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Genetics of Coagulation: Considerations for Cardiac Surgery

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Genetic variants in the coagulation system have been known since antiquity. Today, because of modern improvements in diagnosis and medical management, the clinician is likely to encounter a spectrum of coagulation factor deficiencies and identified polymorphic variants in the surgical population. Because perioperative hemorrhagic and thrombotic complications are potentially serious, it is important to understand the role that these defects and variants may play in predicting risk and optimizing patient management. The implications of coagulation genetics on the perioperative management of the cardiac surgery patient are reviewed.

For purposes of clarity, this review will address 2 rather distinct genetic groups: factor deficiencies and polymorphic genetic variants. First is the group of known, clinically apparent coagulation factor defects or deficiencies. These genetic lesions represent significant disturbances in the function of a gene product, are relatively rare, and are usually, but not always, diagnosed before a patient presents for cardiac surgery. In most cases, replacement of the missing factor and monitoring of levels or activities constitute the mainstay of clinical management. The second group consists of polymorphic variants in coagulation and fibrinolysis genes, which confer much smaller changes in the activities or levels of their corresponding gene products. These variants are often clinically unrecognized and are much more common in the population. Because hemostatic disturbances during cardiac surgery are significant and multifactorial, these genetic variants or groups of variants might interact with environmental factors to affect thrombotic or hemorrhagic risk. Authors have suggested that a significant amount of thrombotic and hemostatic risk could be partially accounted for on the basis of coagulation genetics. If this is true, then optimization of patient management may require preoperative genetic testing.

Coagulation Factor Defects or Deficiencies

This broad topic will be considered in 4 separate subtopics. The more common factor deficiencies (hemophilia A, B, and C, and von Willebrand disease) will be discussed, followed by the less common deficiencies. Inherited thrombophilias (excluding inherited protein C resistance and factor V Leiden) will be discussed in terms of deficiencies of protein C, protein S, and antithrombin. This section will conclude with a discussion of abnormal fibrinogens.

Hemophilias A and B

Factor VIII deficiency (hemophilia A) and factor IX deficiency (hemophilia B) are X-linked recessive diseases occurring in 1 of 50001-3 and 1 of 30,0004 male births, respectively. There is substantial variation in the clinical severity of spontaneous bleeding episodes, and severity correlates well with plasma factor levels.5 Some of the variations in bleeding may be explained

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by the reported 4.4% coinheritance of the factor V Leiden mutation seen in severe hemophilia A, resulting in fewer bleeding episodes and a later onset of first bleeding.6,7

These rare but severe bleeding diatheses, if uncorrected, clearly represent a major risk for perioperative hemorrhage. In cardiac surgery, successful outcomes have been shown by replacing these factors with factor concentrates to obtain levels of 100% preoperatively and then replacing them again to 100% when separating from cardiopulmonary bypass (CPB).8 But these patients require frequent factor replacement and commonly develop neutralizing antibodies (inhibitors), rendering replacement ineffective. Thus, if bleeding is refractory to standard therapy, the use of recombinant activated factor VII should be considered.9

Hemophilia C

Factor XI deficiency (hemophilia C) was first described in 1989 in the Ashkenazi Jewish population, where it is most common.10 In the general population, its incidence is estimated at 1 per million and is inherited in an autosomal pattern.11 At least 39 different mutations have been reported,12 resulting in a spectrum of bleeding disorders.13 Normal levels of factor XI activity range from 70% to 150%, and deficiencies are classified as partial (factor XI activity 20% to 70%) or severe (factor XI activity < 15%).14 Hemophilia C differs from hemophilias A and B in several important regards. First, the disorder is autosomal recessive and occurs in females and males with equal frequency. Also, in contrast to hemophilias A and B, factor XI deficiency does not typically result in spontaneous bleeding into joints and muscles. Factor XI activity also correlates poorly with bleeding tendency, emphasizing the influence of other factors in the phenotype. These factors may include variant factor XI molecules, the presence of other clotting disorders (eg, concomitant von Willebrand disease), or platelet defects.13 In women with severe factor XI deficiency, for example, there is a large variation in the incidence of postpartum hemorrhage.15 Many patients with factor XI deficiency do not suffer from spontaneous bleeding but are at risk for perioperative hemorrhage. In patients with known hemophilia C, factor XI replacement consists of plasma transfusion because factor XI concentrates are not available in the United States and reports suggest that such concentrates might carry thrombotic complications.16-18 Preoperative factor XI activity should be corrected to at least 30% to 45%, but because of the potential thrombotic risk, activity levels should probably be maintained at < 70%.19 Antifibrinolytics might be helpful in reducing bleeding,20 and if concomitant von Willebrand disease is present, the administration of desmopressin may be effective. Unlike hemophilias A and B, inhibitors are rare but do occur, and in this setting recombinant activated factor VII could be considered.21 Successful cardiac surgery in factor XI–deficient patients has been described with plasma transfusion being the mainstay of management.22-24

von Willebrand Disease

von Willebrand factor (vWF) multimers range in size from 600 kd to > 20,000 kd, consisting of dimers of 300-kd vWF subunits. The largest multimers are most effective at mediating platelet adhesion.25 von Willebrand factor is released from endothelial cells and platelets, carries factor VIII, and functions primarily in endothelial platelet adhesion. von Willebrand disease (vWD) is the most common inherited bleeding disorder, occurring in approximately 1% of the population,26 and is characterized by autosomal dominant inheritance in type 1 and 2 and by autosomal recessive inheritance in type 3. Seventy percent of all cases are type 1, which represents a quantitative decrease in vWF, type 2 is characterized by qualitative abnormalities in vWF, and the rare type 3, occurring in 1 per million,27 represents severe disease with nearly undetectable levels of vWF.28

