

Decreased Catecholamine Degradation Associates with Shock and Kidney Injury after Cardiac Surgery

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ABSTRACT

Enzymatic pathways involving catechol-O-methyltransferase (COMT) catabolize circulating catecholamines. A G-to-A polymorphism in the fourth exon of the COMT gene results in a valine-to-methionine amino acid substitution at codon 158, which leads to thermolability and low ("L"), as opposed to high ("H"), enzymatic activity. We enrolled 260 patients postbypass surgery to test the hypothesis that COMT gene variants impair circulating catecholamine metabolism, predisposing to shock and acute kidney injury (AKI) after cardiac surgery. In accordance with the Hardy-Weinberg equilibrium, we identified 64 (24.6%) homozygous (LL), 123 (47.3%) heterozygous (HL), and 73 (28.1%) homozygous (HH) patients. Postoperative catecholamines were higher in homozygous LL patients compared with heterozygous HL and homozygous HH patients ($P < 0.01$). During their intensive care stay, LL patients had both a significantly greater frequency of vasodilatory shock (LL: 69%, HL: 57%, HH: 47%; $P = 0.033$) and a significantly longer median duration of shock (LL: 18.5 h, HL: 14.0 h, HH: 11.0 h; $P = 0.013$). LL patients also had a greater frequency of AKI (LL: 31%, HL: 19.5%, HH: 13.7%; $P = 0.038$) and their AKI was more severe as defined by a need for renal replacement therapy (LL: 7.8%, HL: 2.4%, HH: 0%; $P = 0.026$). The LL genotype associated with intensive care and hospital length of stay ($P < 0.001$ and $P = 0.002$, respectively), and we observed a trend for higher mortality. Cross-validation analysis revealed a similar graded relationship of adverse outcomes by genotype. In summary, this study identifies COMT LL homozygosity as an independent risk factor for shock, AKI, and hospital stay after cardiac surgery. (ClinicalTrials.gov number, NCT00334009)

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Shock and acute kidney injury (AKI) are associated with increased mortality after cardiac surgery.^{1,2} Cardiopulmonary bypass represents a common clinical setting of sympathetic nervous system activation and cardiovascular instability. Postoperative hypotension and vasodilation with increased requirements for catecholamines occur despite adequate intravascular filling, cardiac output, and increased plasma catecholamine concentrations.^{1,3} High circulating catecholamine levels may contribute to persistent vasodilatation via α -adrenoceptor downregulation and desensitization,⁴ depression of vasopressin synthesis, and adenosine triphosphate-

sensitive potassium channel activation in vascular smooth muscle cells.⁵ Circulating catecholamines

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are primarily catabolized through enzymatic pathways involving the enzyme catechol-O-methyltransferase (COMT).⁶ A functional G-to-A polymorphism in the fourth exon of the *COMT* gene results in a valine-to-methionine amino acid transition at codon 158 (*COMT* Val158Met polymorphism), leading to thermolability and lower (L), compared with higher (H) activity of the enzyme.^{7,8} Genetically determined *COMT* activity, among others,^{9,10} influences outcomes in patients with ischemic heart disease.^{11,12} In the kidney, *COMT* is essential for catecholamine degradation along the distal parts of proximal tubules and thick ascending limb of loop of Henle.¹³ We hypothesized that the *COMT* LL genotype coding for low enzyme activity would shift metabolism toward increased plasma catecholamine concentrations and predispose to increased duration of vasodilatory shock and higher AKI incidence after cardiac surgery. To test this notion, we conducted a prospective observational cohort study in cardiac surgery patients.

RESULTS

Patient Population

Patient enrollment is presented in Figure 1. All 260 patients were Caucasian. In accordance with the Hardy-Weinberg equilibrium ($P = 0.85$), genetic analysis detected 64 (24.6%) *COMT* homozygous (LL), 123 (47.3%) *COMT* heterozygous (HL), and 73 (28.1%) *COMT* homozygous (HH) persons. Patients were similar with regard to demographic data, comorbidities, and pre- and intraoperative medication (Table 1).

Intra- and postoperative interventions and hemodynamics were similar among patient groups (Table 2). There was also no difference in median duration of postoperative milrinone infusion when comparing LL patients (7.5 [0.5 to 32.5] hours), with HL patients (8.0 [1.0 to 35.0] hours) and HH patients 6.5 [0.0 to 22.5] hours; $P = 0.67$). Aprotinin was given to 26/64 LL patients, 47/123 HL patients, and 33/73 HH patients ($P =$

0.63). The use of volatile agents, propofol, and other sedatives and analgetics did not differ between the genotype groups.

Plasma Catecholamines

Preoperative plasma levels for epinephrine and norepinephrine were similar among LL, HL, and HH patients (Figure 2, A and B). As monoamine oxidase (MAO)-dependent deamination of epinephrine and norepinephrine leads primarily to production of 3,4-dihydroxyphenylglycol (DHPG), we found a trend for increased plasma DHPG in LL patients, compared with HL and HH patients (Figure 2C).

Postoperative epinephrine levels were not confounded by exogenous epinephrine application because of the small number of patients receiving an intraoperative epinephrine bolus of 1 mg for cardiopulmonary resuscitation (one LL and one HH patient each) and because the interval between bolus and blood sampling is several times larger than is the half life of epinephrine. Postoperative epinephrine levels were already significantly higher in LL compared with HL or HH patients after 6 h. The values increased linearly in LL patients, but not in HL or HH patients. Postoperative norepinephrine levels were also not confounded by exogenous norepinephrine application, because patients receiving norepinephrine infusion were excluded.

Postoperative plasma norepinephrine concentrations increased to a similar extent among genotype groups after 6 h and became significantly higher in LL compared with HL and HH patients after 24 h. Kinetics of postoperative increases in plasma DHPG concentrations were similar to those observed for epinephrine and norepinephrine with the highest concentrations after 24 h in LL patients. *COMT* catalyzes O-methylation, not only of epinephrine and norepinephrine, but also of 3,4-dihydroxyphenylalanine (L-DOPA). L-DOPA was significantly higher at 24 h postoperatively in LL patients compared with HL or HH patients. All groups showed similar early postoperative decrease in L-DOPA after 6 h.

