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Tissue Factor and Platelet Glycoprotein Ib-α Alleles Are Associated with Age at First Coronary Bypass Operation

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Background: **Age is a known risk factor for postoperative complications, but the genetic factors that account for variability in age at presentation for surgery have not been characterized. Because thrombosis is a critical process in the development of coronary syndromes, the authors hypothesized that patients bearing the -1208 insertion allele of tissue factor (TF) and longer glycoprotein Ib-α (GpIbα) variants may come to surgical attention sooner and undergo coronary artery bypass grafting (CABG) at a younger age. The authors tested this hypothesis in a cardiac surgery population.**

Methods: **The impact of the number of TF -1208 insertion alleles and the number of GpIbα repeats on age at first CABG were tested in 424 elective coronary bypass patients. Multivariate regression included traditional risk factors of sex, hypertension, diabetes, hyperlipidemia, and smoking. The authors also tested the hypothesis that these alleles are correlated with age at first noncoronary cardiac surgery in a group of 143 patients undergoing noncoronary cardiac operations.**

Results: **Both the number of TF -1208 insertion alleles and total number of GpIbα repeats were associated with younger age at first CABG in a univariate analysis. In multivariate regression in which traditional risk factors were included, the number of TF -1208 insertion alleles and the total number of GpIbα repeats were independent contributors toward age at first CABG. Neither polymorphism had a significant impact on age at first noncoronary cardiac surgery.**

Conclusions: **Genetic variants in TF and GpIbα are associated with younger age at first CABG, indicating that the younger and older first-time CABG populations are different on the genetic level. How these genetic differences may account for age-associated differences in perioperative risk will be the subject of future investigations.**

AGE is a known risk factor for complications of cardiac surgery. Adverse neurologic outcomes,[1–4] renal dysfunction,[5] atrial fibrillation,[6–8] gastrointestinal complications,[9] and hemostatic complications[10] are all independently associated with advanced age. Although it is clear that patients present for their first coronary artery bypass grafting (CABG) across a wide range of ages, the genetic factors that impact the age at first CABG have not been characterized. Because thrombus formation is a crucial event in acute coronary syndromes, we sought to define the impact of specific polymorphic variants in coagulation system genes on age at first CABG.

Tissue factor (TF), which associates with factor VII to initiate activation of factor X, is present in activated endothelium and atherosclerotic plaque[11,12] and may be responsible for the events leading to coronary occlusion. In addition, the glycoprotein Ib–IX–V complex, responsible for platelet adhesion to the subendothelium *via* von Willebrand factor,[13] also mediates key early events in thrombosis. Both TF and glycoprotein Ib have common genetic variants that may impact their respective activities. TF promoter polymorphisms comprise two common haplotypes, characterized by the presence (-1208 Ins) or absence (-1208 Del) of an 18-bp sequence at position -1208. The -1208 Ins allele has been associated with higher plasma TF concentrations and increased risk for venous thromboembolism.[14] The gene for the glycoprotein Ibα (GpIbα) subunit of the GpIb–IX–V complex contains a 39-bp variable number of tandem repeat (VNTR) polymorphism, resulting in one to four repeats of a 13–amino acid sequence in the carbohydrate-binding region of the peptide. In several studies,[15] the three- and four-repeat alleles have been associated with increased risk for stroke,[16] acute coronary syndromes,[16,17] and sudden cardiac death.[18]

Because these proteins mediate initiation of thrombosis, they represent reasonable candidates for association studies of cardiovascular risk. We were specifically interested in whether these variants could impact coronary disease progression and account for some of the variability in age at first CABG.

Materials and Methods

Patient Enrollment

This study was conducted after approval by the Vanderbilt University Institutional Review Board (Nashville, Tennessee) and in accord with institutional guidelines. We retrospectively examined the records of adult CABG patients in the Vanderbilt Cardiac Surgery Registry for whom the age at first CABG was known (n = 424). This registry is an ongoing repository of cardiac surgery patient data, with DNA storage and clinical data record keeping, to facilitate studies of genetic variants and surgical outcomes. For patients undergoing second or subsequent CABG, the age at first CABG was determined by the date assigned to their first surgery, as listed in the patient history. Patients undergoing emergency surgery and those with unstable hemodynamics were excluded. For a control group, we included all adults from the

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registry who were undergoing first-time noncoronary cardiac surgery (mostly valve repair or replacement, septal defect repairs) and who did not have a history of coronary surgery (n = 143). All patient care providers were blinded to patients' genetic data.