Additional genetic variants of type 2 vWD will be briefly mentioned here and have been reviewed elsewhere.29 Type 2A is the most common subtype of type 2 vWD. It involves the loss of high molecular weight vWF multimers resulting in decreased platelet-dependent function. Type 2B has an increased affinity for the platelet glycoprotein Ib receptor resulting in spontaneous binding of large vWF multimers to platelets facilitating their clearance from the plasma, leaving only smaller, less reactive multimers. Type 2M affects the ability of vWF to polymerize, and type 2N affects the ability of vWF to bind factor VIII. Common genetic variants do not seem to functionally affect vWF, although polymorphisms of the vWF promoter region on genes altering endothelial function and the genotype at the secretor blood group locus can alter circulating levels of vWF.30-32 In addition, variants in platelet glycoproteins that bind vWF (pseudo-vWF or platelet-type vWF) can alter the phenotypic spectrum seen clinically.33
In cardiac surgery, there are few reports detailing the management of inherited and acquired vWD. Acquired vWD might be encountered in cardiac surgery as it is associated with fluoroquinolone antibiotics, hydroxyethyl starch, aortic valve stenosis, mitral valve prolapse, hypothyroidism, and uremia.44-46 In aortic stenosis, for example, acquired vWD is thought to be caused by proteolysis of vWF as it passes through the stenotic valve. In patients with aortic stenosis undergoing corrective surgery, one study found an increase in bleeding in those who had a significantly lower preoperative percentage of high molecular weight vWF multimers.47

As a general guideline based on evidence with noncardiac surgery, treatment with desmopressin (1-deamino-8-D-arginine vasopressin [DDAVP]) 0.3 µg/kg 90 minutes before surgery is effective in vWD type 1, usually effective in acquired vWD, but generally ineffective in types 2 and 3.37 Only single doses of DDAVP are given, as tachyphylaxis is well recognized as is the risk for hyponatremia. For the less common vWD types 2 and 3, vWF replacement with factor VIII concentrates containing vWF (such as Humate-P) or cryoprecipitate is required. Factor concentrates carry a lower risk of infectious transmission and are preferred to cryoprecipitate. Although dosages and frequency of dosing for factor concentrates are not standardized and are based on clinical experience, a dose of 20-30 IU/kg has been recommended to keep vWF levels 50% for 3-10 days or to control bleeding clinically.38

Other Factor Deficiencies

Factor VII deficiency, first described in 1951, is the most common autosomal recessive inherited factor deficiency.49 However, only the rare few with severe deficiency are considered at risk for bleeding because only trace amounts of activated factor VII are required to trigger the coagulation cascade.50 But factor levels do not correlate with bleeding tendencies, so the best predictor of bleeding appears to be a history of bleeding.51,52 Factor VII replacement has been done before surgery, but caution needs to be taken in those patients at low risk for bleeding as thrombosis might occur with factor replacement.53

In cardiac surgery, there is a paucity of literature regarding the management of factor VII deficiency. However, because tissue factor is the main activator of the coagulation system during CPB,54 factor VII may be consumed in this setting and therefore replacement seems reasonable. Although reports are limited, factor VII activity maintained at approximately 15% to 40% by continuous infusion or intermittent administration of factor VIIa has been used safely in cardiac surgery.55,56 Factor VIIa has a short half-life of about 3.5 hours, so intermittent dosing of 20-35 µg/kg was done every 3.5-4 hours during surgery and up to 48 hours postoperatively.

Factor XII, also known as Hageman factor, is the first component of the intrinsic pathway. Its deficiency is inherited in an autosomal recessive pattern and occurs in 2.3% of the general population.39 Preoperatively, the activated partial thromboplastin time is prolonged, but there appears to be no risk of increased bleeding because factor XII is not required for initiation or continuation of coagulation in vivo. In cardiac surgery, factor XII deficiency presents a challenge only from a heparin dosing perspective because the activated clotting time (ACT) requires factor XII to produce accurate values. Several methods have been proposed to deal with this, including empiric heparin dosing without ACT monitoring, assessing for heparin effect based on change from baseline ACT, or modifying ACT samples with donor fresh frozen plasma (FFP).40-43 There is speculation that factor XII deficiency might be a risk factor for thrombotic events because of impaired fibrinolysis, but thrombotic complications in the perioperative period have not been convincingly proven.44-48