Vasodilatory Shock

During their intensive care stay, 69% of LL patients, 57.0% of HL patients, and 46.6% of HH patients met criteria for shock. Besides more significant frequency of vasodilatory shock ($P = 0.033$), LL patients also had a prolonged median shock duration, compared with HL or HH patients (Figure 3). The mean arterial pressure during cardiopulmonary bypass was lower in LL patients compared with HL and HH carriers (Table 2), even although LL patients received vasopressor agents in similar frequency (norepinephrine: $P = 0.54$) and dose (norepinephrine: $P = 0.77$) as did HL and HH patients during that period of time.

Prolonged shock (>48 h) was more common in LL (25.0%) patients compared with HL (13.0%) and HH (6.8%) patients ($P = 0.009$, Pearson's coefficient = 9.5). Those LL patients who received norepinephrine infusions required a higher dose ($0.14 \pm 0.11 \mu\text{g/kg/min}$) during the first 48 postoperative hours, compared with HL patients ($0.10 \pm 0.07 \mu\text{g/kg/min}$)

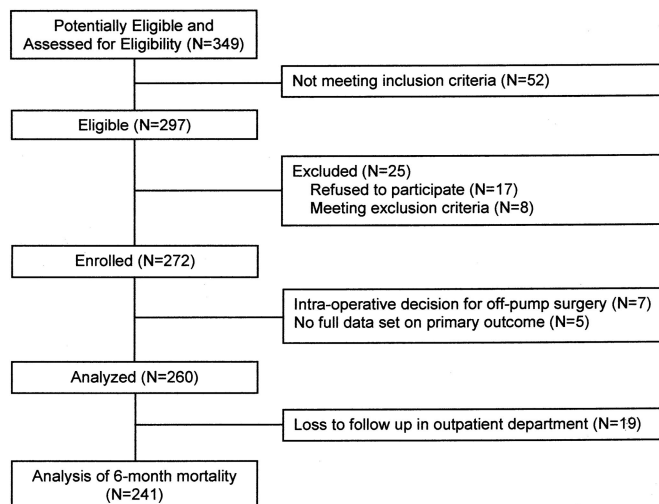


Figure 1. Flow diagram of patient enrollment into the study.

Table 1. Patients' characteristics according to the COMT genotype

Variable	LL (n = 64, 24.6%)	HL (n = 123, 47.3%)	HH (n = 73, 28.1%)	P
Age, y	68.3 ± 8.1	69.2 ± 9.0	68.0 ± 10.0	0.65
Sex, female, n (%)	21 (32.8)	32 (26.0)	19 (26.0)	0.57
BSA, m ²	1.89 ± 0.21	1.93 ± 0.20	1.90 ± 0.23	0.45
Insulin dependent diabetes mellitus, n (%)	5 (7.8)	8 (6.5)	6 (8.2)	0.89
Non-insulin dependent diabetes mellitus, n (%)	11 (17.2)	25 (20.3)	18 (24.7)	0.55
Arterial hypertension, n (%)	56 (87.5)	107 (87.0)	63 (86.3)	0.98
Hypercholesterolemia, n (%)	42 (65.6)	87 (70.7)	49 (67.1)	0.74
Chronic obstructive pulmonary disease, n (%)	13 (20.3)	25 (20.3)	14 (19.2)	0.981
Smoker, n (%)	32 (50.0)	76 (61.8)	40 (54.8)	0.25
Preoperative serum creatinine, μmol/L	92.3 ± 25.4	92.9 ± 28.8	91.2 ± 21.9	0.93
Estimated GFR, ml/min/1.73 m ²	74.3 ± 19.2	76.6 ± 21.7	76.7 ± 19.7	0.73
Peripheral vascular disease, n (%)	6 (9.4)	17 (13.8)	7 (9.6)	0.55
Carotid disease, n (%)	7 (10.9)	13 (10.6)	5 (6.9)	0.64
Atrial fibrillation, n (%)	19 (29.7)	28 (22.8)	18 (24.7)	0.58
Previous myocardial infarction, n (%) ^a	12 (18.8)	25 (20.3)	13 (17.8)	0.91
Left ventricular dysfunction, n (%) ^b	12 (18.8)	16 (13.0)	10 (13.7)	0.55
Left ventricular ejection fraction, %	54.2 ± 9.9	53.5 ± 11.8	54.1 ± 9.9	0.93
Preoperative medication				
platelet inhibitor, n (%)	46 (71.9)	83 (67.5)	54 (74.0)	0.60
ACE inhibitor/AT-II antagonist, n (%)	43 (67.2)	73 (59.4)	51 (69.9)	0.28
beta blocker, n (%)	34 (53.1)	66 (53.7)	37 (50.7)	0.92
calcium channel blocker, n (%)	16 (25.0)	35 (28.5)	17 (23.3)	0.71
HMG-CoA reductase inhibitor, n (%)	38 (59.4)	81 (65.9)	40 (54.8)	0.29
diuretics, n (%)	16 (25.0)	28 (22.8)	17 (23.3)	0.63
CABG surgery, n (%)	30 (46.9)	57 (46.3)	37 (50.7)	0.83
Valvular surgery, n (%)	23 (35.9)	43 (35.0)	23 (31.5)	0.84
CABG and valvular surgery, n (%)	7 (11.0)	14 (11.4)	7 (9.6)	0.93
Thoracic aortic surgery, n (%)	4 (6.3)	9 (7.3)	6 (8.2)	0.91
Redo cardiac surgery, n (%)	3 (4.7)	7 (5.7)	5 (6.9)	0.86
Duration of cardiopulmonary bypass, min	134.3 ± 75.1	137.1 ± 76.7	133.4 ± 54.6	0.93

Numbers denote means ± SD or numbers (%) where appropriate. COMT, catecholamine-O-methyltransferase; BSA, body surface area; ACE inhibitor indicates angiotensin-converting enzyme; AT-II antagonist, angiotensin-II antagonists; HMG-CoA reductase inhibitor, 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitor; CABG, coronary artery bypass graft.

