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Background—The outcome of cardiac surgery is influenced by several factors, but the impact of specific genetic variants has not been systematically explored. Because blood conservation is a pressing issue in cardiac surgery, we tested the hypothesis that factor V Leiden (FVL), a common coagulation factor polymorphism, may protect against blood loss and transfusion in patients undergoing cardiac surgery.

Methods and Results—We enrolled 517 patients undergoing cardiac surgery, including 26 heterozygous FVL carriers, and evaluated the impact of FVL on chest tube output and transfusion by using univariate and multivariate techniques. For patients with FVL, blood loss at 6 (238±131 mL) and 24 hours (522±302 mL) was significantly lower than that for noncarriers (358±259 mL and 730±452 mL; $P<0.001$ and $P=0.001$, respectively). In a multivariate regression analysis, controlling for ethnicity and factors known to affect blood loss, FVL was a significant independent contributor at both time points. Using a similar regression approach, FVL did not have a significant effect on the number of units transfused. However, logistic regression of the risk of receiving any transfusion during hospitalization demonstrated a significant independent protective effect of FVL on overall transfusion risk.

Conclusions—FVL represents a common genetic trait that may protect against blood loss and transfusion in this population. This study demonstrates that genetic variability can affect the outcome of cardiac surgery. **(*Circulation*. 2003;107:1003-1008.)**

Key Words: genetics ■ hemorrhage ■ surgery

Hemorrhagic complications of cardiac surgery represent an important cause of death and resource utilization,[1–3] as patients who undergo cardiac surgery consume 10% to 20% of the nation's blood supply.[3,4] Many factors contribute to blood loss and transfusion after cardiac surgery, such as emergency operation, sex, repeat sternotomy procedures, and duration of cardiopulmonary bypass (CPB).[1,2,5] Despite the improved appreciation of risk, there is often wide variability in hemostatic response to cardiac surgery.[1,2,4,6] Multiple approaches to decrease blood loss in this population have been proposed, with varying degrees of success.[1,2,4,7,8]

The factor V Leiden (FVL) polymorphism consists of a glutamine substitution for arginine-506, producing a variant that is resistant to inactivation by activated protein C.[9] Since its discovery in 1994,[10] FVL has been extensively characterized as the most common known inherited risk factor for deep venous thrombosis.[9] FVL is unlikely to be a significant arterial thrombotic risk factor for adults with vascular disease, as outlined in a recent meta-analysis involving major cardiovascular risk factors,[11] although a few reports have shown increased arterial thrombotic risk associated with FVL in very specific subgroups.[12–14] Population studies estimate the carrier frequency of FVL at 3% to 7% in European populations, with rare occurrence in black and Asian populations.[9]

Investigators have speculated that FVL may be associated with decreased hemorrhagic risk or increased thrombotic risk for patients undergoing CPB.[15–19] Therefore, we characterized the impact of FVL on blood loss and transfusion by analyzing a population of prospectively enrolled patients with cardiac surgery at our institution. We found that when accounting for other known risk factors, FVL had a significant independent protective effect on postoperative blood loss and risk for blood transfusion during hospitalization.

Methods

Patient Enrollment

This study was approved by the Institutional Review Board for Research on Human Subjects at Vanderbilt University. After informed consent was obtained, and in accord with institutional guidelines, we prospectively enrolled 517 adult patients undergoing routine elective cardiac surgery. Patients scheduled for emergency operations, heart or lung transplantation, ventricular assist device placement, and those requiring reexploration for surgical sources of postoperative bleeding were excluded. Patients taking daily aspirin were asked to discontinue aspirin therapy at least 4 days before the

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scheduled date of the operation as part of routine care. All care providers were blinded to patient genotypes.

Patient Treatment

Anesthesia management and CPB were conducted according to our institutional protocol. Briefly, patients received general endotracheal anesthesia, consisting of induction with a combination of thiopental, midazolam, fentanyl, or etomidate and maintenance with isoflurane, pancuronium, and fentanyl. Monitoring included standard modalities (ECG, temperature, invasive blood pressure, pulse oximetry, and gas monitoring) plus central venous pressure or pulmonary artery catheter monitoring and transesophageal echocardiography. Aprotinin was used for repeat sternotomy procedures and those involving more than one open chamber procedure. ε-Aminocaproic acid (ε-ACA) was used for first-time sternotomy operations for patients without a history of venous thrombosis or unstable coronary syndromes.