Factor XIII (fibrin stabilizing factor) is a zymogen composed of 2 α and 2 β peptide subunits when circulating in the plasma and 2 α subunits in platelet granules; the active site is on the α subunit. Thrombin cleaves factor XIII α chains, and the factor XIII α covalently cross-links fibrin molecules leading to clot stabilization. Factor XIII deficiency is very rare, with an estimated incidence of one in several million and is inherited as an autosomal recessive trait with bleeding presenting only in homozygotes.57 Factor XIII levels are related to bleeding after cardiac surgery, and polymorphisms in the factor XIII genes may be related to thrombosis.58-60 Minor alterations of levels or function are likely to be manifested only in settings of tissue injury, because spontaneous bleeding presents only with levels < 2% normal. Replacement of factor XIII consists of plasma transfusion. The plasma half-life of the factor XIII molecule is long (> 96 hours), and full replacement is not needed before surgical intervention in order for normal hemostasis to occur. Determining levels is a complex laboratory test, but modified thromboelastography has been suggested as a simpler monitoring modality.61
Inherited Thrombophilias

Patients with inherited thrombophilias are at lifelong risk of developing venous thrombosis, usually following exposure to a trigger such as pregnancy, estrogen use, trauma, or surgery. Arterial thrombosis caused by these inherited thrombophilias has been reported but is less common. Preoperative screening for inherited thrombophilias is not routinely done in the absence of previous thrombosis, but their unrecognized presence might explain some variation in bleeding seen in cardiac surgery. Hereditary thrombophilias could protect from perioperative bleeding, but they might also predispose to perioperative thrombotic complications. The role of inherited thrombophilias or common polymorphisms in cardiac surgery remains incompletely understood.

Proteins C and S

Protein S, a vitamin K–dependent protein, is an important natural anticoagulant and produces its effect as a cofactor to protein C, which is also vitamin K dependent. Protein C is activated by the binding of circulating thrombin to thrombomodulin, an endothelial receptor, which then causes inactivation of factors V and VIII (Figure 1). In addition, activated protein C increases endothelial release of tissue plasminogen activator (tPA) and inhibits tPA inhibitor. Thus, proteins C and S together promote anticoagulation and fibrinolysis; and deficiency in one or both would therefore produce a procoagulant state.

Protein C Deficiency

Protein C deficiency was first described by Griffin et al in 1981 and occurs in 1 of 200 to 1 of 500 patients. Homozygous deficiency of protein C is associated with life-threatening thrombosis occurring in the neonatal period, whereas those with heterozygous deficiencies have an increased risk of venous thrombosis later in life. Heterozygous protein C deficiency is inherited in an autosomal dominant fashion, and a more severe form is an autosomal recessive disorder. More than 160 different genetic mutations have been identified. Protein C deficiency in cardiac surgery is associated with intraoperative graft thrombosis in off-pump coronary artery bypass graft (CABG) surgery, a higher incidence of perioperative myocardial infarction, and postoperative embolic strokes. The use of full aprotinin doses in patients with protein C deficiency undergoing repeat sternotomies with CPB has been safely done with the preemptive administration of 4 units of FFP to normalize protein C levels in one case, which is similar to what has been reported with protein S deficiency.
Protein S Deficiency

In 1984 an association between protein S deficiency and thrombosis was identified. Like protein C deficiency, it is inherited in an autosomal dominant manner, and a more severe form is the autosomal recessive disorder. It occurs in approximately 1% to 7% of the general population. A case report demonstrated successful management in a patient undergoing on-pump CABG by withholding supplemental antifibrinolytic drugs, but there are also reports of intraoperative and perioperative graft thrombosis. Theoretically, there might be less risk of thrombosis with concomitant aprotinin use in those with protein C deficiency. Spanier et al report the uneventful use of half-dose perioperative aprotinin in repeat sternotomies for heart transplantation in a patient who underwent previous CABG that was performed without perioperative antifibrinolytic drugs and complicated by early postoperative graft thrombosis. During the heart transplantation, however, the patient received 4 units of FFP before heparinization, and intraoperative protein S levels were subsequently measured at 150% of normal, whereas preoperative levels were 36% of normal (normal, 65% to 140%). No additional products were administered, and there were no hemorrhagic or thrombotic complications.

Antithrombin III Deficiency

Antithrombin III (ATIII) is the primary inhibitor of blood coagulation that, when bound to heparin sulfate moieties on endothelial cells, neutralizes the procoagulants thrombin, factor Xa, factor IXa, and factor Xa. It also has a weaker action on trypsin, plasmin, kallikrein, and factor VIIa. Type I ATIII deficiency involves reduced synthesis caused by the deletion of a major segment of the ATIII gene, small deletions/insertions, or single-base substitutions. Type II ATIII deficiency is caused by a single mutation (eg, arginine to cysteine or leucine to phenylalanine), which produces a variant protein with markedly reduced functional activity. Both are autosomal dominant. The association of thrombosis with depressed levels of ATIII was first described in 1965 and occurs in approximately 1 of 2000 cases. Clinical presentation is similar to protein C and protein S deficiency with a predominance of venous thrombosis.