^aWithin 6 months preoperatively. ^bLeft ventricular ejection fraction < 35%.

and HH patients ($0.08 \pm 0.04 \mu\text{g/kg/min}$; $P = 0.001$). Moreover, the LL genotype was independently associated with increased shock duration in the multivariate regression analysis (Table 3).

AKI

AKI occurred more commonly in LL patients compared with HL and HH patients (Figure 4A). The absolute and relative serum creatinine increases from baseline to peak value were higher in LL patients compared with HL and HH patients (Figure 4, B and C). A greater proportion of LL patients developed AKI according to RIFLE criteria R or worse ($n = 20/64$, $n = 23/123$, $n = 10/73$), I or worse ($n = 12/64$, $n = 10/123$, $n = 3/73$), and F ($n = 4/64$, $n = 1/123$, $n = 0/73$) compared with HL and HH patients ($P = 0.038$, $P = 0.011$, $P = 0.014$, respectively). LL patients received higher dose of furosemide (Table 2) and more commonly required renal replacement therapy ($n = 5/64$) compared with HL patients ($n = 3/123$). None of the 73 HH patients needed renal replacement therapy ($P = 0.026$).

The LL genotype was not only independently associated with increased incidence of AKI (Table 3), but also remained independently associated with increased incidence of AKI when length of norepinephrine infusion was included into multivariable model to adjust for potential vasoconstrictive effects ($P = 0.019$; OR 2.9 [1.2 to 7.0]). AKI probably occurred secondary to vasodilatory shock, because patients who developed both AKI and vasodilatory shock concomitantly were equally distributed among genotype groups, namely, 18/20 for LL, 20/24 for HL, and 9/10 for HH patients.

Length of Stay in Intensive Care and in Hospital and Mortality

LL patients had longer 49.8 (31.5 to 91.0 h) median stay in the intensive care unit, compared with 43.8 (25.0 to 67.0 h) for HL patients and 41.0 (26.0 to 48.0 h) for HH patients ($P = 0.036$.) LL patients also had a longer median hospital stay (9.0 [7.0 to 11.5] days) compared with HL patients (8.0 [7.0 to 11.0] days) and HH patients (7.0 [6.5 to 9.0] days; $P = 0.004$). The LL genotype remained independently associated with prolonged

Table 2. Intra- and postoperative fluid balance and hemodynamics

Variable	LL (n = 64, 24.6%)	HL (n = 123, 47.3%)	HH (n = 73, 28.1%)	P
Intraoperative				
fluid intake, ml	3860 ± 1300	3600 ± 1150	3600 ± 1250	0.33
blood products, ml	655 ± 455	585 ± 545	675 ± 515	0.44
urine output, ml	1645 ± 725	1460 ± 655	1570 ± 750	0.21
drain output, ml	80 ± 79	75 ± 77	85 ± 83	0.69
fluid balance, ml	2950 ± 1450	2750 ± 1400	2700 ± 1500	0.56
furosemide, mg	26.3 ± 49.2	13.4 ± 27.3	8.2 ± 14.4	0.003
mean arterial pressure during CPB, mmHg	60 ± 11	63 ± 10	65 ± 12	0.027
Postoperative (0–24 h)				
fluid intake, ml	6540 ± 1490	6290 ± 1220	6200 ± 1450	0.32
blood products, ml	820 ± 680	750 ± 690	835 ± 650	0.45
urine output, mL	4090 ± 1530	4020 ± 1650	4050 ± 1390	0.96
drain output, ml	850 ± 760	780 ± 600	820 ± 660	0.61
fluid balance, ml	2550 ± 1600	2300 ± 1650	2150 ± 1950	0.47
Furosemide, mg	65.7 ± 83.3	35.7 ± 70.1	30.8 ± 38.9	0.003
mean arterial pressure, mmHg	68.9 ± 10.7	70.2 ± 9.8	70.4 ± 10.4	0.64
cardiac index, L/min/m ²	2.27 ± 0.45	2.29 ± 0.42	2.31 ± 0.46	0.89
systemic vascular resistance, dyn/s/cm ⁻⁵	969 ± 183	983 ± 191	1009 ± 187	0.44

Numbers denote means ± standard deviation. CPB, cardiopulmonary bypass.