Definition of Parameters

Ethnicity consisted of patient-reported description, recalling the last two generations. Diabetes and hypertension were defined as present if included in the patient's problem list on the preoperative evaluation. Current tobacco use was defined as the self-reported packs per day at the preoperative evaluation, and smoking history was defined in terms of packs per day and years smoked, the product of which was pack-years. Because recent fasting lipid chemistries were not available for most patients, hyperlipidemia was defined as present if the patient was receiving lipid-lowering therapy at the time of preoperative evaluation. For brevity, we adopted the common nomenclature for the GpIbα alleles, referring to them as A (four repeats), B (three repeats), C (two repeats), and D (one repeat).

Genotype Analysis

Blood was drawn for genotype analysis at anesthetic induction, and DNA was isolated by standard protocols. To evaluate the -1208 I/D polymorphism of the TF promoter, we performed polymerase chain reaction (PCR) amplification of a 99-bp segment (nucleotides -1145 to -1243) of the TF gene. Amplification primers were 5'-GCACAGTTTTATTCTGTTAAAACA-3' and 5'-CCTCTCTCCTTCTTTCCCACGTTT-3'. Amplification was performed in 25-μl volumes containing 100 ng DNA, 25 pmol each primer, 1 U *Taq* polymerase (Roche, Basel, Switzerland), 200 μM each nucleotide, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, and 2.5 μl betaine (Sigma, St. Louis, MO). The reaction was conducted in a GeneAmp 9700 thermal cycler (Roche Molecular Systems, Pleasanton, CA) with a 5-min denaturation step at 94°C, 30 cycles of 95°C for 1 min, 50°C for 1 min, 72°C for 1 min, and finally 7 min at 72°C. Electrophoresis on 3.5% agarose gel revealed the presence or absence of the 18-bp insertion. Direct sequencing was performed on a subset of samples to verify the fidelity of this technique. Patients were classified as bearing zero (homozygous deletion), one (heterozygous), or two (homozygous insertion) insertion (Ins) alleles.

A recent report[19] identified a potentially serious source of genotyping errors arising from a commonly used PCR technique[20] for identifying VNTR alleles. Investigators found that standard PCR conditions may result in selective amplification of only one allele in heterozygous subjects. To avoid these potential errors, we adopted methods outlined by these authors,[19] which include use of dimethylsulfoxide and 7-deaza-dGTP in the PCR reactions. In addition, we selected patients with known B/C, C/D, and B/D heterozygous genotypes, determined by a separate method, to serve as positive controls. Our resulting GpIbα VNTR genotyping method is as follows. Amplification was performed in 25-μl volumes consisting of 100 ng genomic DNA template; 1 U *Taq* polymerase; 10 pmol each primer (described by Kaiser *et al.*[19]); 50 μM each dNTP, with 50% of the dGTP present as 7-deaza-dGTP; 10% betaine (Sigma); 7% dimethylsulfoxide; and buffer containing 10 mM Tris-HCl (pH 8.3 stock), 1.5 mM $MgCl_2$ and 150 mM KCl. Amplification cycles consisted of 10 cycles (94°C: 10 s; 55°C: 20 s; 68°C: 2 min), followed by 25 cycles (94°C: 10 s; 55°C: 20 s; 68°C: 120 s + 15 s/cycle), with +15 denoting an extension of the elongation time by 15 s/cycle, and a final extension time of 7 min at 72°C. Because we predicted that a gene-dose effect may be present regarding the number of VNTRs, we classified patients by the sum total of GpIbα repeats carried on both chromosomes. For example, genotype C/D was classified as three total repeats, genotypes C/C (the most common genotype) and B/D were each classified as four total repeats, genotype B/C was classified as five total repeats, and so on.

