

## Influence of Genotype on Perioperative Risk and Outcome

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MOLECULAR biology has revolutionized medicine by increasing our understanding of the pathophysiologic mechanisms of disease and our ability to assess genetic risk. The use of genetic information from clinical studies to examine the impact of genetic variability on disease characterization and outcome is called *functional genomics*.<sup>1</sup> Functional genomics aims to discover the biologic function of particular genes and to uncover how sets of genes and their products work together in health and disease. It has long been recognized that inherited disease states (e.g., cystic fibrosis, familial hypercholesterinemia, sickle cell anemia, and others) may alter perioperative risk, but it is increasingly evident that specific genotypes may also predict adverse perioperative outcomes in otherwise healthy individuals. Identification of such genotypes may not only provide insight as to why the physiologic response to surgery varies among individuals, but it may also potentially decrease surgical morbidity and mortality through preoperative surgical risk assessment and the administration of prophylactic therapy.

### Basic Molecular Biology Concepts and Terminology

The molecular structure of every protein present in living organisms is encoded by *DNA*. DNA consists of four types of *nucleotides*, each containing a phosphate group, a sugar, and one of four purine or pyrimidine bases: adenine (A), guanine (G), thymine (T), or cytosine

(C). DNA exists as a double helix within the cell nucleus, with base pairing of purines (A and G) to pyrimidines (T and C) between the two backbone strands of phosphate and sugar residues. Variation within these base pairs gives rise to the *genetic code*, with each DNA strand serving as a template for mRNA *transcription* by RNA polymerase within the cell nucleus. A *promoter* region, typically 25–200 base pairs, proximal to the 5' transcription initiation site determines which of the two DNA strands will be replicated by orienting RNA polymerase in a specific direction. DNA transcription is also regulated in part by the binding of various gene-regulatory proteins to specific DNA sequences distant from the promoter region known as *enhancers*. After transcription, the mRNA is modified further within the cell nucleus undergoing splicing, whereby *introns* (areas within the coding gene that do not code for protein) are excised by enzymes called spliceosomes, and the remaining *exons* (areas of DNA that code for protein) are joined together. The final mature mRNA then translocates to the cell cytoplasm, where it undergoes ribosomal *translation* into the various proteins of the body. Each sequential triplet of mRNA bases is called a *codon*, and each codon encodes an amino acid.

A *gene* may be defined as a hereditary coding unit composed of a specific DNA sequence occupying a specific position or *locus* within a *chromosome* (i.e., long DNA molecules and their associated proteins). An *allele* is any of two or more alternative forms of a gene occupying the same chromosomal locus. The most common type of human genetic or allelic variation is the *single nucleotide polymorphism* (SNP), a position at which two alternative bases occur at appreciable frequency (>1%) in the human population.<sup>2</sup> Allelic variation may also occur secondary to other types of *mutations*, including the insertion, deletion, translocation, or inversion of DNA segments. Some mutations are “silent,” but allelic variation may significantly alter the *phenotype* of an organism (i.e., its outward, physical manifestations) and significantly affect the pathogenesis and expression of disease. Although an in-depth primer on molecular biology and genetics is beyond the scope of the present article, the interested reader is referred to several excellent reviews.<sup>3,4</sup>

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### *The Gene Association Study*

A variety of experimental approaches have been advocated to elucidate the effect of hereditary *versus* environmental factors on disease expression. The classic approach to this question has typically involved looking at disease expression patterns within families or in identical *versus* nonidentical twins. Unlike nonidentical twins, identical twins share identical genes. By comparison of their differences and similarities, the influence of hereditary *versus* environmental factors may thus be more easily distinguished. This approach is not without merit, but rapid advances in molecular biology and the human genome project have led to the identification of thousands of novel genes and polymorphisms in recent years. An increasing number of studies are focusing on disease expression or the occurrence of adverse clinical outcomes in individuals expressing a specific genotype. Gene association studies have been used to determine the influence of human genetic variation on the pathogenesis of disease, variability in disease expression, and response to treatment. SNPs, in particular, have been shown to be useful as genetic markers for identifying disease genes by linkage studies in families, linkage disequilibrium in isolated populations, and association analysis of patients and control subjects.<sup>2,5</sup> *Genetic linkage* is the phenomenon whereby alleles at loci close together on the same chromosome tend to be inherited together, because it is rare for crossover to occur between the loci at meiosis (*i.e.*, chromosomal halving). Linkage can be used to map disease genes by typing polymorphic DNA markers to see whether their alleles cosegregate with disease among related subjects. If relative pairs share marker alleles more often than would be expected by chance, this suggests that a susceptibility locus may be linked to the marker. A *haplotype* is a combination of alleles of closely linked loci on a single chromosome that tend to be inherited together. *Linkage disequilibrium* is said to occur when the observed frequencies of haplotypes in a population do not agree with the haplotype frequencies predicted by multiplying together the frequency of individual genetic markers in each haplotype. Linkage disequilibrium thus refers to correlations among neighboring alleles and is thought to reflect haplotypes descended from single ancestral chromosomes.