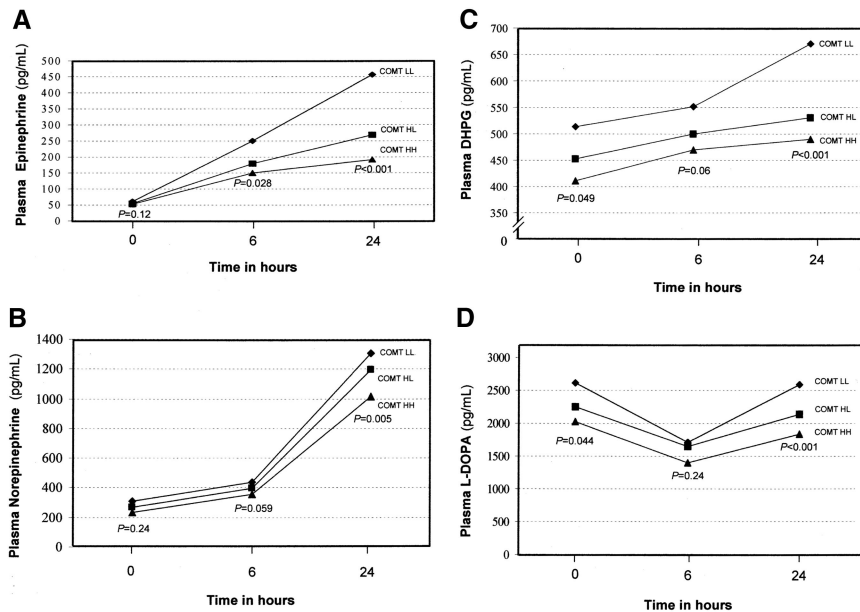


Figure 2. Mean pre- and postoperative concentrations of catecholamines and metabolites in plasma of COMT^{LL} (◆), COMT^{HL} (■), and COMT^{HH} (▲) genotype carriers. (A) Epinephrine concentration with the following standard deviations (pg/mL) applying at 0, 6, and 24 hours in LL (87, 209, and 234), n = 64; HL (60, 202, and 194), n = 123 and HH (56, 175, and 116), n = 73 groups. (B) Norepinephrine concentration in patients not receiving any norepinephrine infusion with the following standard deviations (pg/mL) applying at 0, 6, and 24 hours in LL (117, 41, and 123), n = 16, HL (270, 96, and 289), n = 74, and HH (233, 171, and 449), n = 39 groups. (C) 3,4-dihydroxyphenylglycol (DHPG) concentrations with the following standard deviations (pg/mL) applying at 0, 6, and 24 hours in LL (281, 285, and 288), n = 64 HL (259, 158, and 235), n = 123; and HH (157, 116, and 227), n = 73 groups. (D) 3,4-dihydroxyphenylalanine (L-DOPA) concentration with the following standard deviations (pg/mL) applying at 0, 6, and 24 hours in LL (1471, 1264, and 1126), n = 64, HL (1246, 1133, and 988), n = 123; and HH (1242, 542, and 911), n = 73 groups.

stay in intensive care and in hospital after adjustment for pre- and intraoperative covariates (Table 4). We also observed a trend for higher mortality, as three LL patients (two with cardiogenic shock and one with hemorrhagic shock), two HL pa-

tients (one with cardiogenic shock and one with ischemic hepatitis), and no HH patient died during their hospital stays (P = 0.13). Within 6 mo after surgery, four LL patients, three HL patients, and no HH patients died (P = 0.074).

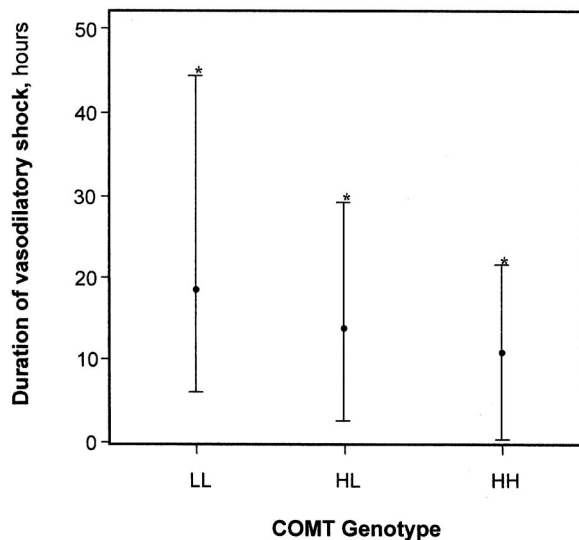


Figure 3. Duration of vasodilatory shock (median with 25th and 75th percentiles) according to catechol-O-methyltransferase (*COMT*) genotype. Black circles indicate medians and black lines indicate 25th and 75th percentiles, * $P = 0.013$.

The findings of the cross-validation analysis (training cohort $n = 190$ and validation cohort $n = 70$) are provided in Table 5 and confirm a progressive worsening of clinical outcomes from HH to HL and LL patients.

DISCUSSION

We found that 25% of 260 elective cardiac surgery patients who were homozygous for the low activity *COMT* L allele (LL patients) presented with increased concentrations of plasma catecholamine and MAO-dependent catecholamine degradation products underlining genotype-phenotype correlations. These patients more commonly developed prolonged vasodilatory shock, AKI, more severe AKI requiring renal replacement therapy, and prolonged hospital stay. After adjustment for important covariates, the LL allele was independently associated with vasodilatory shock, AKI, and prolonged intensive care unit and hospital stay. Nevertheless, preoperatively, LL patients could not be distinguished from HL or HH patients in terms of any other known risk factors.

Cardiac surgery with cardiopulmonary bypass is the most common surgical procedure that is associated with a high risk for vasomotor instability and AKI. At present, there is no intervention known to prevent shock and renal complications after cardiac surgery. On the one hand, achieving an acceptable mean arterial BP with the most frequently used vasopressor agent, norepinephrine, is a standard procedure.¹⁴ On the other hand, systemic hypotension and the concomitant use of vasopressors are considered as key-mediators of AKI.¹⁵ Genotype is an important determinant of both the response to treatment and the susceptibility to adverse reaction for a wide range of drugs. In cardiovascular and renal medicine, *COMT* gene may

be hypothetically involved in pharmacokinetics of exogenous catecholamines, affecting their metabolism and excretion. Preselection of patients based on their molecular profile responsible for regulation of catecholamine metabolism could help to identify patients refractory to norepinephrine or avoid detrimental complications related to adrenoceptor downregulation or desensitization-mediated peripheral vasodilatation that may occur in the face of high catecholamine levels. The use of norepinephrine (and probably vasopressor therapy in general) in critically ill patients with hypotensive vasodilatation despite fluid resuscitation and evidence of renal dysfunction is the subject of controversy, because of a putative worsening of renal vasoconstriction.