Patients with ATIII deficiency could develop inadequate anticoagulation in the setting of CPB, resulting in suboptimal suppression of disseminated intravascular coagulation, thus putting these patients at risk for thrombotic or hemorrhagic sequelae, which might range from subtle postoperative neurocognitive dysfunction to massive thrombosis to consumptive coagulopathy and hemorrhage. Many cardiac surgery patients present with acquired ATIII deficiency. Risk factors for acquired ATIII deficiency include pregnancy, sepsis, recent surgery, disseminated intravascular coagulation, and ongoing heparin infusion. In the setting of cardiac surgery, a relative decrease in ATIII levels before CPB is usually caused by heparin infusion and is known as heparin resistance. Heparin resistance is defined as failure to reach an acceptable activated clotting time suitable for CPB after a standard 300-400 U/kg dose of heparin and is usually identified immediately before initiating CPB in the operating room. The normal physiologic range of ATIII activity is 70% to 130%, whereas ATIII activity in heparin-resistant patients is approximately 50%, similar to the 40% to 50% activity levels seen with hereditary ATIII deficiency.

There is limited evidence regarding the management of hereditary ATIII deficiency during cardiac surgery and related procedures, so this section will focus on the management of acquired ATIII deficiency. In percutaneous transluminal coronary revascularization, acquired ATIII deficiency was associated with thrombotic complications. In cardiac surgery, ATIII deficiency limits the effectiveness of heparin anticoagulation needed for CPB. In this setting, heparin resistance is treated with FFP or recombinant human ATIII at a dose of 75 U/kg, which carries less of an infectious risk. The use of recombinant human ATIII in cardiac surgery appears to be safe with no differences in outcomes compared with controls, including postoperative transfusion requirements, regardless of whether the underlying cause of the heparin resistance was acquired or hereditary.

Abnormal Fibrinogens

Dysfibrinogenemias

These rare abnormal fibrinogens are inherited in an autosomal dominant manner and might manifest as bleeding or recurrent thrombosis, depending on the type of mutation. The dysfibrinogenemias are named for the city in which the abnormal fibrinogen was discovered, for example, Detroit and Cleveland, among others. Mechanisms by which abnormal fibrinogen...
may lead to thrombosis include decreased thrombin binding leading to excessive circulating thrombin, and via the production of fibrin resistant to fibrinolysis. Bleeding occurs when the normal production of fibrin is impaired. There are many dysfibrinogenemia variations, but there is little evidence regarding its impact on cardiac surgery. There are also 3 common fibrinogen polymorphisms, with an incidence of approximately 15%, that have been implicated in coronary artery disease but were not associated with bleeding after CABG surgery (Table 1).

Afibrinogenemia
Congenital afibrinogenemia is an autosomal recessive disease first described in 1920 that results in the inability to synthesize fibrinogen. The defect is localized to the fibrinogen gene cluster on chromosome 4 and occurs in 1-2 per million. Patients can be homozygotes with complete absence of endogenous fibrinogen or heterozygotes with mild to moderate hypofibrinogenemia, the latter of which is often asymptomatic.

In cardiac surgery, caution must be taken because thrombotic complications have been reported as these patients might adjust physiologically to rebalance their hemostatic, coagulation, and fibrinolytic systems, which might make them hypercoagulable when fibrinogen is replaced and/or antifibrinolytic agents are used perioperatively.

Polymorphic Variants in Coagulation and Fibrinolysis Genes
Although the aforementioned defects in the coagulation system place patients at significant lifelong hemorrhagic or thrombotic risks, these problems are usually circumvented in the perioperative period because such defects are frequently identified before surgery. Appropriate management, as outlined herein, can be planned in a relatively straightforward manner by replacement of the missing factor and monitoring of levels or activities. Such is not the case for the broad array of polymorphic coagulation variants. Polymorphisms are defined as genetic variants that occur with > 1% incidence and might or might not contribute to an abnormal phenotype, whereas the inherited diseases such as hemophilia are typically Mendelian or X-linked and occur with an incidence usually < 1%. The impact of a single genetic polymorphism on the function of its gene product might range from zero effect to a very mild impact to a clinically measurable risk. More common polymorphisms might have indirect ties to a disease phenotype and might be dependent on gene-gene or gene-environment interactions, or “epistasis.” The risks associated with specific variants are only beginning to be explored. Even if most patients arrived in the operating room with their genotypes known to their medical teams, optimal management for individual genetic subgroups, if such a management exists, is presently undetermined. The purpose of this discussion is to illuminate the growing field of perioperative coagulation genomics for the practicing anesthesiologist, identifying subjects where research advances can be expected in the coming years. Before discussing the details of where this research is headed, it is important to consider some of the methodological and statistical pitfalls.

Limitations of Genetic Association Studies
When interpreting any genetic association study, several epidemiologic limitations potentially leading to false-positive findings should be considered; these include inadequate sample size, selection of control groups, multiple testing, and population substructure. An exhaustive discussion of this topic is beyond the scope of this review, and the interested reader is referred to several recent articles on the topic. Because the ideal statistical method for these studies has not been determined, the following approach is used.