The SNP Consortium (<http://snp.cshl.org/>; accessed 1/16/03), an international collaboration of academic centers, pharmaceutical companies, and a private foundation, has now mapped and characterized nearly 1.8 million SNPs for biomedical research. However, the impact of only a small fraction of these polymorphisms has been studied in surgical populations. It has been proposed that 30,000–500,000 mapped SNPs could be used to scan the human genome for inherited combinations of alleles associated with common diseases.<sup>6</sup> Several investigators recently advocated building a “haplotype map” of the human genome: a map that will make it easier,

faster, and perhaps cheaper to find disease-causing or disease-predisposing genes. Instead of searching through a giant haystack of millions of SNPs, scientists would be searching through bundles of 10,000 to 50,000 bases each.<sup>7</sup> Haplotype mapping may also greatly increase the sensitivity and specificity of predicting how allotypic variation will affect specific clinical outcomes.

Although an extremely powerful research tool, the gene association study is not without limitations.<sup>1</sup> First, the primary endpoints of a gene association study must be sufficiently powered to account for *genetic admixture* within the study population (*i.e.*, the inclusion of patients originating from many distinct genetic backgrounds). This is especially true for complex diseases with significant heterogeneity (*i.e.*, diseases with multiple genetic origins). Association analyses that rely on the assumption that trait-influencing genes are inherited by descent may fail to identify influential genes, because the association will be divided between multiple loci in the sample of affected individuals. Thus, negative findings in an association study examining only several hundred patients of high genetic admixture should be interpreted with caution. Second, it is crucial for data interpretation that the appropriate statistical analyses are applied, preferably by a statistician experienced in genetic research. Gene association studies typically involve multiple comparisons of many different continuous and noncontinuous variables within different populations. Special care must therefore be taken to avoid identification of spurious gene associations. Moreover, identification of a positive association between a specific genotype and clinical outcome does not necessarily imply causality. The identified genotype may actually be clinically “silent” but be linked to one or more other allotypes that individually or collectively form a disease haplotype. Third, data interpretation of a gene association study correlates directly with the quality of the clinical phenotyping used. Data interpretation is extremely difficult in studies in which the clinical endpoints are not quantifiable or reproducible. Despite these shortcomings, it is increasingly evident that the gene association study has revolutionized our ability to determine the influence of genotype on surgical outcomes. Data obtained from recent studies suggest that specific genotypes are predictive of not only the physiologic response to surgery but also the risk of specific, adverse perioperative clinical outcomes (table 1).

### *Influence of Genotype on Perioperative Inflammation*

Surgery invokes a systemic inflammatory response characterized by complement, leukocyte, and platelet activation and the release of various proinflammatory cytokines. Although inflammation in general is thought to be an adaptive response, in certain individuals the systemic inflammatory response to surgery may be se-