Statistical Analysis

Allele frequencies were evaluated for Hardy-Weinberg equilibrium using a two-sided chi-square test. Because the population was 92.6% white and 93% of the nonwhite patients were African-American (only three patients were neither white nor African-American), ethnicity was classified as white or nonwhite. The effects of the number of -1208 Ins alleles and the total number of GpIbα repeats on age at first CABG were first evaluated using one-way analysis of variance with linear contrast for trend. Bivariate correlations between specific genotypes and age at first CABG were evaluated using the Spearman correlation. To control for traditional cardiovascular risk factors, we used multivariate linear regression, where sex, diabetes, hypertension, current tobacco use, smoking history, and hyperlipidemia were forced into the model as necessary covariates. Ethnicity was included as a necessary covariate to rule out possible confounding by population admixture. Next, the genetic factors (number of -1208 Ins alleles and total number of GpIbα repeats) were added stepwise to the model and retained if their overall contribution was significant at the level of $P < 0.05$. Statistical analysis was performed using SPSS software for Windows, version 10.0 (SPSS, Inc., Chicago, IL).

Results

Study Population

Summaries of the patient populations are shown in table 1. Genotype and allele frequencies are included and were in Hardy-Weinberg equilibrium. Allele frequen-

Table 1. Patient Population

Parameter	CABG Patients	Non-CABG Patients	*P* Value
n	424	143	
Age, yr	60.9 (11.4)	50.3 (15.6)	<0.001
Sex (% female)	31.1	50.3	<0.001
Ethnicity			
White	92.6	91.4	0.492
African-American	6.7	7.9	
Hispanic	0.5	0	
Asian	0.2	1.4	
Medical history:			
Hypertension	72.6	49.7	<0.001
Diabetes	34.7	10.5	<0.001
Hyperlipidemia	48.1	16.8	<0.001
Smoking history	65.1	45.5	<0.001
Pack years (among smokers)	47.0 (37.5)	30.0 (21.2)	0.037
LVEF	0.453 (0.124)	0.481 (0.121)	0.036
Operation (includes CABG)			
Off-pump CABG	11.3	0	<0.001
Repeat sternotomy	10.1	0	<0.001
Valve operation	13.4	69.2	<0.001
Other procedures	3.5	48.3	<0.001
TF allele frequencies:			
-1208 Ins	46.9	39.9	0.038
-1208 Del	53.1	60.1	
GpIbα allele frequencies			
A (four repeats)	0.00	0	0.325
B (three repeats)	9.43	7.69	
C (two repeats)	85.3	85.0	
D (one repeat)	5.31	7.34	
TF genotypes			
Ins/Ins	22.2	17.5	0.093
Ins/Del	49.5	44.8	
Del/Del	28.3	37.8	
GpIbα genotypes			
B/B	0.9	2.10	0.105
B/C	15.6	11.2	
B/D	1.4	0	
C/C	72.9	72.0	
C/D	9.2	14.7	

Data are expressed as mean (SD) or as percent of patients in population.

CABG = coronary artery bypass grafting; Del = deletion allele; GpIbα = glycoprotein Ib-α; Ins = insertion allele; TF = tissue factor; VNTR = variable number of tandem repeats.

cies observed were similar to those reported by other authors.[14,16–18,20,21] Significant differences in age, sex representation, and coexisting disease between the CABG population and the non-CABG population are apparent and illustrate the underlying clinical differences between the two groups. Of note, the TF -1208 Ins/Del allele frequencies in the population presenting for coronary surgery were significantly different from those in the group presenting for noncoronary surgery.