Descriptive statistics, including allele frequency, Hardy-Weinberg equilibrium, and linkage disequilibrium, are calculated for all candidate polymorphisms, which are chosen based on biologic plausibility of the molecule (gene product) being mechanistically involved in the outcome studied. Before analysis, genotypes homozygous for the minor allele (proposed variant associated with disease) were combined with heterozygote carriers of the minor allele. Homozygotes with 2 major alleles are referred to as "wild type." Consequently, analyses are based on 2 genotypic classes for each candidate polymorphism, reflecting the presence (1 or 2 copies) or absence of the minor allele. Clinical models are developed using perioperative and demographic variables previously shown to account for variation in the outcome. Then, separate analyses are performed for each polymorphism to test the null hypothesis of no association between genotype and outcome. Polymorphisms are also combined
to investigate all possible 2-way gene interactions. A multivariate regression model for the outcome is fit using all candidate gene polymorphisms as main effects and all significant pairwise interaction terms identified above a level set at 0.1. Stepwise multiple regression with backward elimination, and a level set at 0.05, is then used to obtain a simpler model. The resulting combination of individual polymorphisms and pairs of polymorphisms constitutes the final genetic model. Finally, a combined model, adding independent variables from the genetic model to those already identified in the clinical model, is also fit to determine the

### Table 1. Candidate Gene Polymorphisms

<table>
<thead>
<tr>
<th>Gene (Synonyms)</th>
<th>Polymorphism</th>
<th>Prothrombotic Allele</th>
<th>Phenotype</th>
<th>Caucasian</th>
<th>African American</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet Membrane Glycoproteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet glycoprotein IaIIa (GPIaIIa, α₂β₁ integrin)</td>
<td>–52C &gt; T</td>
<td>–52C</td>
<td>↑ Surface receptor expression</td>
<td>0.67</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>807C &gt; T</td>
<td>807T</td>
<td>↑ Surface receptor expression and collagen binding</td>
<td>0.38</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Platelet glycoprotein Ibα (GPIbα)</strong></td>
<td>–5T &gt; C</td>
<td>–5C</td>
<td>↑ Surface receptor expression</td>
<td>0.13</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>524C &gt; T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Thr145Met)</td>
<td>Met145 (Ko&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>Unknown</td>
<td></td>
<td>0.09</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Platelet glycoprotein IIIa (β₃ integrin)</strong></td>
<td>1565T &gt; C (Leu33Pro)</td>
<td>Pro33 (P&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>↑ Sensitivity to activation</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Platelet glycoprotein VI (GPVI)</strong></td>
<td>13254T &gt; C (Ser219Pro)</td>
<td>Pro219</td>
<td>Unknown</td>
<td>0.15</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Coagulation Factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin (Factor II, FII)</td>
<td>20210G &gt; A</td>
<td>20210A</td>
<td>↑ Level</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>–1208del/ins</td>
<td>–1208ins</td>
<td>↑ Level</td>
<td>0.46</td>
<td>0.62</td>
</tr>
<tr>
<td>(TF, Factor III, FIII)</td>
<td>–603A &gt; G</td>
<td>–603G</td>
<td>↑ Level</td>
<td>0.49</td>
<td>0.57</td>
</tr>
<tr>
<td>Coagulation factor V (FV)</td>
<td>1691G &gt; A (Arg506Gln)</td>
<td>Gln506</td>
<td>APCR</td>
<td>0.02</td>
<td>0.003</td>
</tr>
<tr>
<td>Fibrinogen-β chain (FGB)</td>
<td>–854G &gt; A</td>
<td>–854A</td>
<td>↑ Level</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>–455G &gt; A</td>
<td>–455A</td>
<td>↑ Level</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>–148C &gt; T</td>
<td>–148T</td>
<td>↑ Level</td>
<td>0.17</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Coagulation Inhibiting Factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue factor pathway inhibitor (TFPI)</td>
<td>–399C &gt; T</td>
<td>–399T [30]</td>
<td>↓ Level</td>
<td>0.11</td>
<td>0.21</td>
</tr>
<tr>
<td>Thrombomodulin (THBD)</td>
<td>1959C &gt; T (Ala455Val)</td>
<td>Val455</td>
<td>Unknown</td>
<td>0.19</td>
<td>0.1</td>
</tr>
<tr>
<td>Fibrinolysis Inhibiting Factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasminogen activator inhibitor type 1 (PAI-1)</td>
<td>–675ins/del (5G/4G)</td>
<td>–675del (4G)</td>
<td>↑ Level</td>
<td>0.54</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylenetetrahydrofolate reductase (MTHFR)</td>
<td>677C &gt; T (Ala222Val)</td>
<td>Val222</td>
<td>↓ Enzyme activity, homocysteine level</td>
<td>0.32</td>
<td>0.19</td>
</tr>
<tr>
<td>Angiotensin Converting Enzyme (ACE)</td>
<td>Intron 16 ins/(I)/del(D)</td>
<td>Intron 16 del</td>
<td>↑ Enzyme activity</td>
<td>0.46</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Note: Minus signs indicate the number of nucleotides upstream from the defined main transcription-initiation site. Major (wild-type) alleles are indicated to the left and minor alleles to the right of each number. For polymorphisms in the encoded protein sequence, the resulting nonsynonymous amino acid substitution is shown in parentheses. APCR = activated protein C resistance. Source: Modified with permission from Welsby IJ, Podgoreanu MV, Phillips-Bute B, et al. Genetic factors contribute to bleeding after cardiac surgery. J Thromb Haemost. 2005;3:1206-1212.²⁶
extent to which genetic polymorphisms account for variation in outcome beyond that explained by clinical variables (gene–environment interaction).

