Influence of *PAI-1* Gene Promoter-675 (4G/5G) Polymorphism on Fibrinolytic Activity After Cardiac Surgery Employing Cardiopulmonary Bypass

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Key Words: *PAI-1 gene polymorphism; fibrinolysis; cardiac surgery; bleeding.*

Summary. *Background and Objective. The plasminogen activator inhibitor type-1 (PAI-1) gene promoter contains 675 (4G/5G) polymorphism. The aim of this study was evaluate the effect of the PAI-1 promoter-675 (4G/5G) polymorphism on the concentrations of PAI-1 and tissue plasminogen activator/PAI-1 (t-PA/PAI-1) complex and bleeding volume after on-pump cardiac surgery.*

Material and Methods. A total of 90 patients were included in the study at Pauls Stradins Clinical University Hospital. Seven patients were excluded due to surgical bleeding. Eighty-three patients were classified according to the PAI-1 genotype: 21 patients had the 4G/4G genotype; 42, the 4G/5G genotype; and 20, the 5G/5G genotype. The following fibrinolysis parameters were recorded: the PAI-1 level preoperatively, D-dimer level at 0, 6, and 24 hours after surgery, and t-PA/PAI-1 complex level 24 hours postoperatively. A postoperative bleeding volume was registered in mL 24 hours after surgery.

Results. The patients with the 5G/5G genotype had significantly lower preoperative PAI-1 levels (17 [SD, 10.8] vs. 24 ng/mL [SD, 9.6], P=0.04), higher D-dimer levels at 6 hours (371 [SD, 226] vs. 232 ng/mL [SD, 185], P=0.03) and 24 hours (326 [SD, 207] vs. 209 ng/mL [SD, 160], P=0.04), and greater postoperative blood loss (568 [SD, 192] vs. 432 mL [168], P=0.02) compared with the 4G/4G carriers. There were no significant differences in the levels of the t-PA/PAI-1 complex comparing different genotype groups.

Conclusions. The carriers of the 5G/5G genotype showed the lower preoperative PAI-1 levels, greater chest tube blood loss, and higher D-dimer levels indicating that the 5G/5G carriers may have enhanced fibrinolysis.

Introduction

Alterations in hemostasis during and after cardiac surgery may have a diversity of etiologies including surgery per se as well as effects of the cardiopulmonary bypass (CPB) on the coagulation and the inflammation cascades, and their cross-reactions with the fibrinolytic and the kinin-kallikrein systems (1, 2). The balance among bleeding, normal hemostasis, and thrombosis is markedly influenced by the aggregation of functioning platelets, the rate of thrombin formation (3), and the fibrinolytic system, which plays a determinant role in this process (4, 5). Additionally, genetic predisposition in the physiopathology of bleeding may influence the body's response to CPB (5–8).

The effects of the fibrinolytic system are mediated by the activation of plasminogen to fibrin-degrading protease plasmin, which then lyses fibrin. Fibrinolytic activity depends on the balance between plasminogen activators and plasminogen activator inhibitors (9). The known plasminogen activators in the vascular system include tissue plasminogen activator (t-PA) and urinary type plasminogen activator (u-PA), while the most important inhibitor is plasminogen activator inhibitor type 1 (PAI-1), a fast acting inhibitor of both t-PA and u-PA (6, 10).

The serine protease inhibitor PAI-1 is a linear glycoprotein with a molecular weight of 48 kDa (11). The active form is synthesized in platelets as well as in endothelium and adipose tissues. PAI-1 binds rapidly to t-PA at a ratio of 1:1 forming a stable complex, which is cleared from circulation by macrophages lining the walls of the liver sinusoids. The formation of t-PA/PAI-1 complexes depends on the function and plasma concentrations of the 2 proteins: the greater t-PA and PAI-1 concentrations are, the higher concentration of the complex will appear in the circulation (12).

The plasma levels of PAI-1 are highly variable and are influenced by environmental and genetic factors (13). The human *PAI-1* gene is located on chromosome 7, and it contains 9 exons and 8 introns

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(14). The functional insertion/deletion (4G/5G) polymorphism has been described in the promoter region at position 675 of the *PAI-1* gene (15). The 4G/4G genotype is associated with the basal PAI-1 levels greater by approximately 25%–30%, therefore, with a greater inhibition of fibrinolysis, and the 5G allele is associated with the lower levels of PAI-1 that could suggest a greater fibrinolytic activity (16, 17). Recently researchers have reported systemic or topical applications of extended half-life PAI-1 in experimental animals to reduce the total blood loss in cases of PAI-1 deficiency (18).

