Gene Polymorphism and Requirement for Vasopressor Infusion After Cardiac Surgery

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Background. **Genes in the class III region of the MHC, encoding proteins involved in inflammation and vascular regulation, were investigated for association with the occurrence of vasodilation and requirement for vasopressor infusion.**

Methods. **A cohort of 236 elective cardiac surgical patients was studied. Hemodynamic and metabolic variables and dosage of vasopressor medications were recorded for the first 12 hours of intensive care unit admission after cardiac surgery on an electronic patient record. Demographic factors and operative details were recorded from other institutional databases. The DNA was extracted from peripheral blood mononuclear cells and genotyped for the presence of polymorphic alleles in genes coding for inflammation-related proteins.**

Results. **Carriage of the dimethylarginine dimethylaminohydrolase II (DDAH II) −449 G allele and the lymphotoxin alpha +252 G allele was significantly less frequent in patients who required infusions of vasopressors after cardiac surgery. On multivariate analysis, prior myocardial infarction, prolonged bypass, and the homozygous carriage of the DDAH II C allele were associated with postoperative vasopressor requirement.**

Conclusions. **Vasopressor requirement after surgery may be related to an interaction of genotype, preoperative morbidity, and prolonged surgery.**

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Systemic inflammation in postoperative cardiac surgical patients manifests as systemic arterial vasodilation with a high cardiac index and a low systemic vascular resistance [1], and may require treatment with vasopressor agents to maintain adequate organ perfusion. In this context, the catecholamines epinephrine and norepinephrine are in common usage. This acute inflammatory response is likely due to surgical trauma and the continuous exposure of heparinized blood to nonendothelial cell surfaces during cardiopulmonary bypass. However, the host response to cardiopulmonary bypass is likely a result of interaction of the intensity and duration of the precipitant with certain innate patient characteristics. These characteristics may have a genetic basis due to carriage of specific functional polymorphic alleles in genes coding for proteins involved in inflammation and vascular regulation.

The major histocompatibility complex encodes the human leukocyte antigen class I and class II molecules, which play an important role in adaptive immunity. Between these two gene clusters is a region densely packed with a selection of genes involved in a variety of biological activities predominantly related to immunity. The best characterized of these is probably tumor necrosis factor alpha (TNFα), a powerful proinflammatory cytokine involved in a wide variety of functions. Altered regulation of this gene due to variation in its regulatory regions has been shown to be associated with a number of diseases including sepsis, autoimmune diseases, rheumatoid arthritis, and cancer [2–5].

Other important genes in this region, in which variation has been implicated in disease, include lymphotoxin alpha (LTA), which is believed to be involved in modulation of immune function. Lymphotoxin alpha polymorphisms have been linked with susceptibility to myocardial infarction and outcome in community-acquired pneumonia [6, 7]. Also in close proximity is another gene of interest, inhibitor of kappa-B-like protein (IkBL or NFkBIL1), which has been associated with both rheumatoid arthritis and ulcerative colitis susceptibility [8, 9]. This region also contains the casein kinase II beta subunit (CSNK2B) gene, a ubiquitous highly conserved enzyme, and the gene encoding dimethylarginine dimethylaminohydrolase II (DDAH II), which regulates cellular methylarginine concentrations, which in turn inhibit nitric oxide synthase activity [10]. Expression of DDAH II predominates in more highly vascularized tissues and in immune tissues.

Common single-nucleotide polymorphisms (SNPs) in some of these genes may be implicated in the occurrence of systemic inflammation with consequent hemodynamic instability after cardiac surgery. To investigate this hypothesis, a study was performed to determine whether there was a relation between requirement for vasopressor infusions in patients after

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Abbreviations and Acronyms

ADMA	= asymmetrical dimethylarginine
CSNK2B	= casein kinase 2 beta subunit
DDAH	= dimethylarginine dimethylaminohydrolase
ICU	= intensive care unit
IkBL, NFkBIL1	= inhibitor of kappa B like protein
LD	= linkage disequilibrium
LTA	= lymphotoxin alpha
SNP	= single nucleotide polymorphism
TNF	= tumor necrosis factor

cardiac surgery and carriage of specific alleles in genes coding for TNFα and the aforementioned adjacent genes on the sixth chromosome.