Control for Population Structure
Genomic control analysis examines for evidence of population stratification in study cohorts, and in its absence, self-reported ethnicity can be used as a covariate in multivariate models to adjust for any race effect. Relative population weights, determined by population structure analysis using > 50 unlinked genetic polymorphisms, are used to test for association between race and outcome variable, as previously described. This confirmatory step excludes subtle effects of racial mixing in diverse populations and justifies using the simpler self-reported ethnicity to account for any possible race effect. Potential bias from population admixture can be investigated in 3 ways. Self-reported race (provided racial groups are sufficiently represented) and interaction between race and each genetic polymorphism are tested directly as predictors of the outcome. Race can then be tested as a covariate in later multivariable models.

Population Size and Study Power
Typically, studies require a relatively large population of patients and a prospective cohort design that reduces the selection bias inherent in case-control designs. Power analyses need to be performed accounting for the variability of the outcome variable and the frequency (incidence) of the minor allele in the population to be studied. Specialist software is available, such as nQuery Advisor Release 4.0 (Janet D. Elashoff, Statistical Solutions Ltd., Los Angeles CA, 2000).

Linkage Disequilibrium
Single nucleotide polymorphisms identified as associated with outcome might be in linkage disequilibrium with other functional (causal) variants not included in the analysis; only larger studies incorporating more single-nucleotide polymorphisms could delineate this effect. Other important alleles, not considered as primary candidate polymorphisms or in linkage disequilibrium with studied polymorphisms, might not be tested in a given study. For example, an explanation for the interaction observed between 2 polymorphisms (A and B) in linkage disequilibrium might rely on genetic structure rather than effect modification. It is possible that the observed variance in postoperative bleeding associated with the interaction term is not caused by A, B, or the combination of the two, but rather another genetic effect (polymorphism C) that tends to travel with this particular combination of alleles, or haplotype.

Multiple Comparisons
Finally, it is necessary to adjust for multiple comparisons using several different techniques (permutation testing, Bonferroni correction, false discovery rate) and present all data simultaneously rather than focusing on any one specific finding. The best test for a true association is for multiple authors to confirm the association. By the nature of examining numerous multilocus interactions and including clinical factors, multiple comparisons are necessary even when a limited subset of primary candidate genes is prospectively chosen. When candidate polymorphisms are selected based on previously published scientific evidence, it is likely that findings are rooted in biology and not spurious testing. In this setting, exploratory studies often make no adjustment for multiple comparisons. However, additional prospective studies will be required to confirm such results. Although analysis of gene-gene interactions does increase the number of tests performed, it is important to explore these relationships between common genetic variants, accepting that this approach is hypothesis generating rather than definitive.

Employing haplotype analysis can reduce the number of comparisons. For example, if there are 3 polymorphisms that exist together in a gene each with 3 allelic combinations (AA, Aa or aa), there are 27 (3^3) possible genotypes. In practice, however, there might be only 5 actual genotypes that have ever been determined in humans; therefore, these 5 haplotypes can be considered as covariates in a model rather than considering every possible allelic combination of the individual polymorphism. This could confer some error in that these 5 haplotypes account for only 99% of the observed genotypes; if they accounted for only 70%, then haplotype analysis would not be an appropriate technique. This decision is at the investigators’ discretion, but any study should provide this information to the reader.

Factor V Leiden Gene Mutation G1691A
Resistance to activated protein C (APC) is the most common cause of hereditary thrombophilia, and 90%
of those with APC resistance have the mutation termed factor V Leiden.\textsuperscript{104-106} This arginine-506 to glutamine-506 substitution in factor V, at the site where APC normally cleaves activated factor V, produces a variant that is resistant to inactivation by APC.\textsuperscript{106} Factor V Leiden is reported to be the most commonly inherited risk factor for thrombosis,\textsuperscript{105} occurring in up to 15\% in Greece, Sweden, Lebanon, and Syria,\textsuperscript{107} approximately 5\% in Europe and Canada,\textsuperscript{108} 2\% to 4\% in Holland,\textsuperscript{109} and < 1\% in Asia and Africa.\textsuperscript{110} In a North Carolina cardiac surgical population, the incidence of heterozygotes was < 2\%.\textsuperscript{96} There might be a blood-sparing effect attributable to the factor V Leiden in cardiac surgery. In recent studies, patients undergoing cardiac surgery with the factor V Leiden mutation had less perioperative blood loss and were less likely to receive transfusion.\textsuperscript{111,112} In one of these analyses, the impact of factor V Leiden persisted in a multivariate analysis accounting for other clinical risk factors.\textsuperscript{111} However, a larger study involving analysis of multiple genetic variants did not reveal an independent blood-sparing effect of the factor V Leiden mutation.\textsuperscript{96} The thrombotic risk of factor V Leiden in surgery is unclear, but it is probably small.\textsuperscript{113} There is a well-established risk of thrombosis associated with the presence of the factor V Leiden mutation, and although early studies in pediatric cardiac surgery did not show adverse outcomes,\textsuperscript{89} thrombotic complications could occur in cardiac and vascular surgery. For example, in CABG surgery there was a trend toward complications such as saphenous venous graft occlusion.\textsuperscript{114} In vascular surgery, patients with factor V Leiden were more prone to graft thrombosis at one month and one year,\textsuperscript{115} but these data have not been duplicated.