The aim of this study was to investigate the effect of the *PAI-1* promoter-675 (4G/5G) polymorphism on the plasma concentrations of PAI-1 and t-PA/PAI-1 and bleeding volume after on-pump cardiac surgery.

Material and Methods

The study protocol and the informed consent form were approved by the Ethics Committee (No. 151209-4L) of Pauls Stradins Clinical University Hospital, Riga, Latvia. Written informed consent was obtained from each patient.

Between May 1 and November 30, 2011, 90 adult patients scheduled for cardiac surgery using CPB were enrolled into a prospective observational study. For every patient, the predicted operative mortality was calculated using the European System for Cardiac Operative Risk Evaluation (EuroSCORE) (19).

The inclusion criteria were as follows: age of at least 18 years, first-time coronary artery bypass grafting (CABG) and/or valve replacement under CPB, EuroSCORE of <10%, values of coagulation tests within the reference ranges at baseline (prothrombin time [PT], 70%–120%, or international normalized ratio [INR], 0.8–1.2; fibrinogen plasma concentration 1.5–3.5 g/L; platelet count [PLT], 150–400×10^9/L; hemoglobin [Hb] concentration, >135 g/L for men and >120 g/L for women), and no anticoagulant, antiaggregant, or nonsteroidal anti-inflammatory therapies for at least the last 5 days prior to surgery in order to disclose drug-induced platelet dysfunction. The last dose of low-molecular-weight heparin (LMWH) was administered in the evening before the surgery.

The exclusion criteria included emergency and redo operations, preoperative hemostatic disorders with a history of hemorrhagic events or coagulopathy (PT below 50% or INR greater than 1.5, fibrinogen plasma concentration less than 1.5 g/L, and PLT less than 100×10^9/L), and severe renal and/or hepatic dysfunctions.

Perioperative Management. The same anesthetic protocol was used in all patients. Anesthesia was induced with fentanyl (Fentanyl-Kalceks® 0.05 mg/mL, JSC Kalceks, Latvia), 0.2–0.3 mg midazolam (Dormicum®, F. Hoffman-La Roche AG, Basel, Switzerland), 0.1–0.3 mg/kg etomidate (etomidate injections 2 mg/mL, Sagent Agila, India), and 0.2 mg/kg cisatracurium (Nimbex 2 mg/mL, GlaxoSmithKline Manufacturing S.p.A, Italy) and maintained with sevoflurane (Sevoflurane Piramal, Piramal Healthcare Ltd, United Kingdom) at MAC 0.8–1.2. The patients did not receive antifibrinolytic drugs during and after the surgery. During CPB, anesthesia was maintained with fentanyl, propofol, and cisatracurium at dosages of 0.03–0.06 μg/(kg·min), 3–5 mg/(kg·h), and 0.1 mg/(kg·h), respectively. Before the beginning of CPB, heparin (Pan-Heparin Sodium®, Panpharma S.A./Rotexmedica Gmbh, Germany) was administered at a dose of 300 to 400 units/kg initially and 5000 to 10 000 units to achieve and maintain an activated coagulation time (ACT) above 480 seconds during CPB.

Standard pulsatile CPB with an extracorporeal circuit consisting of a polypropylene membrane oxygenator (Admiral®, Eurosets TM, Italy) with moderate hypothermia (bladder temperature, 34°C–35°C) in combination with hemodilution was used. Myocardial protection was achieved by using St. Thomas 4:1 cardioplegia (AlleMan®, Germany). Weaning off CPB after the surgical procedure was performed after rewarming the patient to a bladder temperature of at least 36°C. After separation from CPB, protamine (Protamin Meda®, Meda Pharma, Wien, Austria) at a dose of 1 mg per 100 units of heparin was administered initially, followed by additional doses until ACT had returned to baseline.