Hemodynamic effects of circulating norepinephrine are irrelevant at concentrations below 500 pg/ml and of circulating epinephrine at levels below 80 pg/ml.¹⁶ Concentrations of circulating catecholamines in our patients were far beyond those thresholds already 6 h after cardiopulmonary bypass. This further supports the notion that the clearance of circulating catecholamines appears to be dependent on *COMT* activity. The enzyme *COMT* is of relatively minor importance at the synaptic cleft and a low *COMT* activity probably does not significantly influence intrasynaptic norepinephrine concentrations which are rather depending on MAO and the norepinephrine transporter.¹⁷

Irrespective of their *COMT* genotype, preoperative catecholamine levels in our patients were similar with those previously reported in healthy volunteers.¹⁸ We found significantly elevated postoperative plasma epinephrine levels in all LL individuals and elevated norepinephrine levels in LL patients who were not receiving any norepinephrine infusions. Despite higher postoperative plasma catecholamine levels, patients with low *COMT* activity remained vasopressor dependent. Furthermore, a greater proportion of LL patients received continuous norepinephrine infusions and were exposed to a higher additive norepinephrine dose over the first 24 h postoperatively. We noticed a pattern of progressive increase in the vasodilatory shock duration, AKI incidence, and plasma catecholamine concentrations that was least common in HH patients, intermediate in HL patients, and highest in LL patients. The observed pattern provides plausibility for the notion that *COMT* genotype influences the vasomotor responses and patient outcomes. *COMT* genotype affects the amount of the *COMT* protein and determines the level of cytosolic enzymatic activity.¹⁹ The *COMT* LL genotype might contribute to decreased removal of excess plasma epinephrine and norepinephrine levels that could occur during and after cardiac surgery, as well as during exogenous catecholamine administration. Increased compensatory deactivation via monoamine oxidase is indicated by increased levels of the intraneuronal deaminated metabolite of norepinephrine, 3,4-dihydroxyphenylglycol,²⁰ as we observed in LL patients. Similar observations can be obtained in patients treated with *COMT* inhibitors.²¹ Beside nervous system and erythrocytes, *COMT* can be found in kidney and heart.²² *COMT* has an important role in preventing the

Table 3. Association of *COMT* genotype with duration of vasodilatory shock and incidence of acute kidney injury

Variable	β Coefficient	95% CI	P Multivariable
Dependent: Duration of vasodilatory shock ^a			
Duration of CPB	0.37	0.25 to 0.50	<0.001
<i>COMT</i> LL genotype	26.2	7.3 to 45.2	0.007
Chronic obstructive pulmonary disease	26.7	5.2 to 48.2	0.015
Simultaneous CABG and valvular surgery	31.8	4.2 to 59.4	0.024
Dependent: Incidence of postoperative AKI ^b			
	Odds ratio	95% CI	P Multivariable
Duration of cardiopulmonary bypass	1.008	1.003 to 1.014	0.004
Age	1.07	1.02 to 1.13	0.006
<i>COMT</i> LL genotype	3.20	1.37 to 7.46	0.007
Peripheral vascular disease	2.96	1.12 to 7.83	0.029

CPB, cardiopulmonary bypass; *COMT*, catechol-O-methyltransferase; CABG, coronary artery bypass graft; CI, confidence interval; AKI, acute kidney injury (defined as increase in serum creatinine >50% from baseline to peak value within the first five postoperative days).

^aMultivariable linear regression analysis included *COMT* LL and HH genotype, age, arterial hypertension, diabetes mellitus (including non-insulin dependent diabetes and insulin-dependent), peripheral vascular disease, atrial fibrillation, preoperative ACE-inhibitors, chronic obstructive pulmonary disease, smoking, preoperative estimated glomerular filtration rate, carotid disease, left ventricular ejection fraction, duration of cardiopulmonary bypass, and valvular surgery and simultaneous CABG.

^bMultivariable linear regression analysis included: *COMT* LL and HH genotype, age, arterial hypertension, diabetes mellitus, preoperative estimated glomerular filtration rate, diuretics, peripheral vascular disease, left ventricular ejection fraction, duration of cardiopulmonary bypass, cardiac reoperation, simultaneous CABG and valvular surgery and recent acute myocardial infarction.

formation of oxidants derived from catechol-dependent semiquinones and quinines.^{23–25} Increased levels of plasma catecholamines lead, in part, to increased degradation via non-enzymatic auto-oxidative pathways.²⁶ In this setting, increased generation of reactive oxygen species have been observed, which in turn reduces the biologic and therapeutic efficacy of catecholamines on vasomotor tone^{24–26} and may contribute to renal tubular cell injury. However, adult mice lacking *COMT* activity do not have increased oxidative stress in the liver or brain.²⁷ Experimental studies indicate that the inhibition of cardiac *COMT* activity decreases the removal of accumulated catecholamines evoked by myocardial ischemia that may augment cellular injury²⁸; however, data in the renal setting are lacking.

*COMT*LL individuals are predisposed to a spectrum of human pathologies ranging from neuropsychiatric and hormonal disorders⁹ to cardiovascular diseases.^{11,12} The *COMT* LL genotype may lend susceptibility to various stressors. Heavy coffee intake, for example increased the incidence of acute coronary events in LL patients, but not in HH patients.¹² Finnish *COMT*LL men also had increased risk of acute coronary events in interaction with high homocysteine levels.¹¹ The observed genotype-phenotype correlations may be even more pronounced in women, as estrogens may influence catecholamine pharmacodynamics,²⁹ and *COMT*-deficient mice display sexual dimorphism in catecholamine and catecholamine degradation product concentrations.³⁰ We were not able to explore this issue because of the limited number of female patients in our study and their postmenopausal age.

We are aware that the observational nature of the study precludes any causal proof or direct mechanistic insights. There are, however, several potential explanations as to why LL individuals more frequently failed to adequately respond to norepinephrine and developed AKI. Compared with wild type, *COMT*-deficient mice showed increased vascular relaxation as

well as enhanced dopaminergic tone and resistance to salt-induced hypertension.³¹ *COMT* inhibitors induce dopamine-dependent natriuresis and inhibition of Na⁺/K⁺/ATPase,³² an effect easily counteracted by fluid administration. L-DOPA is filtered at the glomerulus, absorbed, and then converted to dopamine in the proximal tubule. We cannot exclude that *COMT* may be of importance in the regulation of renal dopaminergic tone potentially involved in AKI. We have observed pre- and postoperative differences in L-DOPA according to *COMT* genotype in our patient cohort. Unfortunately, we were not able to investigate whether or not adverse renal outcomes of our LL patients may be related to impaired dopaminergic tone, as measurements of urinary catecholamine excretion were not feasible. The role of *COMT* in regulation of vascular tone is complex, as are endothelium-dependent vasorelaxation and sensitivity of arterial smooth muscle cells to nitric oxide altered in *COMT*-deficient mice and the arterial responses to norepinephrine.³¹

Our study has limitations. The cross-validation analysis did not reproduce all statistical significances, yet found a progressive relationship of adverse outcomes according to *COMT* genotypes. Nonetheless, we do not wish to imply that *COMT* is the last word in renal catecholamine metabolism. Renalase is a recently-discovered secreted amine oxidase that metabolizes catecholamines.³³ The gene encoding this novel renal hormone has a polymorphism that has been associated with hypertension.³⁴ An interaction between *COMT* and renalase would be an important question for further studies.

