factors in young survivors of myocardial infarction. *Blood* 94:46-51, 1999
- Ishida F, Ito t, Takei M, Shimodaira S, Kitano K, Kiyosawa K: Genetic linkage of Kozak sequence polymorphism of the platelet glycoprotein Ib $\alpha$  with human platelet antigen-2 and variable number of tandem repeats polymorphism, and its relationship with coronary artery disease. *Br J Haematol* 111:1247-1249, 2000
- Ozelo MC, Origa AF, Aranha FJP, Mansur AP, Annichino-Bizzacchi JM, Costa FF, Pollak ES, Arruda VR: Platelet glycoprotein Ib $\alpha$  polymorphisms modulate the risk for myocardial infarction. *Thromb Haemost* 92:384-386, 2004
- Murata M, Matsubara Y, Kawano K, Zama T, Aoki N, Yoshino H, Watanabe G, Ishikawa K, Ikeda Y: Coronary artery disease and polymorphisms in a receptor mediating shear stress-dependent platelet activation. *Circulation* 96:3281-3286, 1997
- Gonzalez-Conejero R, Lozano ML, Rivera J, Corral J, Iniesta JA, Moraleda JM, Vicente V: Polymorphisms of platelet membrane glycoprotein Ib $\alpha$  associated with arterial thrombotic disease. *Blood* 92:2771-2776, 1998
- Mikkelsen J, Perola M, Penttila A, Karhunen PJ: Platelet glycoprotein Ib $\alpha$  HPA-2 Met/VNTR B haplotype as a genetic predictor of myocardial infarction and sudden cardiac death. *Circulation* 104:876-880, 2001
- Faraday N, Martinez EA, Scharpf RB, Kasch-Semenza L, Dorman T, Pronovost PJ, Perler B, Gerstenblith G, Bray PF: Platelet gene polymorphisms and cardiac risk assessment in vascular surgical patients. *Anesthesiology* 101:1291-1297, 2004
- Carlsson LE, Greinacher A, Spitzer C, Walther R, Kessler C: Polymorphisms of the human platelet antigens HPA-1, HPA-2, HPA-3, and HPA-5 on

- the platelet receptors for fibrinogen (GPIIb/IIIa), von Willebrand factor (GPIb/IX), and collagen (GPIa/IIa) are not correlated with an increased risk for stroke. *Stroke* 28:1392–1395, 1997
29. Sonoda A, Murata M, Ito D, Tanahashi N, Ohta A, Tada Y, Takeshita E, Yoshida T, Saito I, Yamamoto M, Ikeda Y, Fukuuchi Y, Watanabe K: Association between platelet glycoprotein Iba genotype and ischemic cerebrovascular disease. *Stroke* 31:493–497, 2000
  30. Sonoda A, Murata M, Ikeda Y, Fukuuchi Y, Watanabe K: Stroke and platelet glycoprotein Iba polymorphism. *Thromb Haemost* 85:573–574, 2001
  31. Mazzucato M, Pradella P, De Angelis V, Steffan A, De Marco L: Frequency and functional relevance of genetic threonine<sup>145</sup>/methionine<sup>145</sup> dimorphism in platelet glycoprotein Iba in an Italian population. *Transfusion* 36:891–894, 1996
  32. Li CQ, Garner SF, Davies J, Smethurst PA, Wardell MR, Ouwehand WH: Threonine-145/methionine-145 variants of baculovirus produced recombinant ligand binding domain of GPIba express HPA-2 epitopes and show equal binding of von Willebrand factor. *Blood* 95:205–211, 2000
  33. Jilma-Stohlwetz P, Homoncik M, Jilma B, Knechtelsdorfer M, Unger P, Mannhalter C, Santoso S, Panzer S: Glycoprotein Ib polymorphism influence platelet plug formation under high shear rates. *Br J Haematol* 120:652–655, 2003
  34. Boncler MA, Golanski J, Paczuski R, Watala C: Polymorphisms of glycoprotein Ib affect the inhibition by aurointricarboxylic acid of the von Willebrand factor dependent platelet aggregation. *J Mol Med* 80:796–801, 2002
  35. Yee DL, Wood J, Bergeron A, Sun C, Dong JF, Bray PF: Thr-145 and VNTR C/D polymorphisms on glycoprotein Iba demonstrate increased platelet reactivity to shear stress and ristocetin (Abstract). *Blood* 102:783a, 2003
  36. Ulrichs H, Vanhoorelbeke K, Cauwenberghs S, Vauterin S, Kroll H, Santoso S, Deckmyn H: Von Willebrand factor but not  $\alpha$ -thrombin binding to platelet glycoprotein Iba is influenced by the HPA-2 polymorphism. *Arterioscler Thromb Vasc Biol* 23:1302–1307, 2003
  37. Porto I, Leone AM, Nanni L, Sciahbasi A, De Vita M, Lanza GA, Andreotti F: Interplay of platelet polymorphisms, risk factors, and von Willebrand factor, and flow-mediated conditions in determining collagen-adenosine diphosphate PFA-100 results in patients with coronary artery disease. *Blood Coagul Fibrinolysis* 16:97–104, 2005
  38. Matsubara Y, Murata M, Hayashi T, Suzuki K, Okamura Y, Handa M, Ishihara H, Shibano T, Ikeda Y: Platelet glycoprotein Ib alpha polymorphisms affect the interaction with von Willebrand factor under flow conditions. *Br J Haematol* 128:533–539, 2005
  39. de Jager J, Dekker JM, Kooy A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CDA: Endothelial dysfunction and low-grade inflammation explain much of the excess cardiovascular mortality in individuals with type 2 diabetes. *Arterioscler Thromb Vasc Biol* 26:1086–1093, 2006
  40. Krauss RM: Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care* 27:1496–1504, 2004
  41. Sudhir K: Clinical review: lipoprotein-associated phospholipase A<sub>2</sub>, a novel inflammatory biomarker and independent risk predictor for cardiovascular disease. *J Clin Endocrinol Metab* 90:3100–3105, 2005
  42. Zalewski A, Macphee C: Role of lipoprotein-associated phospholipase A<sub>2</sub> in atherosclerosis. *Arterioscler Thromb Vasc Biol* 25:923–931, 2005
  43. Zalewski A, Nelson JJ, Hegg L, Macphee C: Lp-PLA<sub>2</sub>: a new kid on the block. *Clin Chem* 52:1645–1650, 2006
  44. Caslake MJ, Packard CJ: Lipoprotein-associated phospholipase A<sub>2</sub> (platelet-activating factor acetylhydrolase) and cardiovascular disease. *Curr Opin Lipidol* 14:347–352, 2003
  45. Tselepis AD, John Chapman M: Inflammation, bioactive lipids and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A<sub>2</sub>, platelet activating factor-acetylhydrolase. *Atheroscler Suppl* 3:57–68, 2002
  46. Gazi I, Lourida ES, Filippatos T, Tsimihodimos V, Elisaf M, Tselepis AD: Lipoprotein-associated phospholipase A<sub>2</sub> activity is a marker of small, dense LDL particles in human plasma. *Clin Chem* 51:2264–2273, 2005