**Table 1. Example Genotypes Linked to Adverse Perioperative Outcomes**

Gene	Gene Symbol	Chromosomal Location	Polymorphism	Allele Frequency	Phenotype	Linked Perioperative Outcome
Angiotensin-converting enzyme	ACE	17q23	ins/del intron 16	0.57/0.43	↑ ACE	↑ Vascular reactivity <sup>19,21</sup>
APOE	APOE	19q13.2	Allele ε4	0.1–0.4	↑ TNF-α and IL-8; ↓ IL-1ra	Neurocognitive dysfunction <sup>34,35</sup>
Chymase A	CMA1	14q11.2	G-1905A	0.46/0.54	↑ Conversion of angiotensin I to angiotensin II?	CABG restenosis <sup>33</sup>
Coagulation factor V	F5	1q23	G1691A	0.98/0.02	Activated protein C resistance	CABG restenosis <sup>26</sup> , thromboembolism <sup>25</sup> ; renal allograft thrombosis <sup>27</sup>
Cytotoxic T-lymphocyte antigen 4	CTLA4	2q33	Alleles 1, 3, 4, 7	0.81/0.04/ 0.04/0.11	Altered T-cell activation?	Transplant rejection <sup>58</sup>
Endothelial NO synthase	NOS3	7q36	G894T	0.09	↓ NO	↑ Vascular reactivity <sup>18</sup>
Integrin β-3 (glycoprotein IIIa)	ITGB3	17q21.32	PIA <sup>2</sup> (C12548T)	0.02–0.19	↓ Platelet activation threshold	CABG thrombosis, MI, neurocognitive dysfunction, and death after cardiac surgery <sup>32,39</sup>
Interferon-γ	IFNG	12q14	Allele 2	0.75	↑ IFN-γ	Transplant fibrosis and rejection <sup>51,62</sup>
IL-1ra	IL1RN	2q14.2	IL1RN*2	0.36–0.49	↓ IL1RN	Pouchitis <sup>16</sup> ; sepsis <sup>67</sup>
IL-6	IL6	7p21	G-174C G-572C	0.59/0.41 0.94/0.06	↑ IL-6	↑ Inflammatory response after CPB <sup>9</sup>
IL-10	IL10	1q31-q32	G-1082A	0.51/0.49	↓ IL10	Transplant rejection <sup>44–46,51</sup>
PAI-1	SERPINE1	7q21.3-q22	4G/4G	0.27	↑ PAI1	Catheter thrombosis <sup>30</sup>
Coagulation factor 2 (prothrombin)	F2	11p11-q12	G20210A	0.02	↑ Factor 2 (prothrombin)	Renal allograft thrombosis <sup>28</sup>
Transforming growth factor β1	TGFB1	19q13.1	G915C T869C	0.90/0.10 0.65/0.35	↑ TGF-β1	Accelerated coronary vasculopathy and lung fibrosis after transplantation <sup>59–61</sup>
TNF-α	TNF	6p21.3	G-308A	0.82/0.18	↑ TNF-α	Need for prolonged mechanical ventilation <sup>12</sup> ; transplant rejection <sup>44–48</sup> ; sepsis <sup>65,66</sup>
TNF-β	LTA	6p21.3	NcoI RFLP 1064–1069 intron 1	0.35/0.65	↑ TNF-α	Sepsis <sup>14,15</sup>
Vascular endothelial growth factor	VEGF	6p12	G-1154A C-2578A	0.43 (G/A) 0.48 (G/G) 0.29 (C/C) 0.50 (C/A)	↑ VEGF	Transplant rejection <sup>52</sup>

Nomenclature system for polymorphisms: The gene names and symbols used in table 1 are taken from Online Mendelian Inheritance in Man (OMIM; <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>; accessed 1/16/03). The nomenclature system used for polymorphisms is as follows. Polymorphisms in the 5' region are numbered either from the start of transcription or from the start of translation (specified for each gene). Thus, G-261A indicates a G/A substitution affecting the 261st nucleotide upstream from the transcription (or translation) start. Polymorphisms in translated exons are designated by the codon number and the single letter amino acid codes. Thus, K198N indicates a lysine at codon 198 substituted by an asparagine; L191L indicates a silent mutation at codon 191 (leucine/leucine). Polymorphisms in untranslated exons and located upstream from the start of translation are numbered from the start of the exon: T+55/ex4C indicates a T/C substitution affecting the 55th nucleotide of exon 4. Polymorphisms in the 3' region are numbered after the stop codon: A+39C indicates an A/C substitution affecting the 39th nucleotide after the stop codon. This designation also concerns polymorphisms in untranslated exons located after the stop codon. Polymorphisms in introns are numbered either positively from the start of the intron or negatively from the start of the following exon: C+8/in7A indicates a C/A substitution affecting the eighth nucleotide of intron 7; C-32/in1T indicates a C/T substitution affecting the 32nd nucleotide before exon 2. Insertion/deletion polymorphisms are designated ins/del: +238ins/del indicates an insertion/deletion at nucleotide 238 after the stop codon. Repeat polymorphisms are designated rpt.