In conclusion, our hypothesis that impaired metabolism of circulating catecholamines might predispose to an unfavorable outcome after cardiac surgery was confirmed in a blinded prospective study. Our findings provide a conceptual framework for further studies in vasodilatory shock and AKI in sepsis. Perhaps more suitable hemodynamics could be achieved in LL patients were they given vasopressin rather than cat-

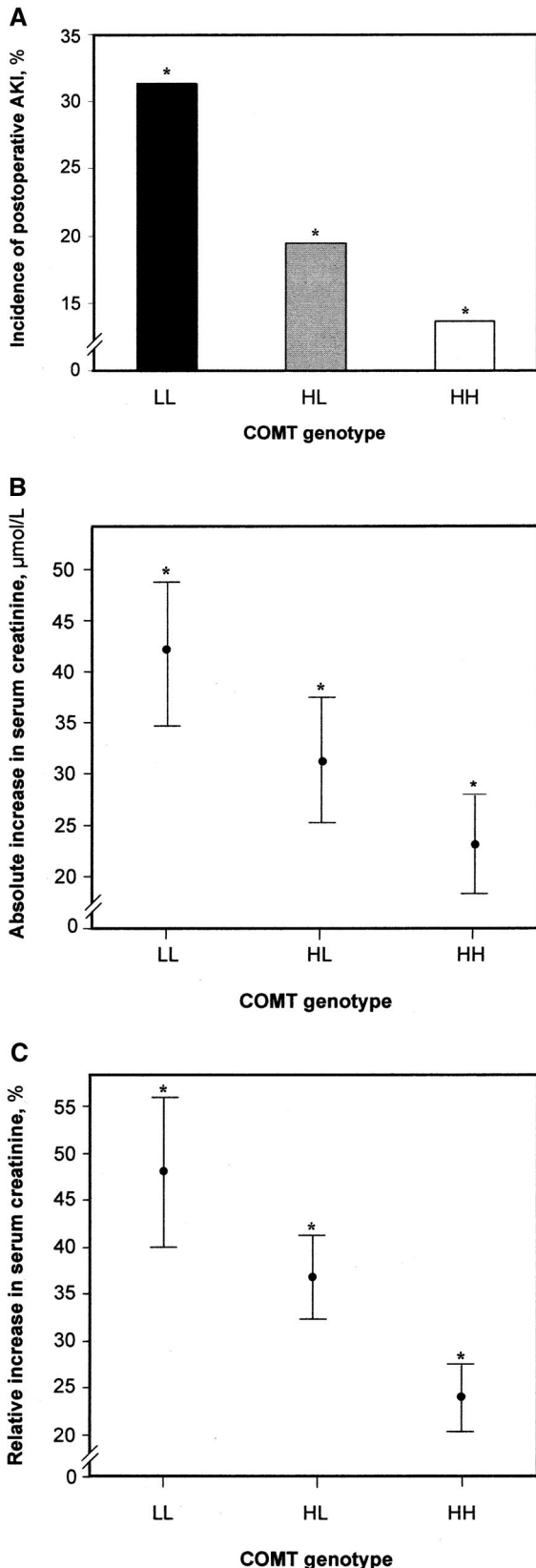


Figure 4. Influence of the catechol-O-methyltransferase (COMT) genotype on acute kidney injury (AKI). (A) Incidence of postoperative AKI in patients with different COMT genotypes; * $P =$

echolamines, and acute kidney injury might be attenuated by avoidance of cardiopulmonary bypass³⁵ and nephrotoxic medications. If confirmed in larger trials, preoperative COMT genotyping together with known clinical risk factors could aid in preoperative risk stratification to anticipate occurrence of circulatory and renal dysfunction, and prolonged recovery. Patients thus identified may benefit from an individualized treatment directed at prevention of hemodynamic instability and organ dysfunction.^{36–39}

CONCISE METHODS

Study Population and Data Collection

We prospectively enrolled a total of 260 consecutive patients who underwent elective cardiac surgery with the use of cardiopulmonary bypass at the Austin University Hospital, Melbourne. Procedures included isolated coronary artery bypass grafting, isolated valve surgery, simultaneous coronary artery bypass grafting and valve surgery, and thoracic aortic surgery. We excluded patients taking COMT inhibitors, monoamine oxidase inhibitors type A and B, or high to moderate doses of steroids (>10 mg prednisone or equivalent); emergency patients (operation performed within 24 h after cardiac symptoms commenced); patients with chronic renal impairment (preoperative serum creatinine >300 $\mu\text{mol/L}$); patients receiving intravenous nitrates/nitroprusside sodium; and patients under the age of 18. To exclude ascertainment bias, investigators and clinical personnel were blinded to outcomes or COMT genotype, respectively.

Clinical practice during operation and intensive care was not changed or modified for the purpose of the study. The use of antibiotics, volatile agents, propofol and other sedatives, antifibrinolytics, and fluid management was left to the discretion of the anesthesiologist or intensive care physician and was documented. Analgesia was achieved with fentanyl or morphine intraoperatively and with acetaminophen or tramadol postoperatively. Antihypertensive medication including angiotensin-converting enzyme inhibitors was withdrawn on hospital admission (generally 1 d before surgery).