Data Collection and Analysis. The following pre- and perioperative variables were taken into account: age, sex, body mass index (BMI), ejection fraction (EF), type of surgery, extracorporeal circulation time (min), aortic clamp and reperfusion times (min), and preoperative coagulation parameters such as Hb, PLT, PT, and fibrinogen. The following parameters of fibrinolysis were analyzed: plasma concentrations of PAI-1 preoperatively and t-PA/PAI-1 complex 24 hours after the surgery. In order to assess the fibrinolytic activity, D-dimer concentrations were determined at 3 time points: after the surgery on admission to the intensive care unit, and 6 and 24 hours postoperatively.

Fibrinolysis was assessed by an enzyme-linked immunosorbent assay (ZYMUTEST®, HYPHEN BioMed, France) for the quantification of PAI-1 (reference range, 1–25 ng/mL) and t-PA/PAI-1 complex (reference range, <5 ng/mL). Moreover, the immunoturbidimetric test (D-dimer PLUS®, Dade Behring, Marburg, Germany) was used for the quantitative analyses of cross-linked fibrin degradation products (D-dimer, reference range, <300 ng/mL).

Preoperatively, fibrinogen plasma concentration, PT, PLT, and Hb were recorded to allow the comparison among 3 groups of patients. Fibrinogen

plasma concentrations were determined as described by Clauss (20). Briefly, citrated plasma was brought to coagulation by the administration of an excessive amount of thrombin (Multifibren U reagent, Siemens Healthcare Diagnostics, USA). The reference value is 1.8–3.5 g/L. PT was analyzed with a prothrombin complex assay (Lyophilized Dade® and Innovin® reagent, Siemens Healthcare Diagnostics, USA).

All the coagulation parameters were determined using Sysmex® CA-1500 (Siemens Healthcare Diagnostics, Marburg, Germany). The Hb concentration and PLT were analyzed by a Beckman Coulter LH 750 hematology analyzer. The Coulter LH 750 uses an impedance technology to measure the PLT count, and the hemoglobin cyanide method was used to measure Hb concentrations.

Genomic DNA was extracted from whole blood using the standard phenol-chloroform extraction method. The region harboring the PAI-1 4G/5G polymorphism was amplified by the polymerase chain reaction (PCR) using sequence specific primers. The PCR products were then purified using Sap/Exo I (Thermo Scientific® Fermentas, Lithuania) and sequenced on an Abi Prizm 3130xl genetic analyzer. Sequence analysis was done using the Finch TV software.

A postoperative bleeding volume was recorded as chest tube drainage in mL at 24 hours postoperatively. Surgical bleeding was diagnosed at the time of re-exploration if one or more specific bleeding sites were identified. The patients with surgical bleeding were excluded from the further study.

Statistical Analysis. All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS® 17.0, Chicago, USA). Continuous variables were described as mean and standard deviation (SD) and categorical variables as percentages (%). Statistical significance was defined as a $P<0.05$. The sample size was determined based on the number of cases hospitalized during the study period. The baseline data of the study groups were checked by an appropriate analytical test according to the data distribution. The Pearson correlation coefficient was calculated between fibrinolytic parameters and the volume of postoperative bleeding. The Mann-Whitney *U* test and the Kruskal-Wallis *H* test were used to compare continuous parameters in study groups for nonparametric variables and the Student *t* test or ANOVA for parametric variables. The ranges of PAI-1, complex of t-PA/PAI-1, D-dimer, and postoperative 24-hour blood loss were compared between the different genotypes of the *PAI-1* polymorphism.

Results

Clinical Course. In total, 90 consecutive adult cardiac surgical patients (47 men and 43 women) with a mean age of 66 years (SD, 10) were considered for inclusion. Seven patients (7.8%) required reoperation between 10 minutes and 32 hours after the surgery because of excessive bleeding or hemipericardium, but none of them died. Moreover, during the reoperation, it was discovered that it was surgical bleeding. As shown in Table, 83 patients were subject to the further analysis and were classified into 3 groups according to the PAI-1 genotype: 4G/4G group (n=21), 4G/5G group (n=42), and 5G/5G group (n=20). As Table shows, there were no significant differences in the demographic characteristics such as mean age, gender, BMI, and EF and preoperative parameters (Hb, PLT, PT, fibrino-

Table. Characteristics of Patients According to Plasminogen Activator Inhibitor Type-1 Gene Promoter -675 (4G/5G) Polymorphism