Anticoagulation for CPB consisted of 400 U/kg unfractionated porcine heparin. Temperature management involved cooling to 28° to 30°C, temperature uncorrected blood gas management, and cold anterograde and retrograde cardioplegia techniques. At the conclusion of CPB, anticoagulation was reversed with 250 mg protamine, with an additional 50 mg administered in the following 10 minutes in the presence of ongoing microvascular bleeding. Autologous blood transfusion strategies such as normovolemic hemodilution were not used in any of these patients.

Transfusion decisions in the operating room and intensive care unit at our institution account for patient age, medical history, coexisting vascular disease, myocardial performance, and presumed cause of bleeding. Transfusion guidelines were as follows: packed red blood cells were transfused in sets of ≥2 units for patients >65 years of age with hematocrit <22, ongoing bleeding not likely to resolve with present interventions, CPB time >2.5 hours, or evidence of end-organ dysfunction. Platelets were transfused in 4-U sets for microvascular hemorrhage continuing after normalization of activated clotting time or for platelet dysfunction or low platelet count in the setting of ongoing clinical bleeding. Platelets were not administered simply for low platelet count in the absence of bleeding. Plasma was transfused in sets of 2 or more units when bleeding continued after platelet transfusion.

Clinical Parameters

We examined preoperative, intraoperative, and postoperative variables by chart review. Preoperative medications were defined as those medications the patient was receiving on a regular basis at the time of preoperative evaluation. Chest tube output was measured at 6 and 24 hours after intensive care unit arrival. Transfusion was recorded as the number of allogeneic units of blood components (red cells, platelets, plasma, and cryoprecipitate) and total units administered from the time of operating room entry until hospital discharge.

Genetic Analysis

We tested patient DNA for FVL by using a modified technique of Ridker et al.[20] Briefly, genomic DNA was isolated from blood obtained near the time of anesthetic induction. A 223-bp fragment of the factor V gene containing the G1691A substitution of exon 10 was amplified using primers 5′-ACCCACAGAAAATGATGCCCAG-3′ and 5′-TGCCCCATTATTTAGCCAGGAG-3′. Amplification reaction consisted of 20 ng template DNA, 200 μmol/L each dNTP, 25 pmol each primer, and 2 U Taq polymerase (Roche) in a buffer containing 10 mmol/L Tris-HCl (pH 9.2), 1.5 mmol/L $MgCl_2$, and 25 mmol/L KCl in a volume of 50 μL. Amplification consisted of denaturing at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with final extension of 72°C for 5 minutes. Amplified fragments were size-verified on 2% agarose gels and then digested for 2.5 hours at 37°C with *Mnl*I. Electrophoresis of the digested product yielded bands at 37, 82, and 104 bp for the wild-type allele and bands at 82 and 141 bp for FVL. Validation was confirmed by sequencing.

Mathematical Models

Statistical tests were performed with SPSS software 10.0 (SPSS Inc). Comparisons between patients with FVL and noncarriers were performed with the use of a *t* test for continuous data and χ^2 test or Fisher's exact test for discrete data. Univariate analysis of chest tube output and number of units of blood component transfusion was conducted with the use of a *t* test after Levene's test for homogeneity of variances. Significance was defined as $P<0.05$.

For multivariate analysis, we evaluated chest tube output at 6 and 24 hours and number of units of blood component transfusion as dependent variables by using stepwise linear regression. The following were entered stepwise as independent variables for chest tube output: carriership for FVL, age, ethnicity (white versus nonwhite), sex, repeat sternotomy, use of aprotinin, use of ε-ACA, open chamber procedures, preoperative platelet count, duration of CPB, and preoperative use of the following drugs: aspirin, coumarin, heparin, antiplatelet drugs, and nonsteroidal anti-inflammatory drugs (other than aspirin). For transfusion of blood products, preoperative hematocrit was also included as an independent variable. Risk for transfusion was evaluated by logistic regression, whereby patients were classified as either having received or not received transfusion of any blood product between arrival to the operating room and hospital discharge.