were enough to be associated with significant perioperative and long-term clinical morbidity, including impaired hemostasis, ventricular failure, myocardial infarction, stroke, and multisystem organ dysfunction. Recent evidence suggests that the degree and severity of surgery-induced inflammation may be significantly influenced by genotype. Thus, modulation of the perioperative immune response may represent one mechanism by which allotypic variation may influence the incidence of adverse postoperative outcomes. For example, in patients undergoing primary coronary artery bypass graft

(CABG) surgery requiring cardiopulmonary bypass (CPB), protamine-induced complement activation and the postoperative pulmonary shunt fraction are significantly increased in patients expressing the complement component C4a null allele.<sup>8</sup> In a study examining several common SNPs within the promoter region of the proinflammatory cytokine interleukin (IL)-6 gene, significantly higher plasma IL-6 levels were observed after CPB in cardiac surgical patients carrying the G-572C allele and in patients homozygous for the G-174C allele, even after control for possible confounding factors, such as the

duration of CPB, the aortic cross-clamp, and surgery.<sup>9</sup> Recently, Grocott *et al.*<sup>10</sup> demonstrated that expression of the apolipoprotein (APO)  $\epsilon 4$  polymorphism is associated with significantly lower IL-1 receptor antagonist (IL-1ra) concentrations, along with unchanged levels of IL-1 $\beta$ , suggesting a proinflammatory cytokine imbalance after CPB. The APO  $\epsilon 4$  genotype has also been associated with elevated tumor necrosis factor (TNF)- $\alpha$  and IL-8 levels in patients undergoing CABG surgery after removal of the aortic cross-clamp.<sup>11</sup>

Increasing evidence suggests that allotypic variation may also increase the risk of adverse perioperative outcomes by altering the proinflammatory *versus* antiinflammatory cytokine balance. For example, the G  $\rightarrow$  A transitions at the -308 site within the promoter region of the TNF- $\alpha$  gene and the +250 site within the first intron of the TNF- $\beta$  gene are associated with elevated levels of the proinflammatory cytokine TNF- $\alpha$ .<sup>12-15</sup> In contrast, the G  $\rightarrow$  A transition at the -1082 site within the promoter region of the IL-10 gene is associated with lower levels of the antiinflammatory cytokine IL-10. Recently, the +250G/-308G (TNF GG) haplotype was demonstrated to be associated with the need for prolonged mechanical ventilation, and the GG genotype at the -1082 IL-10 site with increased mortality after CABG surgery.<sup>12</sup> A second example involves the surgical treatment of ulcerative colitis, which includes colectomy and ileal pouch-anal anastomosis. The most frequent complication of ileal pouch-anal anastomosis is pouchitis, occurring in approximately 30% of patients. Carter *et al.*<sup>16</sup> and Casini-Raggi *et al.*<sup>17</sup> found that a variable number of tandem-repeat polymorphisms in the IL-1ra gene (IL-1ra\*2) predicts the development of pouchitis after ileal pouch-anal anastomosis, possibly because of an imbalance in the IL-1/IL-1ra ratio. These data suggest that allotypic variation may significantly influence the incidence of adverse perioperative outcomes by altering surgery-induced inflammation.

#### *Influence of Genotype on Perioperative Vascular Reactivity*

Recently, several genes predictive of the vascular response to surgical and/or pharmacologic manipulation have been identified. These studies demonstrate another potential mechanism by which allotypic variation may influence the incidence of adverse perioperative outcomes. Philip *et al.*<sup>18</sup> studied the vascular response to phenylephrine in patients undergoing CABG surgery using constant, nonpulsatile pump flow during CPB. Vascular responsiveness to  $\alpha$ -adrenergic stimulation was significantly increased in individuals expressing the endothelial nitric oxide synthase G894T gene polymorphism, suggesting altered release of the potent vasodilator NO. Vascular reactivity to phenylephrine and plasma angiotensin-converting enzyme levels are also increased in patients homozygous for the angiotensin-converting