Role of TF and GpIbα Alleles in Age at First CABG

Figure 1 shows mean age at first CABG as a function of the number of -1208 Ins alleles and the total number of GpIbα repeats. Significant differences were found by analysis of variance in each case ($P = 0.003$ for TF alleles and $P = 0.009$ for GpIbα alleles). These *P* values were

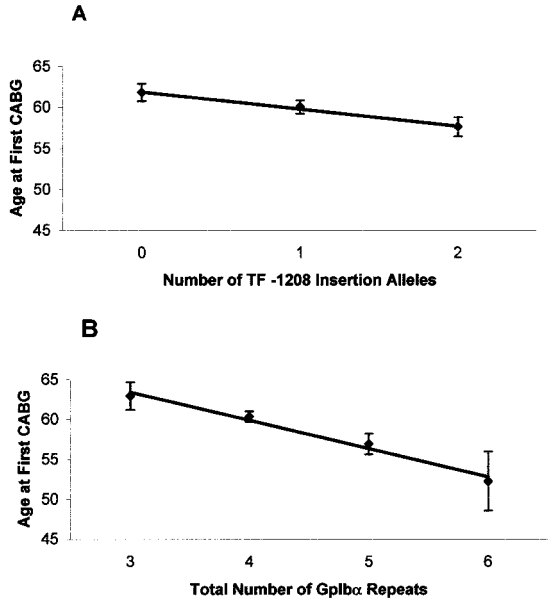

Fig. 1. Mean age at first coronary artery bypass grafting (CABG) by patient genotype. *Data points* represent mean for each genotype group in the CABG population (n = 424); *error bars* represent SEM. (*A*) Impact of the number of tissue factor (TF) -1208 insertion alleles on age at first CABG. Linear regression yielded an average of 2.1-yr-younger age for each insertion allele. (*B*) Impact of the total number of glycoprotein Ib-α (GpIbα) repeats on age at first CABG. Linear regression yielded an average of 3.2-yr-younger age for each GpIbα repeat.

determined using weighted linear contrast for trend, a sensitive method of detecting ordinal tendencies. In addition, significant bivariate correlations were determined using the Spearman correlation ($r_s = -0.127$, $P = 0.009$ for TF; $r_s = -0.144$, $P = 0.003$ for GpIbα). The gene dose effects of the number of -1208 Ins alleles and total number of GpIbα repeats on age at first CABG were quantified using simple linear regression, shown in table 2. Each -1208 Ins allele was associated with a 2.07-yr-younger age, and each GpIbα repeat was associated with a 3.21-yr-younger age at first CABG. Interestingly, the genotype groups for both TF and GpIbα were no different regarding coronary disease severity, as indicated by number of vessels bypassed, history of myocardial infarction, or history of class 3 or 4 angina, or regarding baseline myocardial function as indicated by left ventricular ejection fraction derived from intraoperative echo-

Table 2. Univariate Linear Regression of Age at First CABG on Number of TF Insertion Alleles and Number of GpIbα Repeats

Variable	Coefficient	SE	*P* Value
TF model			
No. of TF insertion alleles	−2.07	0.79	0.009
Y-intercept	61.92	0.93	<0.001
GpIbα model			
No. of GpIbα repeats	−3.21	1.05	0.002
Y-intercept	73.08	4.34	<0.001

CABG = coronary artery bypass grafting; GpIbα = glycoprotein Ib-α; TF = tissue factor.

Table 3. Multivariate Linear Regression of Age at First CABG

Variable	Coefficient	SE	*P* Value
Y-intercept	69.56	4.42	<0.001
Female sex	1.79	1.19	0.132
Hyperlipidemia	−1.39	1.03	0.177
Hypertension	*3.74*	*1.22*	*0.002*
Diabetes	0.74	1.14	0.518
Tobacco history	*0.0696*	*0.0189*	*<0.001*
Current tobacco use	*−4.88*	*0.96*	*<0.001*
Nonwhite ethnicity	−2.64	2.1	0.211
GpIbα repeats	*−2.74*	*1.03*	*0.008*
TF ins alleles	*−1.59*	*0.76*	*0.037*

Variables and values in italics are significant contributors.

CABG = coronary artery bypass grafting; GpIbα = glycoprotein Ib-α; Ins = insertion; TF = tissue factor.

cardiography (chi-square test with linear association and analysis of variance with linear contrast for trend; data not shown).