To accommodate for ethnic admixture, ethnicity was entered first as a necessary covariate in all regression models, since FVL is unevenly distributed between different ethnic populations. Intraoperative use of ε-ACA was less frequent among patients with FVL than noncarriers (Table 1). Therefore, use of ε-ACA was also entered into each regression model as a necessary covariate before entry of the remaining variables. Variables were then entered individually, beginning with the most significant contributor, and maintained in the model if their contribution was significant at the level of $P<0.05$. This method proceeded until none of the remaining variables was significant.

Results

Patient Population

The patient population is shown in Table 1. Of the 517 patients enrolled, 26 were heterozygous for FVL (referred to as "carriers"). Patients homozygous for factor V wild type were referred to as "noncarriers." We found no patients homozygous for FVL. FVL carriers were less likely to receive ε-ACA during surgery, possibly a result of our clinical guidelines to avoid its use in patients with a history of thrombosis. There were nonsignificant trends toward younger age, coumarin use, and history of congestive heart failure among FVL carriers.

FVL and Hemostasis After CPB

For patients with FVL, blood losses at 6 (238±131 mL) and 24 hours (522±302 mL) were significantly lower than for noncarriers (358±259 mL and 730±452 mL; $P<0.001$ and $P=0.001$, respectively). These results are shown in Figure 1. Next, multivariate linear regression was performed by using known independent variables as possible contributors (Table 2). At both the 6-hour and 24-hour time points, controlling for ethnicity and use of ε-ACA, FVL was a significant contributor to blood loss, with the listed variables contributing 21.3% and 19.1% of the variability in blood loss, respectively. The impact of FVL was similar to that of the antifibrinolytic drugs, aprotinin, and ε-ACA.

Factor V Leiden and Transfusion After Cardiac Surgery

Mean transfusion, in units of blood products, for carriers and noncarriers, is shown in Figure 2. No significant differences

TABLE 1. Patient Population

Parameter	Noncarriers (n=495)	Factor V Leiden (n=26)	P
Age	57.7 (14.3)	53.4 (12.1)	0.133
Sex, % women	38.3	42.3	0.682
Ethnicity			
White	91.5	100.0	0.250
Black	7.5	0.0	
Asian	0.6	0.0	
Hispanic	0.4	0.0	
Operation			
CABG	70.3	57.7	0.174
Valve	34.0	30.8	0.733
Non-CABG, nonvalve	10.4	11.5	0.745
Repeat sternotomy	13.2	11.5	0.999
Preop medications			
Aspirin	56.8	61.5	0.636
Antiplatelet drugs	6.3	0.0	0.391
NSAIDs	8.8	3.8	0.715
Coumarin	7.1	15.4	0.123
Heparin	9.8	11.5	0.734
Medical history			
Hypertension	64.8	64.5	0.949
Diabetes	28.5	30.8	0.804
LVEF	0.462 (0.118)	0.445 (0.140)	0.520
History of CHF	24.2	38.5	0.102
History of MI	33.6	30.8	0.765
Intraoperative treatment			
Aprotinin	22.0	23.1	0.897
ϵ-ACA	36.7	15.4	0.034*
CPB duration, min	120 (46)	124 (53)	0.666
Intra-aortic balloon pump	7.1	11.5	0.428

Numbers denote means (with standard deviation) or percent where appropriate.

NSAIDs indicates nonsteroidal antiinflammatory drugs; LVEF, left ventricular ejection fraction; CHF, congestive heart failure; MI, myocardial infarction; ϵ-ACA, ϵ-aminocaproic acid; CPB, cardiopulmonary bypass.

Significance determined by Student's *t* test for continuous data or χ^2 or Fisher's exact test for discrete data where appropriate.

were found between the two groups for any of the blood products. Table 3 shows multivariate analyses of transfusion for each blood component. FVL was not a significant contributor in any of these models.