Characteristic	4G/4G n=21	4G/5G n=42	5G/5G n=20	*P* value
Demographic data				
Age, years	65.2 (11)	66 (10)	64 (12)	0.6
Male sex, n (%)	11 (52)	19 (45)	12 (60)	0.2
BMI, kg/m^2	26.6 (4.5)	28 (4.9)	28 (5)	0.5
EF, %	56.7 (7)	55 (7.8)	57 (8)	0.2
Type of surgery				
Coronary, n (%)	8 (38)	17 (41)	9 (45)	0.1
Valve, n (%)	10 (47)	14 (33)	7 (35)	0.3
Mixed, n (%)	3 (15)	11 (26)	4 (20)	0.04
Preoperative parameters				
Hemoglobin, g/dL	136 (18)	136 (15)	135 (15)	0.9
Platelet count, $\times 10^9$/L	218 (63)	216 (53)	215 (65)	0.9
Prothrombin time, %	92 (13)	90 (13)	84 (16)	0.08
Fibrinogen, g/L	4.5 (1.7)	4.5 (1.3)	4.2 (0.8)	0.3
Surgical parameters				
CPB duration, min	95 (36)	106 (41)	113 (43)	0.2
Aorta occlusion time, min	59 (27)	66 (24)	72 (32)	0.2
Reperfusion time, min	32 (15)	34 (16)	34 (12)	0.7
Blood loss, mL per 24 h	432 (168)	568 (192)	609 (321)	0.1

Values are mean (SD) unless otherwise stated.
BMI, body mass index; EF, ejection fraction; CPB, cardiopulmonary bypass.

gen level) comparing the genotype groups. The only one significant difference was found for a mixed type of surgery: the 4G/5G carriers significantly more frequently underwent the surgery of this type that those with the 4G/4G or 5G/5G genotypes.

PAI-1 Polymorphism. The distribution of patients by the PAI-1 polymorphism was as follows: 21 patients (25%) had the 4G/4G genotype, 42 (51%) had the 4G/5G genotype, and the 5G/5G genotype was found in 20 (24%) of the 83 patients studied. All alleles were in the Hardy-Weinberg equilibrium.

Relationship Between Genotype and Plasma Concentrations of PAI-1, t-PA/PAI-1 Complex. Significant differences in the preoperative PAI-1 plasma concentrations were found comparing the patients' groups by genotypes: the carriers of the 4G/5G and 4G/4G genotypes had significantly higher preoperative PAI-1 plasma concentrations than those with the 5G/5G genotype (27 [SD, 13] and 24 [SD, 9.6] versus 17 ng/mL [SD, 10.8]; P=0.004 and P=0.04, respectively) (Fig. 1). A significant correlation was found between the preoperative PAI-1 plasma concentration and the postoperative 24-hour blood loss in the 4G/5G group (r=–0.4, P=0.01).

The mean plasma concentrations of t-PA/PAI-1 complex 24 hours after the surgery were as follows: 5G/5G, 3.6±2.4 ng/mL; 4G/5G, 3.9±2.1 ng/mL; and 4G/4G, 3.1±1.8 ng/mL. However, there were no significant differences in the t-PA/PAI-1 complex levels comparing the genotype groups. Correlation analysis showed a significant relationship between the t-PA/PAI-1 complex level and the postoperative 24-hour blood loss only in the 4G/5G carriers (r=–0.32, P=0.04).

Relationship Between Genotype and D-Dimer Levels. The highest D-dimer levels were shown by the 5G/5G genotype group postoperatively at all 3 time points after surgery: 334 [SD, 224], 371 [SD, 226], and 326 ng/mL [SD, 206] at 0, 6, and 24 hours, respectively. There were significant differences in the D-dimer level between the 5G/5G and 4G/4G groups at 6 hours (371 [SD, 226] vs. 232 ng/mL [SD, 185], P=0.03) and 24 hours (326 [SD, 207] vs. 209 ng/mL [SD, 160], P=0.04) (Fig. 2).

Relationship Between Genotype and Postoperative 24-Hour Blood Loss. As expected, there were differences in the postoperative 24-hour blood loss comparing all the 3 PAI-1 genotypes. There was a significant difference in the blood loss volume at 24 hours after surgery between the 5G/5G and 4G/4G genotypes (568 [SD, 192] vs. 432 mL [SD, 168], P=0.02). However, the greatest postoperative blood loss was shown by the 4G/5G carriers (609 mL [SD, 321] per 24 hours), and it also reached a statistically significant difference in comparison with the 4G/4G group (609 [SD, 321] vs. 432 mL [SD, 168], respectively; P=0.02).