To control for possible confounding by traditional cardiovascular risk factors, we used multivariate linear regression. In this model, sex, diabetes, hypertension, current tobacco use (in packs per day), smoking history (in pack-years), hyperlipidemia, and ethnicity were necessary covariates. Next, the number of -1208 Ins alleles and the total number of GpIbα repeats were added to the model stepwise and included if their contributions were significant. As shown in table 3, significant contributors included hypertension, smoking history, current tobacco use, number of GpIbα repeats, and number of -1208 Ins alleles. Each GpIbα repeat and each -1208 Ins allele were associated with 2.74-yr-younger and 1.59-yr-younger ages at first CABG. In this model, the independent variables accounted for approximately 11.6% of the variability in the dependent variable.

Role of TF and GpIbα Alleles in Age First Noncoronary Surgery

As a negative control, we examined the dependence of age at first noncoronary surgery on TF and GpIbα genotypes in a cohort of patients undergoing first-time noncoronary cardiac surgery. Age at first noncoronary surgery as a function of TF Ins alleles and GpIbα repeats is shown in figure 2. There were no significant effects of TF or GpIbα alleles on age at first noncoronary surgery, using both analysis of variance with weighted linear contrast for trend ($P = 0.241$ and 0.983, respectively), and Spearman bivariate correlation ($r_s = -0.098$, $P = 0.243$; $r_s = -0.017$, $P = 0.844$, respectively). To control for possible confounding by traditional cardiovascular risk factors, we then constructed a multivariate model as we did for CABG patients with the same necessary covariates, followed by stepwise entry of genetic variables. As shown in table 4, significant contributors included smoking history, current tobacco use, and hyperlipidemia. There were no significant contributions of the genetic variables on age at first noncoronary cardiac surgery.

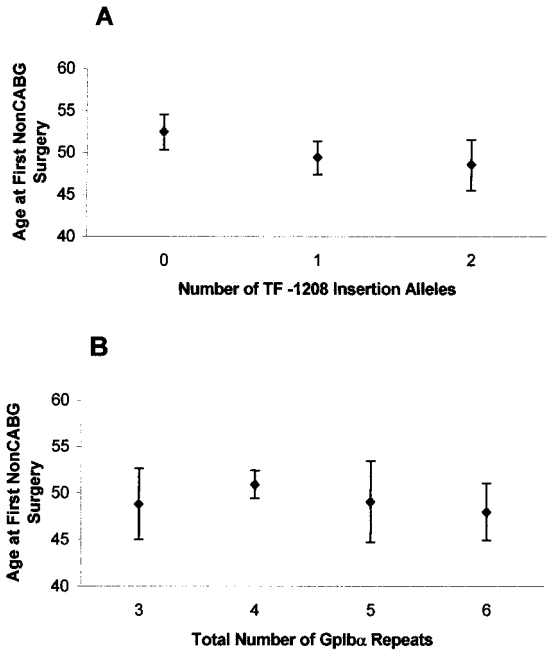

Fig. 2. Mean age at first noncoronary cardiac surgery by patient genotype. *Data points* represent mean for each genotype group in the noncoronary surgery population (n = 127); *error bars* represent SEM. (*A*) Impact of the number of tissue factor (TF) -1208 insertion alleles on age at first noncoronary surgery. No effect was found by analysis of variance, Spearman bivariate correlation, or linear regression. (*B*) Impact of the total number of glycoprotein Ib-α (GpIbα) repeats on age at first noncoronary surgery. As above, no effect was found by analysis of variance, Spearman correlation, or linear regression. CABG = coronary artery bypass grafting.

Genotype Frequency in Population Strata

The above findings indicate that the number of TF -1208 Ins alleles and the total number of GpIbα repeats are independently associated with younger age at first CABG. The distribution of allele and genotype frequencies across the different age groups presenting for first-time CABG is shown in figure 3. Here, we have divided

Table 4. Multivariate Linear Regression of Age at First Noncoronary Surgery

Variable	Coefficient	SE	*P* Value
Y-intercept	45.21	2.19	<0.001
Female sex	0.56	2.41	0.817
Hyperlipidemia	*6.71*	*3.35*	*0.047*
Hypertension	3.68	2.53	0.149
Diabetes	0.47	4.05	0.908
Tobacco history	*0.25*	*0.061*	*<0.001*
Current tobacco use	*−7.04*	*3.04*	*0.022*
Nonwhite ethnicity	−6.17	4.25	0.149
No. of TF -1208 Ins alleles			0.147
No. of GpIbα repeats			0.599

Variables and values in italics are significant contributor.