Because transfusion typically occurred in defined sets (2 or more units of red cells, 4 U of platelets, see Methods section), we evaluated overall risk of receiving any transfusion. This was performed by classifying patients as having received or not received any transfusion between operating room arrival and hospital discharge. We found that 46.2% of FVL carriers and 28.3% of noncarriers were discharged from the hospital without transfusion ($P=0.051$ by χ^2 analysis). To account for possible confounding variables, logistic regression was then performed with known risk factors as possible contributors. As before, ethnicity and use of ϵ-ACA were included as necessary contributors before entry of any remaining variables. Table 4 shows the logistic regression model, in which FVL was found to exert a significant protective effect independent of known risk factors ($P=0.010$). In summary, FVL may not affect a number of individual units transfused

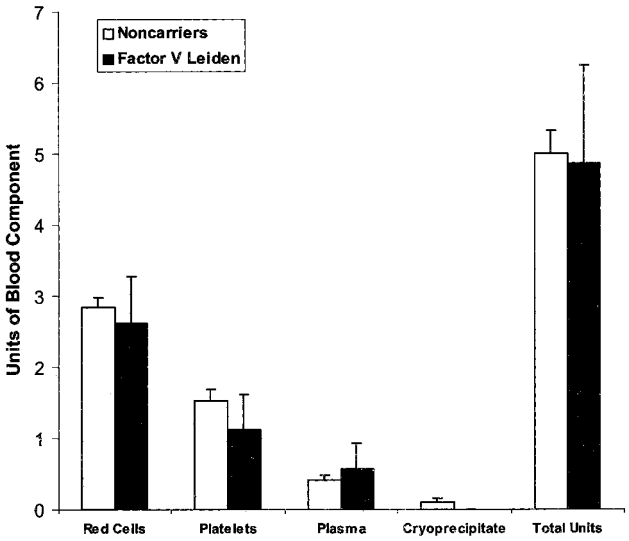

Figure 1. Effect of FVL on postoperative blood loss: Univariate analysis. Bars denote mean blood loss by chest tube output, error bars indicate SEM. *Significant difference between noncarriers and FVL carriers at $P<0.05$.

TABLE 2. Linear Regression Models for Blood Loss

Time Point/Parameter	Coefficient	SEM	P
6 Hours			
Constant	555.5	71.8	<0.001
Nonwhite ethnicity	40.34	40.16	0.315
Female sex	−85.26	22.73	<0.001
Use of ϵ-ACA	−144.2	24.5	<0.001
Use of aprotinin	−167.7	28.9	<0.001
Duration of CPB	1.057	0.247	<0.001
Preop platelet count	−0.5996	0.1561	<0.001
Preop NSAIDs	135.4	38.1	<0.001
Preop aspirin	79.12	22.17	<0.001
Factor V Leiden	−145.6	49.9	0.004
Age	−1.842	0.788	0.020
24 Hours			
Constant	882.8	94.3	<0.001
Nonwhite ethnicity	75.78	71.39	0.289
Female sex	−152.7	40.7	<0.001
Preop platelet count	−0.9235	0.2760	<0.001
Preop aspirin	97.65	40.39	0.016
Duration of CPB	2.141	0.444	<0.001
Use of aprotinin	−260.9	51.9	<0.001
Use of ϵ-ACA	−218.7	43.6	<0.001
Factor V Leiden	−238.5	88.6	0.007
Open chamber procedures	−85.83	42.74	0.045

ϵ-ACA indicates ϵ-aminocaproic acid; CPB, cardiopulmonary bypass; Preop, preoperative; NSAID, nonsteroidal antiinflammatory drug.