In the presence of antifibrinolytic drugs, the thrombotic risk of factor V Leiden still appears to be small or unmeasurable in the current literature. Regarding the concomitant use of lysine analogs in cardiac surgery, such as epsilon aminocaproic acid, reports of catastrophic thrombosis have been published.\textsuperscript{113,114} With aprotinin, there is a theoretical risk favoring thrombosis when combining the APC resistance of factor V Leiden with the APC inhibitory effects of aprotinin.\textsuperscript{112} However, prospective studies have reported no adverse thrombotic events in patients with the factor V Leiden mutation when using epsilon aminocaproic acid in first-time sternotomies, without a history of venous thrombosis, or aprotinin in repeat sternotomies.\textsuperscript{111,112}

### Prothrombin Gene Mutation 20210A

First described in 1996, the prothrombin gene mutation is reported to be the second most common inherited thrombophilia,\textsuperscript{105} occurring in approximately 2\% of healthy controls.\textsuperscript{116} The mutation is a guanine to adenine substitution at position 20210 in the 3'-untranslated region of the prothrombin gene leading to increased hepatic synthesis of plasma prothrombin,\textsuperscript{117} resulting in an increased risk of venous thrombosis. Studies are limited, but the prothrombin gene mutation has been associated with less bleeding after CABG surgery.\textsuperscript{96}

### Inherited Hyperhomocysteinemia, Methylenetetrahydrofolate Reductase Gene Mutation

Severe hyperhomocysteinemia is a rare inborn error of metabolism (cystathionine beta-synthase deficiency), whereas mild to moderate hyperhomocysteinemia occurs in up to 15\% of patients.\textsuperscript{118} In cardiac surgery, this hypercoagulable state may lead to thrombosis or altered hemostasis, and unlike the other inherited thrombophilias, hyperhomocysteinemia can be associated with arterial thrombosis. Although the exact mechanism is poorly elucidated, homocysteine has effects on the vascular endothelium that can lead to thrombosis. Hyperhomocysteinemia is an example of gene-environment interaction being determined in part by genetic factors and also by dietary intake of vitamins B\textsubscript{6} and B\textsubscript{12}, concomitant renal failure, hypothyroidism, increasing age, and smoking. Furthermore, hyperhomocysteinemia is not caused by a single genetic problem but instead might result from defects in several genes encoding different enzymes involved in the metabolism (Figure 2). Genetic polymorphisms affecting methylenetetrahydrofolate reductase include C677T and A1298C. The prothrombotic minor allele of the C677T polymorphism is found in up to 30\% of cardiac surgical patients but was not found to be associated with bleeding after CABG surgery.\textsuperscript{96} Data on thrombotic outcomes are awaited.

### Tissue Factor Pathway Inhibitor (TFPI)

The initial steps of coagulation are regulated by a protease inhibitor known as tissue factor pathway inhibitor (TFPI), which binds to and inhibits factor
Xa, and the resulting TFPI/ factor Xa complex inhibits factor VIIa/tissue factor. Tissue factor pathway inhibitor is predominantly expressed on the endothelium but is also found to a lesser extent in platelets, on monocytes, and as a soluble plasma protein. Tissue factor pathway inhibitor levels increase some 10-fold on initiation of CPB, as heparin displaces it from endothelial cell surface binding sites, freeing the molecule into plasma. However, there is a wide variability in TFPI antigen and activity levels upon heparinization for CPB, then TFPI levels fall rapidly as TFPI reattaches to the endothelium upon protamine neutralization of heparin but staying elevated for at least 5 hours postoperatively. Tissue factor pathway inhibitor is cleaved by proteases including factor Xa, neutrophil elastase, plasmin, and matrix metalloproteases (MMPs), especially MMP-12. A laboratory phenotype of TFPI resistance has been proposed as a possible risk factor for venous thrombosis. The TFPI gene contains several known genetic polymorphisms: a C>T transition in intron 7 (–33C/T), a Val264Met substitution in exon IX near the C-terminus, a –287C>T substitution near the C-terminus of the gene encoding glycoprotein IIIa (GP IIIa), and a homozygous 807T (873A) polymorphism allied with increased density of platelet GP Ia/IIa collagen-receptor gene, and (5) genetic variation in the platelet surface adenosine 5-diphosphate receptor gene P2Y1. Because of the

Thrombin-Activatable Fibrinolysis Inhibitor (TAFI)

Thrombin-activatable fibrinolysis inhibitor levels are under strong genetic control, but whether this translates into a clinically relevant impact of TAFI variants on cardiac surgery outcome remains to be demonstrated. Investigators have reported mild or equivocal impact of TAFI variants on thrombotic risk in other clinical settings.