Patients and Methods

Patients having elective and uneventful cardiac surgery between July 2001 and December 2002 were considered for inclusion in this study, which was approved by the Institutional Ethics Review Committee, which also waived the need for consent for data publication.

All patients had similar cardiopulmonary bypass, with heparin-coated circuits and roller pumps. After surgery all patients were admitted to a dedicated cardiac surgical intensive care unit (ICU).

A clinical information system (Care Vue, Phillips Medical, Eindhoven, the Netherlands) in the cardiac surgical ICU captures data from physiologic monitoring, mechanical ventilators, and blood gas analyzers, and from laboratory-based servers storing biochemical and hematology results. Intensive care unit admission was timed from the timing of the first recorded heart rate in the ICU, and this time point was used to calculate the interval between ICU admission and subsequent events. Patient demographics, historic comorbidity, and operative details were retrieved from a separate institutional database.

Patients who required support with potent vasopressors such as epinephrine or norepinephrine in the first 12 hours after ICU admission were identified from the electronic archive, and the dosage of vasopressor infusion was retrieved. Hemodynamic variables, including cardiac index and systemic vascular resistance, and arterial blood gases, including lactic acid levels and hemoglobin were retrieved for the study interval.

The first 12 hours after ICU admission was divided into four 3-hour-long periods. Mean arterial pressure was averaged for each period, and the relation between mean arterial pressure and requirement for vasopressor infusion was analyzed to determine whether vasopressor use was appropriate. Similarly, mean arterial pressure in patients who were administered dopamine infusion for renal preservation without vasopressor agents was compared with mean arterial pressure in patients with no vasopressor or dopaminergic infusions. Clinical practice was not changed or modified for the purpose of the study.

On enrollment to the study, blood was drawn on all study patients and frozen at −70C for subsequent genomic analysis. Genomic DNA was extracted using the QIAmp DNA Midi kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

The SNPs used in this study comprised both polymorphisms that were previously identified in the literature, and associated with disease, as in the case of variations in TNFα, LTA, and IkBL. In addition two SNPs were selected from CSNK2B, one of which, CSNK2B+2054 (rs805256), causes a synonymous nucleotide change, whereas the other, CSNK2B+4151 (rs4569) occurs in the 3 prime (3′) untranslated region. One SNP was also chosen in the second intron of DDAH II. This variation has been designated DDAH II -449 (rs805305), relative to the translation start site because this gene has multiple transcription start sites [11]. Allelic variation for the SNPs was assayed using Amplifluor technology by Kbiosciences (Hoddeston, Herts, United Kingdom; http://www.kbioscience.co.uk/). Primer sequences are given in the Appendix for each of the assays used in this study.

Data were analyzed by *t* test, χ^2 test, and Fisher's exact test where appropriate. Serial measures of mean arterial pressure was analyzed with analysis of variance for repeated measures. Factors that were significantly associated with vasopressor requirement on univariate analysis were included in a stepwise logistic regression analysis with the JMP (SAS Institute, Cary, North Carolina) statistical pack. All p values of less than 0.05 were considered significant. Haplotype frequency estimation was performed using the expectation maximation method as implemented by HITAGENE software (www.hitagene.com) and Phase 2.0 [12]. The significance of haplotype frequency differences between the two groups was determined using a modification of the haplotype association test described by Zaykin and associates [13] and implemented by HITAGENE.

Results

The study included 236 patients, with 56 (23%) requiring either epinephrine or norepinephrine infusion in the first 12 hours after ICU. Two patients died, 1 from each group. Forty-nine patients received epinephrine and 28 patients received norepinephrine infusions; 21 patients received both infusions. The details of these vasopressor infusion rates are in Table 1. The relation between postoperative vasopressor requirement, preoperative demographic factors, preexisting comorbidity, and operative details are listed in Table 2. Prior myocardial infarction and duration of aortic cross-clamping were significantly associated with requirement for vasopressor infusion after cardiac surgery.

Mean arterial pressure was significantly lower in the group of patients who required vasopressor infusion. The average central venous pressure over the 12-hour study period was significantly greater in patients who required infusion of vasopressor substances. Heart rate was