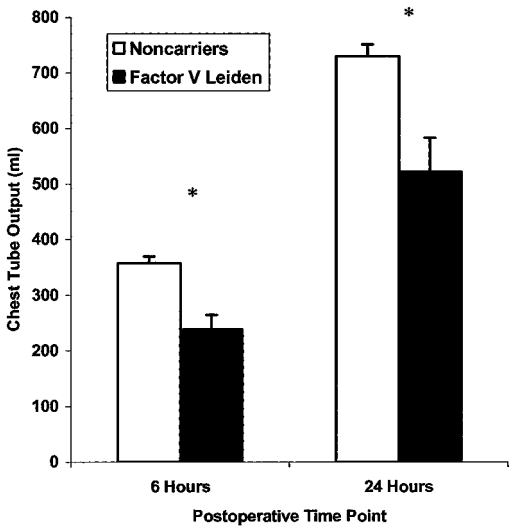

Figure 2. Effect of FVL on postoperative transfusion: Univariate analysis. Bars denote mean number of units transfused from beginning of surgery until hospital discharge. Error bars indicate SEM. No significant difference was found between FVL carriers and noncarriers for any of the blood components.

but appears to affect blood loss and overall risk for receiving transfusion in our population.

Discussion

Through the use of a multivariate method to account for known risk factors, FVL carriers in this study had decreased blood loss and were more likely to leave the hospital without a transfusion. This constitutes the first report of a significant effect of FVL on a relevant hemostatic outcome after cardiac surgery.

Improved hemostasis associated with FVL in cardiac surgery has been implied by Sweeney et al,[18] who report a trend toward decreased transfusion for patients with FVL. Likewise, increased thrombosis risk associated with FVL in cardiac surgery has been suggested, but the risk is unclear. Activated protein C resistance is exacerbated by aprotinin, both in vitro[18] and ex vivo,[16] strengthening concerns regarding thrombotic risk of antifibrinolytic drugs. Moor et al[17] reported increased coronary graft occlusion in patients with FVL, a result falling just short of significance ($P=0.06$). Fanashawe et al[15] described massive thrombosis after circulatory arrest with ε-ACA in 2 patients, one of whom carried FVL. Conversely, a Canadian study[19] reported no increased risk of postoperative thrombosis associated with FVL in 200 pediatric patients who had cardiac surgery. Overall, current inferences regarding the risk of FVL in cardiac surgery are provocative yet inconclusive. Our study is the first to report a significant independent effect of FVL on postoperative blood loss and transfusion and highlights the need for future investigations to characterize FVL as a possible thrombotic risk factor in this population.

The multivariate analysis of the transfusion data (Table 3) failed to demonstrate previously reported associations: repeat sternotomy, female patients, and open chamber procedures,[1,5] possibly because of the lack of an objective transfusion algorithm.[4] These associations are further hampered by underlying associations between independent variables. For example, repeat sternotomy procedures are routinely performed with aprotinin, making it difficult to separate their contributions. FVL was not found to affect the units of blood products transfused, possibly because the impact of FVL on blood loss in the first 24 hours (238 mL) is less than 1 unit of red cells. FVL did affect risk for receiving any transfusion (Table 4), so the overall effect of FVL on transfusion appears to be clinically relevant.

Notably, ε-ACA was used significantly less in FVL carriers (15.4%) than noncarriers (36.7%), possibly because our clinical practice includes ε-ACA for patients undergoing first-time CPB without a history of thrombosis or unstable coronary syndromes. However, this would be expected to increase blood losses in FVL carriers, attenuating the effect

TABLE 3. Linear Models for Transfusion of Blood Components

Blood Component/Significant Variables	R^2	Coefficient	P
Red cells	0.210		
Constant		5.24	<0.001
Nonwhite ethnicity		0.890	0.067
Use of ε-ACA		−1.37	<0.001
Preop hematocrit		−0.152	<0.001
Duration of CPB		−0.0179	<0.001
Age		0.0361	<0.001
Platelets	0.106		
Constant		1.07	0.160
Nonwhite ethnicity		0.0389	0.945
Use of ε-ACA		−0.983	0.004
Duration of CPB		0.0210	<0.001
Preop platelet count		−0.00676	0.003
Plasma	0.056		
Constant		0.224	0.471
Nonwhite ethnicity		−0.0893	0.704
Use of ε-ACA		−0.236	0.088
Duration of CPB		0.00661	<0.001
Preop platelet count		−0.00193	0.041
Cryoprecipitate	0.032		
Constant		0.376	0.075
Nonwhite ethnicity		−0.0795	0.605
Use of ε-ACA		−0.121	0.182
Preop platelet count		−0.00148	0.017
Preop aspirin		−0.211	0.015
Duration of CPB		0.00205	0.029
Total units	0.166		
Constant		9.22	<0.001
Nonwhite ethnicity		0.770	0.491
Use of ε-ACA		−2.20	0.001
Duration of CPB		0.0527	<0.001
Preop platelet count		−0.0134	0.003
Preop hematocrit		−0.159	0.006

ε-ACA indicates ε-aminocaproic acid; CPB, cardiopulmonary bypass; Preop, preoperative.