Aspirin Resistance: Platelet Glycoprotein Variants

The phenomenon of platelet unresponsiveness to the expected antiaggregatory effect of aspirin (ASA) is complex and multifactorial but potentially has major clinical implications in up to 25% of patients with coronary artery disease (CAD) or undergoing CABG surgery. Prothrombotic genetic variations contribute to ASA resistance, and increased risk of cardiovascular events might involve (1) polymorphisms on the cyclooxygenase-1 gene, (2) overexpression of cyclooxygenase-2 mRNA on platelets and endothelial cells, (3) polymorphism PLA1/A2 of the gene-encoding glycoprotein IIIa (GP IIIa), (4) the homozygous 807T (873A) polymorphism allied with increased density of platelet GP Ia/IIa collagen-receptor gene, and (5) genetic variation in the platelet surface adenosine 5-diphosphate receptor gene P2Y1. Because of the
possible increased risk of ischemic vascular events, carriers of these genetic polymorphisms might be resistant to the antithrombotic effects of ASA and should be considered for additional or alternative treatment.

Genetic variation in response to antiplatelet drugs is not limited to aspirin, as a hyperreactive platelet may "resist" the effect of numerous antiaggregatory agents despite apparent sensitivity to these agents in vitro. The most widely studied polymorphism is the PlA polymorphism (substitution of proline for leucine at amino acid 33) on the IIIa chain of the glycoprotein (GP) IIb/IIIa fibrinogen receptor. Approximately 75% of patients with CAD, and 85% presenting for CABG are PlA1/A1 homozygotes; the remainder are typically heterozygotes or carriers with the PlA1/A2 genotype. Carriers of the PlA2 allele have a higher degree of GPIIb/IIIa activation during acute events, a reduced response to ASA (P = .05) and P-selectin expression (P = .02) during the overall study time course, and a lower antiplatelet effect to a 300-mg clopidogrel loading dose up to 24 hours following intervention (P < .05) and IIb/IIIa inhibitors.

### Common Genetic Variants: A Genomic Analysis

In a study of 780 CABG surgery patients, the impact of multilocus prothrombotic genetic polymorphisms on postoperative bleeding using a candidate gene approach was investigated (Table 1). As is common with complex genetic associations, the differences in outcome associated with individual polymorphisms are relatively small, but certain combinations of the 7 genetic variants may cause exaggerated bleeding after CABG surgery. Of the 19 functional polymorphisms in 13 candidate genes that were prospectively chosen, 7 polymorphisms demonstrate significant association with postoperative bleeding independently (ACE deletion/insertion D/I) or in interaction with each other (Table 2). These include GPIaIIa (α2β1 integrin) -52C > T, platelet glycoprotein IaIIa 807C > T, platelet glycoprotein IaIIa -52C > T × tissue factor -603A > G, platelet glycoprotein Ib? 524C > T × tissue factor -603A > G, prothrombin 20210G > A × TFPI –399C > T, tissue factor –603A > G, tissue factor pathway inhibitor –399C > T.

Data linking these polymorphisms to thrombotic outcomes are awaited, but Golanski et al have identified polymorphisms of platelet glycoproteins GPIa(807)C/T and GPIIIa PlA(1/A2) to be associated with postoperative ischemic episodes and platelet hyperreactivity. Interestingly, the periprocedural effect of the prothrombotic allele of GPIa(807)C/T in CABG surgery was to promote bleeding. Also, there was no association with the PlA polymorphism and bleeding, whereas in the more complex surgery for insertion of a ventricular assist device, PlA1/A1 homozygotes...
had more bleeding,\textsuperscript{152} and others found the effect to differ dependent on aspirin use.\textsuperscript{145} This biphasic effect of some polymorphisms might be related to intraoperative platelet activation, as platelets activated during CPB are immediately dysfunctional but could once again become hyperreactive in the postoperative period. Platelet transfusion might also confound this genetic effect depending on the genotype of transfused platelets.

Summary

Although coagulation factor deficiencies and identified polymorphic variants are relatively rare, their undiagnosed presence in cardiac surgery could affect a spectrum of outcomes ranging from postoperative bleeding and transfusion to postoperative thrombosis. Fortunately, most clinically apparent factor deficiencies are usually diagnosed before patients present for cardiac surgery. However, this is not usually the case for the polymorphic variants. But preoperative screening for these variants might become the standard of care in the future, and the presence of these abnormalities will need to be dealt with by the anesthesiologist. Currently, the magnitude of this problem in general is small, but when the impact of common genetic variants on outcome is more fully understood, preoperative genetic screening might be employed in the future.

At this time, there are limited management strategies for those with coagulation factor deficiencies and polymorphic variants undergoing cardiac surgery. For this reason, an in-depth understanding of each of them and review of the literature are essential for their safe management. For now, only the following suggestions can be made. If factor defects or deficiencies (including von Willebrand disease, antithrombin III deficiency, and protein C or protein S deficiency) have been diagnosed preoperatively, factor concentrates or FFP should be administered before incision, subsequent documentation of normalized factors should be done, standard anticoagulation should be maintained during surgery, and antibrinolytic agents can be considered on a case-by-case basis (eg, repeat sternotomies). Conversely, if a polymorphic variant is known preoperatively, routine management should not be changed at this time. In all cases, particularly those associated with thrombophilia, attention should be paid to early postoperative antithrombotic strategies to combat venous thromboembolism and graft thrombosis.

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