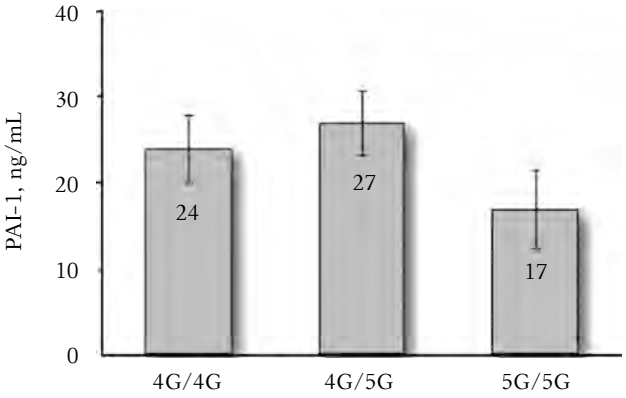

Fig. 1. Preoperative concentration of plasminogen activator inhibitor type 1 (PAI-1) according to the PAI-1 genotype
P=0.04, 5G/5G carriers vs. 4G/4G, and
P=0.004, 5G/5G carriers vs. 4G/5G. Values are means.

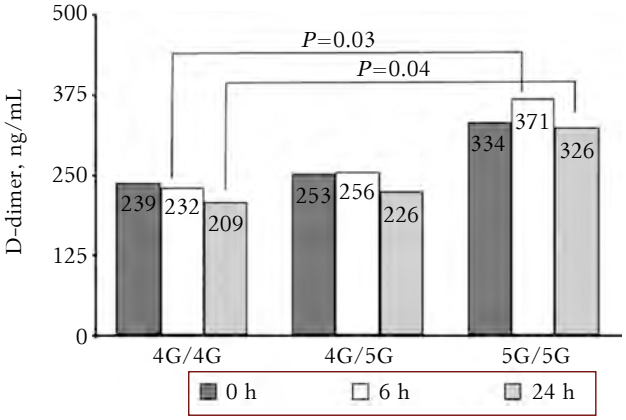

Fig. 2. D-dimer level postoperatively at 3 time points (0, 6, and 24 hours after surgery) according to the plasminogen activator inhibitor type 1 genotype
Values are means.

Discussion

The PAI-1 gene-675 (4G/5G) polymorphism is known to influence the plasma levels of PAI-1, which is considered as the main regulator of fibrinolysis (21–24). Much attention is given to the 4G/4G polymorphism regarding to coronary artery disease; however, there are much fewer reports in the literature of the importance of the 5G/5G polymorphisms and their influence on the plasma PAI-1 and t-PA/PAI-1 complex concentrations and fibrinolytic activity.

The present study has revealed that the 5G/5G polymorphism may be associated with a lower preoperative plasma concentration of PAI-1, with higher levels of D-dimer, and greater bleeding after on-pump cardiac surgery. These results are consistent with several recent investigations showing that the 4G/5G polymorphism of the *PAI-1* gene can affect fibrinolytic activity and can be associated with increased postoperative bleeding (5, 24, 25).

Since PAI-1 is a more stable indicator of fibrinolysis, which is usually present at higher concentrations in comparison with t-PA, the PAI-1 level before the operation and the t-PA/PAI-1 complex level 24 hours after surgery and their associations with the postoperative 24-hour blood loss were determined. The reaction between PAI-1 and t-PA, which usually results in the formation of the t-PA/PAI-1 complex, continues until t-PA is consumed. Thus, the complex is considered an indicator of the concentration and function of active PAI-1 in blood (26).

Regarding the preoperative PAI-1 plasma concentration, our study showed that PAI-1 levels differed according to the patients' genotypes. The 5G/5G carriers had the lowest levels of PAI-1 before the operation. Other recent studies have also observed such associations between the 5G/5G genotype and the PAI-1 plasma concentration (22, 27). Burzotta et al. (24) investigated the *PAI-1* 4G/5G polymorphism association with the basal PAI-1 levels including 111 patients undergoing elective coronary bypass surgery. The PAI-1 levels were measured before surgery, daily up to 72 hours, and at discharge. They concluded that the PAI-1 levels were higher in the carriers of the 4G-allele than 5G/5G homozygotes because of higher baseline values.