GpIbα = glycoprotein Ib-α; Ins = insertion allele; TF = tissue factor.

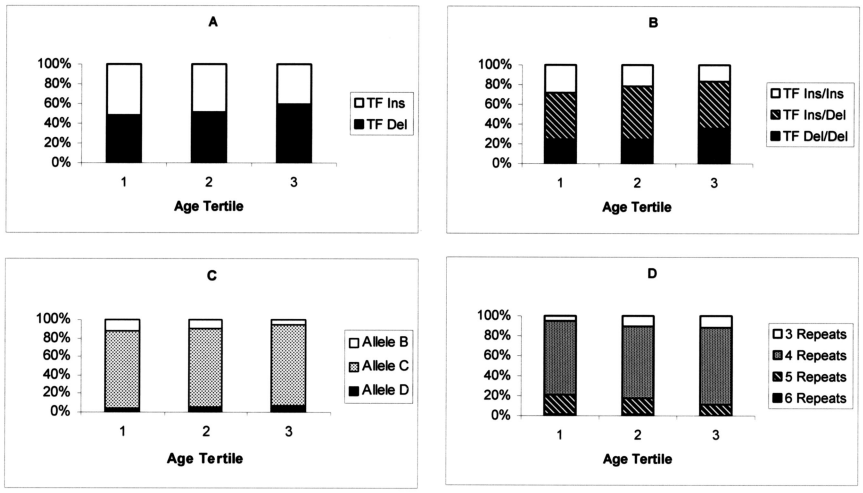

Fig. 3. Allele and genotype frequencies by age at first coronary artery bypass grafting (CABG) tertile. The CABG population was stratified into equal tertiles by age at first CABG (first tertile = age 54 years and younger; second tertile = age 55–65 years, inclusive; third tertile = age 66 years and older). *Bars* denote relative allele and genotype frequencies in each tertile as indicated. (*A* and *B*) Tissue factor (TF) allele and genotype frequency by age at first CABG tertile, respectively. Statistically significant differences across the three age groups were demonstrated by chi-square test with linear-by-linear association (P = 0.007 and 0.008, respectively). (*C* and *D*) Glycoprotein Ib-α variable number of tandem repeat allele and genotype frequency by age at first CABG tertile, respectively. Statistically significant differences across the three age groups were demonstrated by chi-square test with linear-by-linear association (P = 0.005 and 0.004, respectively). Del = deletion allele; Ins = insertion allele. Alleles B, C, and D are defined in the text.

the population into tertiles based on age at first CABG (first tertile = age 54 years and younger; second tertile = age 55–65 years, inclusive; third tertile = age 66 years and older). Allele and genotype frequency for TF and GpIbα were significantly different across the groups, using chi-square test with linear-by-linear association.

Discussion

Because patients present for their first CABG across a wide range of ages, we were interested in finding genetic variables that could account for some of this variability. Our selection of these candidate genes was based on (1) physiologic evidence for a role of these genes in pathogenesis, (2) existing epidemiologic reports of cardiovascular risk with variable conclusions, and (3) lack of reports showing the impact of these alleles on the age of clinical presentation. Here, we present evidence that polymorphisms of TF and GpIbα account for some of the variability in age at first CABG, independent of traditional cardiovascular risk factors. These genetic variables seem to play no role in age at first noncoronary surgery in a cohort of patients without coronary disease. Also, there was no difference in coronary artery disease severity (measured by number of vessels bypassed, history of myocardial infarction or severe angina, or left ventricular ejection fraction) across the genotype groups. We conjecture that these polymorphisms may be associated with earlier progression of subclinical disease to symptomatic disease, resulting in earlier surgical intervention. This is consistent with findings of Newman *et al.*,[22] who observed increased frequency of the apolipoprotein E4 allele in younger CABG patients, independent of traditional risk factors. The genetic risk interactions associated with cardiac surgery outcomes, however, are likely to be more complex because it is the older CABG population that is more at risk for postoperative complications.[1–9] The next set of genetic studies must address how these alleles may directly impact both immediate surgical risks (such as perioperative infarction or stroke) and longer-term surgical risk (such as need for repeat CABG). An assessment of these risks may provide the framework for exploring the possible benefit of preoperative genotyping and targeted patient treatment.