enzyme insertion/deletion polymorphism of intron 16 (DCP1).<sup>19,20</sup> The pressure-flow curve in patients undergoing CPB is shifted upward (*i.e.*, higher pressures as flow increases) in patients homozygous for the angiotensin-converting enzyme insertion/deletion polymorphism, suggesting impaired flow-mediated vasodilation.<sup>21</sup> Recently, the pressor response to laryngoscopy and tracheal intubation was shown to be associated with genetic variability in the  $\beta 2$  adrenoceptor gene.<sup>22</sup> After control for age, sex, weight, baseline blood pressure, heart rate, and rate-pressure product, patients possessing the glutamic acid homozygote of  $\beta 2$  adrenoceptor-27 produced significantly greater changes in mean arterial pressure and rate-pressure products than patients with the glutamine  $\beta 2$  adrenoceptor-27 homozygote.<sup>22</sup> These studies demonstrate that vascular reactivity in response to surgical or pharmacologic manipulation is influenced by genotype.

#### *Postoperative Thrombotic Outcomes*

Much effort in recent years has been devoted to the identification of genes associated with "hypercoagulable states."<sup>23</sup> One of the best known of these polymorphisms is the factor V Leiden genotype, which is a result of a point mutation in factor V (A1691G). Factor V Leiden is the most common genetic defect associated with the occurrence of primary venous thrombosis and is present in 10-50% of cases of venous thromboembolism.<sup>23</sup> Highly prevalent in Caucasians (up to 15%), factor V Leiden is associated with an increased incidence of postoperative venous thromboembolism, stroke, and CABG thrombosis.<sup>23-26</sup> In addition, the factor V Leiden and prothrombin G20210A mutations have been linked to an increased risk of primary allograft thrombosis after renal transplantation.<sup>27,28</sup> The number of genetic polymorphisms linked to a preoperative hypercoagulable state are too numerous to review here, but mutations within the antithrombin III, protein C, protein S, prothrombin, and factor V genes are believed to be associated with more than 60% of the cases of superficial and deep venous thrombosis.<sup>29</sup>

Several studies have identified genotypes associated with an increased risk of acute or delayed restenosis after CABG surgery. Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor of tissue plasminogen activator and is an important negative regulator of fibrinolytic activity. Circulating PAI-1 levels are regulated in part by a guanidine insertion/deletion (4G/5G) polymorphism in the promoter region of the PAI-1 gene. PAI-1 blood concentrations are increased in individuals expressing the 4G/4G PAI-1 genotype, suggesting reduced fibrinolysis.<sup>30</sup> Furthermore, PAI-1 activity has been positively correlated with venous and arterial graft occlusion after cardiac surgery.<sup>31</sup> Platelets are another important mediator of acute thrombosis. The human platelet antigen-1b (HPA-1b or Pl<sup>A2</sup>) polymorphism is a

risk factor not only for thrombotic CABG occlusion but also for myocardial infarction and death after cardiac surgery.<sup>32</sup> Finally, delayed restenosis after CABG surgery has been linked to homozygosity for the G allele of heart chymase (CMA-1905), which increases the conversion of angiotensin I to angiotensin II and is an independent risk factor for accelerated CABG atherosclerosis.<sup>33</sup> The mechanism by which the CMA-1905 polymorphism exerts an increased risk of accelerated CABG atherosclerosis is unclear, suggesting that this polymorphism may be part of a larger haplotype associated with increased postoperative risk.

#### *Postoperative Neurocognitive Dysfunction*

Neurocognitive dysfunction after CPB is a common complication, occurring in up to 75% of patients.<sup>34</sup> Increasing evidence suggests an association between the APO  $\epsilon 4$  genotype and neurocognitive dysfunction after CPB.<sup>34,35</sup> The APO  $\epsilon 4$  allele has also been associated with worsened neurologic dysfunction in the setting of closed head trauma, nonaneurysmal intracranial hemorrhage, thromboembolic stroke, and Alzheimer disease.<sup>36,37</sup> The exact mechanism by which APO  $\epsilon 4$  genotype influences perioperative neurocognitive dysfunction has yet to be identified, but the APO  $\epsilon 4$  allele does not affect global cerebral blood flow or the cerebral metabolic rate for oxygen during CPB.<sup>38</sup> The  $PI^{A2}$  polymorphism of the glycoprotein IIIa constituent of the platelet integrin receptor glycoprotein IIb/IIIa has also been linked to worsened neurocognitive dysfunction after CPB, suggesting possible exacerbation of platelet-dependent thrombotic processes associated with plaque embolism.<sup>39</sup> Together, these data suggest that allotypic variation may influence the severity of neurocognitive dysfunction after CPB.