To the best of our knowledge, the literature is scanty on the reports focusing on the importance of the t-PA/PAI-1 complex and its relationship with the *PAI-1* gene-675 (4G/5G) polymorphism. We expected to find the association between the t-PA/PAI-1 complex level and the *PAI-1* genotype as well because the clearance rate of the t-PA/PAI-1 complex is slower than that of free t-PA. It follows consequently that the lower the plasma PAI-1 level, the smaller amount of the t-PA/PAI-1 complex will be generated in the circulation (12). Unfortunately, we could not find a statistically significant difference in the complex plasma concentrations comparing 3 *PAI-1* genotypes. Even more, the 4G/4G carriers demonstrated the lowest plasma concentrations of the t-PA/PAI-1 complex 24 hours after surgery, and the 4G/5G carriers, the highest. We speculate that the values of t-PA/PAI-1 complex could be still influenced by CPB as it is well known that the PAI-1 and t-PA/PAI-1 concentrations are increased after CPB as part of the "fibrinolytic shut down," and they start to rise immediately after surgery and slowly decrease in the following days after surgery (28, 29). The carriers of the 4G/5G genotype showed also the highest preoperative plasma PAI-1 concentrations. In the 4G/5G group, a significant correlation was found between the preoperative PAI-1 and t-PA/PAI-1 complex levels at 24 hours after the surgery, and the postoperative 24-hour blood loss. We propose that the lack of such a correlation could be explained by the fact that the 4G/5G genotype group was the biggest as to the patients' sample size. Other researchers also observed associations between the PAI-1 level and the postoperative bleeding volume (5, 25).

The *PAI-1*-675 (4G/5G) gene polymorphism affects the PAI-1 plasma concentration in circulation and therefore fibrinolytic activity, which can be confirmed by higher levels of D-dimer (30). In our study, D-dimer postoperatively reached a higher level in the 5G/5G group in comparison with the 4G/5G and 4G/4G genotype groups. Additionally, the 4G/5G and 5G/5G carriers had a greater bleeding volume 24 hours after the surgery compared with the 4G/4G genotype group. It is difficult to isolate only one factor affecting blood loss after CPB. We speculate whether the greater blood loss after surgery in the 4G/5G group could be influenced by hypothermia, hemodilution, and other factors, not only by the PAI-1 genotype. Furthermore, only in the 5G/5G group bleeding was confirmed with the increased D-dimer levels postoperatively indicating enhanced fibrinolysis. Our finding is consistent with the recent studies where an association between the *PAI-1* gene 4G/5G polymorphism and postoperative bleeding volume was also found (1, 23). Jimenez Rivera et al. (5) studied genetic factors associated with excessive bleeding after cardiopulmonary bypass. They performed a study on 26 patients. Excessive bleeding was observed in 1 (20%) of the 5 4G/4G genotype carriers, 5 (42%) of the 12 4G/5G carriers, and 7 (78%) of the 9 5G/5G carriers. They concluded that excessive bleeding was significantly associated with the 5G homozygote for the *PAI-1* polymorphism.

The following limitations should be acknowledged. The main limitation is a relatively low sample size because of financial limitations for the determination of the *PAI-1* gene polymorphism and DNA extraction as well as for the determination of a fibrinolytic marker. It also would be advantageous to determine preoperatively the t-PA and PAI-1 plasma concentrations separately and simultaneously with the t-PA/PAI-1 complex concentrations 24 hours after the surgery. This would allow us to better understand the relationship between free t-PA, free PAI-1 antigen, and their complexes as the t-PA/PAI-1 complex. Although the sample size is the main limitation of this study, the association of the *PAI-1* gene-675 (4G/5G) polymorphism with the plasma levels of PAI-1 and D-dimer, and postoperative 24-hour blood loss of patients after cardiac surgery employing cardiopulmonary bypass was assessed.

Conclusions

Associations between the *PAI-1* promoter-675 (4G/5G) polymorphism, preoperative PAI-1 plasma concentrations, and postoperative 24-hour blood loss were observed. The carriers of the 5G/5G genotype showed the lower preoperative PAI-1 levels,

greater tendency to chest tube blood loss, and higher D-dimer levels after cardiac surgery employing cardiopulmonary bypass indicating that the 5G/5G carriers may have an enhanced fibrinolytic activity.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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