Genetic association studies have recently reported cardiovascular risks associated with many polymorphisms in candidate genes involved in coagulation and fibrinolysis.[23,24] Currently, the cardiovascular risk associated with TF variants is unclear. Arnaud *et al.*[14] reported only mildly increased TF plasma concentrations and mildly increased risk for venous thrombosis associated with -1208 Ins. Because their study was conducted in a European population and TF variants in other ethnic groups have yet to be reported, ethnicity was included as a necessary covariate in the regression models. The biology of TF is complex. TF with factor VIIa converts factors IX and X to their activated forms,[12,25,26] serving a crucial role in thrombosis.[25] Induction of TF on cell surfaces is associated with the hypercoagulability of obesity,[27] sepsis,[28] antiphospholipid antibody syndrome,[29,30] surgery,[31] and hyperhomocysteinemia.[32] TF is expressed in atherosclerotic plaque[33,34] and correlates with thrombin generation in blood flowing across diseased vessels.[35] TF may be an important signaling receptor, initiating angiogenesis through p38 and p42–p44 mitogen-activated protein kinases.[36] The relevance of the -1208 Ins/Del polymorphism on TF gene function is unclear, although the current study provides indirect evidence that the -1208 Ins allele may be associated with increased TF activity.

The cardiovascular risks associated with GpIbα polymorphisms have been reported but remain controversial.[16–18,20,21] These conflicting conclusions may be explained by differing study populations, study design, and

definitions of clinical endpoints. Where risk has been identified, the odds ratio for coronary endpoints associated with the larger alleles is in the range of 2.2–3.5 and seems to be highest in Asian populations.[17] The VNTR polymorphism is in strong linkage disequilibrium with two other loci. One is a Thr145Met substitution, with the Met-145 allele in concordance with the A or B VNTR alleles.[17] Some, but not all,[21,37–39] association studies have found increased prevalence of Met-145 in cases of arterial thrombosis relative to controls.[16,18] However, binding studies have not shown differences in von Willebrand factor binding between the Met-145 and Thr-145 variants.[40] The other locus in linkage disequilibrium with the VNTR locus is a C/T substitution at position -5, near the Kozak translation start site.[38,41,42] Although additional association studies showed increased stroke[21] and coronary[43,44] risk associated with the -5C allele, an American[45] and a British study[46] did not find an association with myocardial infarction. Expression studies *in vitro* have shown increased translational efficiency of constructs containing the -5C allele,[41] and the -5C allele exhibited increased collagen-stimulated thrombus formation at low shear rates.[47] These data provide mechanistic support for the role of GpIbα alleles in cardiovascular risk, but it is unclear exactly which locus may be responsible. Finally, the possibility exists that the polymorphisms measured are simply in linkage disequilibrium with a larger, inherited disease haplotype elsewhere in the genome, a consideration for this and for all genetic association studies in general. This may account for inconsistencies in previous studies of function associated with these variants.

Recently, the ability of the PCR method to detect VNTR alleles has been scrutinized.[19] These authors demonstrated that alterations in magnesium concentration could produce selective amplification of only one allele in a heterozygous subject, resulting in the subject being misclassified as homozygous. This problem can be resolved by addition of 7-deaza-dGTP or by a combination of *Taq* and *Pwo* polymerases. To address whether selective amplification produced misclassification in our population, we confirmed that our PCR technique produced dependable results using positive controls. Also, each amplification run produced at least one of the three alleles known to exist in the white population (B, C, or D), so methodologic reasons for failure to amplify one allele are unlikely.