#### *Postoperative Renal Dysfunction*

Acute renal injury after CABG surgery occurs in approximately 8% of patients, with up to 1% requiring perioperative dialysis.<sup>40</sup> Acute renal failure is an independent predictor of mortality after cardiac surgery,<sup>41</sup> with rates exceeding 60% in patients requiring dialysis.<sup>42</sup> Even minor degrees of postoperative renal dysfunction are associated with significant in-hospital increases in mortality, morbidity, and costs.<sup>42</sup> In insulin-dependent diabetic patients, the APO  $\epsilon 2$  allele is a negative predictor of creatinine clearance and a positive predictor of urinary albumin, immunoglobulin G, and  $\alpha 1$ -microglobulin excretion.<sup>43</sup> Consistent with this observation is a recent report demonstrating that inheritance of the APO  $\epsilon 2$  or  $\epsilon 3$  allele is predictive of increased postoperative serum creatinine levels after cardiac surgery in patients with normal preoperative renal function compared with the  $\epsilon 4$  allele.<sup>40</sup> Identification of genotypes predictive of acute renal impairment may potentially allow preoperative risk stratification and administration of targeted ther-

apy to enhance perioperative renal perfusion/function in patients at increased risk.

#### *Transplant Outcomes*

During the past decade, significant effort has been directed toward the identification of genotypes predictive of poor transplant outcomes, such as allograft rejection, atherosclerosis, or fibrosis. Many studies have focused on genotypes that alter the balance between proinflammatory and antiinflammatory cytokines. For example, the incidence of rejection after cardiac or renal transplantation is increased in individuals expressing both the TNF- $\alpha$  G-308A and the IL-10 G-1082A SNPs, which are associated with elevated levels of the proinflammatory cytokine TNF- $\alpha$  and decreased levels of the antiinflammatory cytokine IL-10, respectively.<sup>44-47</sup> Homozygous expression of either the TNF- $\alpha$  G-308A or IL-10 G-1082A SNP has also been shown to confer an increased risk of rejection after heart, kidney, or liver transplantation,<sup>45-48</sup> although this has not been confirmed in all studies.<sup>49,50</sup>

Other cytokine or growth factor polymorphisms linked to an increased incidence of allograft rejection include interferon- $\gamma$ , vascular endothelial growth factor, and IL-1. Circulating levels of the proinflammatory cytokine interferon- $\gamma$  are regulated in part by a variable dinucleotide (CA)<sub>n</sub> repeat polymorphism of the interferon- $\gamma$  gene. Inheritance of allele 2 (12 repeats) of this polymorphism is associated with elevated interferon- $\gamma$  levels and an increased incidence of renal allograft rejection.<sup>51</sup> Vascular endothelial growth factor may also modulate posttransplantation inflammation by enhancing endothelial permeability and augmenting leukocyte migration into the allograft.<sup>52</sup> Recently, two SNPs of the vascular endothelial growth factor gene, G-1154A and C-2578A, were shown to be associated with increased vascular endothelial growth factor levels and to confer an increased risk of acute renal allograft rejection.<sup>52</sup> Finally, the incidence of gingival graft failure for the treatment of periodontal disease has been linked to polymorphisms within the IL-1 gene.<sup>53</sup>

Allotypic variation may also alter the cellular immune response to transplantation. T-cell activation is mediated in part by binding of the CD28 receptor to B7-1 and B7-2 ligands on antigen-presenting cells. In contrast, binding of cytotoxic T-lymphocyte antigen 4 to these same ligands inhibits T-cell activation.<sup>54</sup> The dinucleotide (AT)<sub>n</sub> repeat polymorphism in exon 3 of the cytotoxic T-lymphocyte antigen 4 gene has been linked to autoimmune diseases such as insulin-dependent diabetes mellitus, Graves disease, Hashimoto thyroiditis, and Addison disease.<sup>55-57</sup> Recently, the liver and kidney allograft rejection rate was shown to be significantly increased in individuals expressing allele 3, 4, or 7 of the cytotoxic T-lymphocyte antigen 4 AT repeat polymorphism,

whereas patients carrying allele 1 had a tendency toward lower rejection rates.<sup>58</sup>