Hemodynamic management targeted a mean arterial pressure of >70 mmHg, a cardiac index of >2.5 L/min/m² as measured with use of pulmonary artery catheter, and a central venous pressure between 12 and 16 cm H₂O. Intraoperative cardiac index during cardiopulmonary bypass was preset at the cardiopulmonary bypass machine (2.5 L/min/m²). During cardiopulmonary bypass, in addition to norepinephrine, other vasopressor agents such as metaraminol, ephedrine, or phenylephrine were administered. Postoperatively, norepinephrine was the only vasopressor used in our patient cohort. None of the patients received vasopressin, dopamine, or dobutamine. None of the

0.038. (B) Absolute increase in serum creatinine from baseline to peak value during the first five postoperative days (mean and SEM) according to COMT genotype. Circles indicate mean and lines indicate SEM, * $P = 0.016$. (C) Relative increase in serum creatinine from baseline to peak value within the first five postoperative days (mean and SEM), * $P = 0.008$.

Table 4. Association of *COMT* genotype with length of stay in intensive care and length of stay in hospital

Variables	β Coefficient	95% CI	P Multivariable
Dependent: Length of stay in intensive care ^a			
duration of CPB	0.49	0.35 to 0.65	<0.001
<i>COMT</i> LL genotype	42.8	20.5 to 65.2	<0.001
diabetes mellitus	42.4	18.3 to 66.4	0.001
simultaneous CABG and valvular surgery	48.8	16.7 to 80.9	0.003
chronic obstructive pulmonary disease	33.4	7.7 to 59.0	0.011
Dependent: Length of stay in hospital ^b			
duration of CPB	0.03	0.02 to 0.04	<0.001
<i>COMT</i> LL genotype	3.0	1.1 to 4.8	0.002
simultaneous CABG and valvular surgery	4.6	2.0 to 7.3	0.001
BSA	0.2	0.1 to 0.4	0.017
preoperative atrial fibrillation	2.3	0.3 to 4.3	0.024
diabetes mellitus	2.0	0.2 to 4.0	0.048

CPB, cardiopulmonary bypass; *COMT*, catechol-O-methyltransferase; CABG, coronary artery bypass graft; BSA, body surface area; CI, confidence interval.
^aMultivariable linear regression analysis included: LL and HH genotype, diabetes mellitus (including non-insulin dependent diabetes and insulin-dependent), chronic obstructive pulmonary disease, preoperative atrial fibrillation, ventricular dysfunction, preoperative use of ACE inhibitor, duration of cardiopulmonary bypass, valvular surgery and simultaneous CABG and valvular surgery.
^bMultivariable linear regression analysis included: LL and HH genotype, BSA, diabetes mellitus, chronic obstructive pulmonary disease, preoperative atrial fibrillation, preoperative use of ACE inhibitor, duration of cardiopulmonary bypass and simultaneous CABG and valvular surgery.

Table 5. Cross-validation outcomes according to *COMT* genotypes

Training data (n = 190)	LL (n = 64, 24.6%)	HL (n = 123, 47.3%)	HH (n = 73, 28.1%)	P
Duration of vasodilatory shock, h	18.3 (4.8–36.5)	13.0 (0.5–30.0)	11.0 (0.0–22.5)	0.14
Incidence of vasodilatory shock > 48 h, n (%)	10 (21.7)	12 (13.2)	5 (9.4)	0.21
Increase in serum creatinine, $\mu\text{mol/L}^a$	35.8 \pm 46.9	30.5 \pm 41.3	19.9 \pm 32.8	0.11
Incidence of AKI, n (%)	12 (26.1)	15 (16.5)	6 (11.3)	0.16
Dose of furosemide 0–24 h, mg	46.9 \pm 49.7	36.5 \pm 48.3	30.5 \pm 41.3	0.19
Incidence of RRT, n (%)	3 (6.5)	1 (1.1)	0 (0.0)	0.058
Length of stay in ICU, hrs	48.5 (26.8–80.8)	44.0 (24.0–67.6)	40.0 (23.0–67.8)	0.10
Length of stay in hospital, days	9.0 (7.0–11.0)	8.0 (7.0–10.0)	7.0 (7.0–8.5)	0.029
In hospital mortality, n (%)	2 (4.4)	1 (1.1)	0 (0.0)	0.57
Validation data (n = 70)	LL (n = 18, 25.7%)	HL (n = 32, 45.7%)	HH (n = 20, 28.6%)	P
Duration of vasodilatory shock, h	19.5 (6.3–55.3)	13.5 (5.0–27.8)	9.5 (0.0–14.8)	0.039
Incidence of vasodilatory shock > 48 h, n (%)	6 (33.3)	4 (12.5)	0 (0.0)	0.013
Increase in serum creatinine, $\mu\text{mol/L}^a$	50.0 \pm 49.1	33.7 \pm 38.6	30.4 \pm 35.1	0.029
Incidence of AKI, n (%)	8 (44.4)	8 (25.0)	4 (20.0)	0.14
Dose of furosemide 0–24 h, mg	97.8 \pm 113.5	42.9 \pm 66.5	31.6 \pm 32.6	0.019
Incidence of RRT, n (%)	2 (11.1)	2 (6.3)	0 (0.0)	0.31
Length of stay in ICU, hr	65.0 (39.3–181.3)	43.5 (39.0–83.3)	40.0 (38.0–63.0)	0.12
Length of stay in hospital, days	10.0 (7.0–14.8)	8.5 (7.0–12.8)	8.0 (7.0–10.8)	0.49
In hospital mortality, n (%)	1 (5.6)	1 (3.1)	0 (0.0)	0.55

Numbers denote mean \pm SD, median (25th to 75th percentiles), or numbers (%). *COMT*, catecholamine-O-methyltransferase; AKI, acute kidney injury defined as defined; RRT, renal replacement therapy; ICU, intensive care unit.
^aFrom baseline to peak value within the first five postoperative days.

patients simultaneously received infusion of norepinephrine and nitroglycerine. The postoperative use of vasopressor agents and inotropic medications (typically milrinone at this center) was recorded hourly until patients were discharged from the intensive care unit.

This study adhered to the Declaration of Helsinki. The institutional review board of the Austin Hospital approved this study. This study was registered with clinicaltrials.gov (NCT00334009). Written informed consent was obtained before patient enrollment. Clinicians and investigators engaged in pre-, intra-, and postoperative data col-

lection were blinded to patient’s genotype (as this was determined after enrollment of patients was complete).