TABLE 4. Logistic Regression Model for Transfusion Risk

Parameter	Coefficient	SEM	*P*
Constant	2.77	1.27	0.028
Nonwhite ethnicity	−0.0889	0.422	0.833
Use of ϵ-ACA	−1.01	0.247	<0.001
Age	0.0307	0.0085	<0.001
Female sex	0.524	0.259	0.043
Preop hematocrit	−0.114	0.025	<0.001
Duration of CPB	0.0121	0.0030	<0.001
Factor V Leiden	−1.25	0.49	0.010

ϵ-ACA indicates ϵ-aminocaproic acid; CPB, cardiopulmonary bypass; Preop, preoperative.

observed. In addition, withholding ϵ-ACA from patients with thrombosis who are FVL noncarriers may increase the observed blood loss in the noncarrier group. To control for this potential confounding, the use of ϵ-ACA was included as a necessary covariate in regression models. Also, care providers were not blinded to patient history, which included history of venous thrombosis, thus creating a partial unblinding among the clinicians to FVL and a possible source of an unknown measure of confounding for which we have not controlled.

The mechanism of how FVL decreases postoperative blood loss needs to be investigated. Activated protein C resistance may increase thrombin at the injury site, which may also increase platelet reactivity. Hemodilution occurs during CPB, and factor V levels fall as low as 30% to 40% of normal.[21,22] Whether FVL is affected by hemodilution to a greater or lesser extent than wild-type factor V is unknown and worthy of speculation. Also, it is not known how protein C resistance conferred by FVL is affected by dilution of CPB, since dilution alone significantly alters coagulation.[21,22]

Preoperative nonsteroidal anti-inflammatory drugs and aspirin were associated with increased postoperative bleeding, a finding reported by others,[1,23] and consistent with a generally accepted model that platelet dysfunction accounts for a considerable fraction of bleeding.[24] Interestingly, preoperative coumarin use was not associated with increased blood loss in our study, a result also reported by others.[25] Dietrich et al[26] strikingly describe a decrease in blood loss after cardiac surgery associated with coumarin. This may be explained by lower prothrombin levels in coumarin-treated patients, since thrombin generation during CPB has been associated with postoperative bleeding.[27] Also, because our providers were not blinded to coumarin therapy, patients receiving coumarin may have received more plasma transfusion before surgery, supplementing coagulation factor levels, specifically antithrombin, and possibly limiting blood loss.[27] An interesting question is whether lack of increased blood loss associated with coumarin is limited to FVL noncarriers or if it extends to FVL carriers, since patients with FVL may have increased thrombin generation during CPB.

Some authors have suggested preoperative screening for FVL.[15] On the basis of current data, in an effort to decrease risk, data from prospective trials are needed to recommend screening of patients who have cardiac surgery. However, should selective screening, based on patient history or family history of venous thrombosis, be adopted, as recommended for medical patients in whom the diagnosis of FVL has been considered?[28] In our population, such selective screening based on history or family history would exclude 75% of the patients with FVL from being tested. Furthermore, neither history nor family history of venous thrombosis, when presented to the model, made a significant contribution to blood loss (data not shown), whereas FVL did. Therefore it is unlikely that selected screening would appreciably identify patients with FVL, or those at decreased risk for postoperative blood loss.

To our knowledge, this is the first report that FVL protects against blood loss and transfusion after cardiac surgery. This was observed while accounting for other factors known to affect hemorrhagic risk and therefore demonstrates that genetic variability can have an independent, relevant impact on outcome after complex surgical procedures.

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