We emphasize the difference between risk factors for coronary disease and risk factors for age at first CABG in this population. Hypertension, for example, was associated with older age at first CABG. This may be explained by considering that every patient has significant coronary disease, but we have defined hypertensive patients as those with that diagnosis (and therefore more likely to be receiving treatment) at the time of preoperative evaluation. In fact, we observed that hypertensive patients were more likely to be receiving β blockers (60.4% *vs.* 45.7%; $P = 0.018$ by chi-square test) and angiotensin-converting enzyme inhibitors (48.4% *vs.* 24.1%; $P < 0.001$). Therefore, in this population with coronary disease, patients with the diagnosis of hypertension are receiving different medical therapy than those without and are presenting later for surgery. Diabetes and hyperlipidemia did not appear as significant contributors, possibly because the study was underpowered or because the effects of these risk factors were also partly abrogated by medical therapy. Newman *et al.*[22] also did not find a significant effect of diabetes on age at first CABG in a similar cardiac surgery population, but they did report a significant impact of sex (which we did not observe). Also, other authors have sometimes not found associations between postoperative ischemic syndromes and either diabetes[48] or sex,[49] which underscores differences between these surgical populations, in which all patients have significant vascular disease, and the general medical population. It is also unclear why current tobacco use (defined as packs per day at the time of presurgical evaluation) was associated with younger age at first CABG, whereas smoking history (in pack-years) was associated with older age at first CABG. To speculate, heavier current smokers may experience symptoms related to tobacco use, prompting evaluation for concurrent cardiac disease, whereas those with heavy tobacco history may be less likely to seek medical attention and therefore present later for surgery. Such health behaviors, rather than pathophysiologic effects of smoking, may be at work here because we observed similar associations for the non-CABG population as well (table 4). These speculations regarding patient behavior and medical surveillance would need to be tested in studies designed to address them more appropriately.

In the noncoronary surgery cohort, significant contributors included hyperlipidemia, smoking history, and current tobacco use. For reasons that are unclear, hyperlipidemia (assessed by use of lipid-lowering therapy) was associated with increased age at noncoronary surgery. Antilipid therapy could serve as a marker for better medical surveillance, delaying surgical intervention. It is also possible that antiinflammatory effects of statins and other lipid-lowering drugs may suppress development of symptoms in patients with valve disease, although this has yet to be demonstrated. It is also unclear why *smoking history* was associated with older age at first noncoronary surgery, whereas *current tobacco use* was associated with younger age, a finding also observed in the coronary surgery population and possibly a result of similar health behaviors.

As mentioned indirectly above, the most important limitations of this study involve the surgical study population and how it differs from the general population. Conclusions and assumptions regarding coronary disease risk factors in the general population may not be

applicable when considering age at first CABG in this study group because all patients in this population by definition have significant coronary disease. Patients present for CABG for many reasons, which include unstable coronary syndromes, severe angina, or advanced asymptomatic disease found incidentally. Referral patterns and third-party payer mix also impact in patient selection. The finding that the severity of cardiac disease was constant across the genetic groups, whereas the age at first CABG was different, suggests that a certain level of disease severity and symptomatology warrants surgery, regardless of the age at which that severity is reached. In patients with specific at-risk alleles, that threshold is reached earlier. Also, although our group of noncoronary surgery patients was smaller than our CABG population (n = 143 *vs.* n = 424), a significant effect is unlikely to be found in a larger noncoronary population because of the low Spearman correlation observed in the nonparametric analysis. In addition, our findings may only be relevant to white patients because our population was 92.6% white. Future studies should focus on extending these observations to other populations and ethnic groups.

In conclusion, we report consistent, additive gene dose effects of the number of TF -1208 Ins alleles and the total number of GpIbα tandem repeats on age at first CABG, independent of traditional cardiovascular risk factors. Therefore, these GpIbα and TF variants may serve a more important role in influencing age for development of symptoms rather than lifetime ischemic risk and could account for conflicting findings in previous epidemiologic studies. Furthermore, we have demonstrated that the older and younger first-time CABG populations are different on the genetic level. How these genetic factors may impact age-related differences in surgical outcomes must be addressed in future studies.

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