Genetic Analysis and Catecholamine Measurements

A single-nucleotide polymorphism (472 G/A) in exon 4, the coding region of the *COMT* gene, causes an amino acid change from valine to methionine at position 108/158 (V108/158M), with the valine allele (472G) being associated with a three- to four-fold higher *COMT* enzymatic activity as compared with the methionine allele (472A).⁴⁰ The

COMT gene has been mapped to chromosome 22 (22q11.21-q11.23; 22q11.21) (GenBank accession number Z26491). Polymorphism in the *COMT* gene is trimodal in the human population,⁸ with approximately 25% of Caucasians being homozygous for low enzyme activity (*COMT* LL), 50% heterozygous (*COMT* HL), and 25% homozygous for high activity (*COMT* HH).^{41,42}

We tested patient DNA for the G-to-A transition in codon 158 (membrane-bound form)/108 (soluble-form) of the *COMT* gene using a PCR-based restriction fragment length polymorphism assay essentially as described previously.⁴³ Genomic DNA was prepared from 200- μ l blood samples by AGOWA's DNA extraction service (AGOWA GmbH, Berlin) using the AGOWAmag Maxi DNA Isolation Kit on KingFisher KF96 system. A 210 bp fragment of the *COMT* gene containing the polymorphic site was amplified using the following pair of primers: 5'-TCG TGG ACG CCG TGA TTC AGG-3' and 5'-ACA ACG GGT CAG GCA TGC A-3'. After digestion with the restriction endonuclease *Nla*III (2 h at 37°C), the PCR products were run on 4% agarose gel and visualized by ethidium bromide staining. The results were documented using the BioDocAnalyze system (Biometra, Goettingen, Germany). The PCR product derived from the Val158/108 encoding allele contained two *Nla*III sites (at position 83 and 197) and the Met158/108 encoding allele three *Nla*III sites (at position 83, 179 and 197). Accordingly, diagnostic bands had a size of 114 (Val158) and 96 bp (Met158), and both alleles shared an 83 bp fragment. Ambiguous or unidentifiable results were reamplified and rescanned. The laboratory investigators were unaware of the sample sources and clinical outcomes until the end of the study.

Concentrations of plasma catecholamines (norepinephrine, epinephrine, and 3,4-dihydroxyphenylalanine - the precursor of dopamine) and the deaminated metabolite of epinephrine and norepinephrine - 3,4-dihydroxyphenylglycol were measured at baseline, 6 h, and 24 h after commencement of cardiopulmonary bypass. Catecholamines and 3,4-dihydroxyphenylglycol were extracted from 1 ml of plasma with alumina adsorption, separated by reverse-phase HPLC, and detected using colorimetric detection according to previously described methods,⁴⁴ without knowledge of genotype and patient outcomes.

Outcome Measures

Vasodilatory shock was defined as persistent hypotension (mean arterial pressure less than 70 mmHg) occurring in the early postoperative period (within 6 h after weaning from cardiopulmonary bypass) in the setting of the targeted cardiac index and central venous pressure requiring pharmacologic support by norepinephrine as previously published.⁴⁵ We measured vasodilatory shock as the duration of norepinephrine infusion. Norepinephrine was commenced after volume depletion was corrected. AKI was defined as increase in serum creatinine concentration >50% from baseline to peak value within the first five postoperative days. Other outcomes included absolute and relative increase in serum creatinine from baseline to peak value during the first five postoperative days, indicating severity of AKI, dose of furosemide to maintain urine output 0.5 to 1.0 ml/kg/h, requirement for renal replacement therapy, length of stay in intensive care and in hospital, and mortality (in-hospital and at 6 mo postoperatively

through information from the outpatients department). At our center, renal replacement therapy was initiated if the patient fulfilled at least one of the following clinical criteria: oliguria (urine output <100 ml/6 h) that has been unresponsive to fluid resuscitation measures, hyperkalemia ($[K^+] >6.5$ mmol/L), severe acidemia (pH < 7.2), or clinically significant organ edema (eg, lung) in the setting of renal failure.

Statistical Analysis

The genotype frequencies were tested for Hardy Weinberg equilibrium using a standard chi-square test. All data were assessed for normal distribution using histograms. We present variables as means \pm SD when data were normally distributed and used ANOVA for comparison of all genotypes. When data were not normally distributed, we present variables as medians with 25th to 75th percentiles and use Kruskal-Wallis test for comparison of all genotypes. For categorical data, we performed three group comparisons using chi-square test or Fisher exact test when the expected value was less than 5. All demographic, genetic, preoperative, and intraoperative variables (Tables 1 and 2) were tested in univariate regression analysis for their relationship to the predefined postoperative clinical outcomes (duration of vasodilatory shock, incidence of AKI, length of stay in the intensive care unit, and length of stay in hospital). We included all covariates with a univariate $P < 0.10$ and those of clinical importance in each setting into multivariable linear regression analysis assessing each of these outcome variables. Variables with a significant P value in the last step of the multivariate regression analysis are presented in Tables 3 and 4. Logarithmic transformations were applied when necessary before multivariable linear regression analyses were performed. GFR was estimated using the Modification of Diet in Renal Disease Study equation re-expressed for use with the serum creatinine values standardized to isotope dilution mass spectroscopy.⁴⁶ In a secondary *post hoc* analysis, we assessed the performance characteristics of renal outcomes according to the RIFLE criteria.⁴⁷ We performed a common type of cross-validation analysis (holdout validation) to test the robustness of the study findings.⁴⁸ The observations were chosen randomly from the initial sample by using a random number generator (Microsoft Corp. Redmond, WA) to form the validation data, and the remaining observations were retained as the training data. For multiple testing, Bonferroni correction was applied using adjusted cut-offs for P values.⁴⁹ We used SPSS Version 15.0 (SPSS Inc, Chicago, IL) and MedCalc Version 9.3.9.0 (MedCalc Software, Mariakerke, Belgium).

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DISCLOSURES

None.

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