One of the major limitations to long-term survival after cardiac transplantation is the development of accelerated coronary vasculopathy, characterized by diffuse intimal accumulation and proliferation of inflammatory cells, smooth muscle cells, ground substance, and lipids. One important mediator of vascular neointimal formation is transforming growth factor- $\beta$ 1. Recently, Densem *et al.*<sup>59</sup> found a correlation between the recipient's transforming growth factor- $\beta$ 1 genotype and the development of accelerated coronary vasculopathy after cardiac transplantation. Carriers of the G-allele of the G915C SNP, who express higher transforming growth factor- $\beta$ 1 levels than C-homozygotes, developed coronary vasculopathy on average nearly 3 yr earlier than the C-homozygotes.<sup>59</sup> The transforming growth factor- $\beta$ 1 G915C SNP has also been associated with the development of hypertension, fibrotic lung disease, and graft fibrosis after lung transplant rejection.<sup>60,61</sup> Similarly, inheritance of allele 2 of the dinucleotide (CA)<sub>n</sub> repeat polymorphism of the interferon- $\gamma$  gene is associated with increased interferon- $\gamma$  levels and the development of lung graft fibrosis.<sup>62</sup> These data demonstrate that allotypic variation significantly influences acute and chronic transplant outcomes.

### Infection

Sepsis resulting in the development of multiorgan failure remains a leading cause of morbidity and mortality in the intensive care unit.<sup>63</sup> Recent evidence suggests a genetic predetermination of the inflammatory cytokine response to infection. One key mediator is TNF- $\alpha$ .<sup>63</sup> Numerous studies have shown a direct correlation between TNF- $\alpha$  levels and mortality in septic patients.<sup>64</sup> An *Nco*I restriction fragment length polymorphism within the first intron of the TNF- $\beta$  gene, TNFB2, correlates with increased TNF- $\alpha$  plasma concentrations and is an independent risk factor for death caused by septic shock.<sup>14,15</sup> Similarly, the G-308A TNF- $\alpha$  gene polymorphism (TNF 2 allele) is associated with increased TNF- $\alpha$  plasma concentrations and is also associated with an increased susceptibility to sepsis and sepsis-induced mortality.<sup>65,66</sup> Patients homozygous for the TNF2 allele have a 3.7-fold increased risk of death.<sup>65</sup> The frequency of the IL-1ra A2 polymorphism, which has previously been linked to an increased incidence of lupus erythematosus and ulcerative colitis, is also significantly increased in patients with severe sepsis, suggesting a possible infection susceptibility allele.<sup>67</sup> Together, these data imply that allotypic variation may influence the risk of perioperative infection.

### Summary

Rapid progress in molecular biology has revolutionized our ability to assess the impact of genetic variability on

disease characterization and outcome. Despite these encouraging results, a few words of caution are warranted for the clinician trying to critically evaluate the increasingly large body of literature linking various allotypes to specific, adverse outcomes. First, it is important to recognize that gene association studies do not imply causality. The identified genotype may actually be clinically "silent" but be linked to one or more other allotypes that individually or collectively form a disease haplotype. Second, because gene association studies typically involve multiple comparisons of many variables within different populations, there is always the potential for the identification of spurious gene associations that may or may not prove significant or causal. Third, one cannot extrapolate positive gene association findings to other populations with different genetic backgrounds. Finally, it is important to recognize that environmental factors may influence gene association findings even in individuals of homogenous genetic backgrounds. For example, clinically significant polymorphisms in gene promoter regions influenced by the induction of CPB might otherwise go unrecognized in patients having off-pump cardiac surgery. Thus, at present, a strong need remains for prospective, sufficiently powered, gene association studies conducted in well-defined, highly phenotyped populations. Only then can we begin to critically evaluate the relative importance or clinical significance of various gene associations. Continued identification of allotypes and haplotypes predictive of adverse perioperative events may not only further our understanding of the pathophysiologic response to surgery but also potentially decrease surgical morbidity and mortality *via* preoperative risk assessment and the administration of prophylactic therapy.

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