The Heart Surgery Forum #2007-1117
10 (6), 2007 [Epub October 2007]
doi: 10.1532/HSF98.20071117

Online address: http://cardenjennings.metapress.com

# Genetic Polymorphisms Contribute to Acute Kidney Injury after Coronary Artery Bypass Grafting

**Selim C. Isbir**,[1] Atike Tekeli,[2] Arzu Ergen,[3] Hulya Yilmaz,[3] Koray Ak,[1] Ali Civelek,[1] Umit Zeybek,[3] Sinan Arsan[1]

[1]Department of Cardiovascular Surgery, Marmara University School of Medicine, Istanbul, Turkey; [2]Department of Cardiovascular Surgery, Academic Hospital, Istanbul, Turkey; [3]Department of Molecular Medicine, Institute for Experimental Medical Research, Istanbul University, Istanbul, Turkey

## ABSTRACT

**Background**. Acute kidney injury is one of the most serious complications after cardiac surgery. Genetic polymorphisms are reported to be associated with postoperative renal impairment. The aim of this study was to investigate the relationship between selected gene polymorphisms and acute kidney injury after cardiac surgery.

**Methods.** Two hundred forty-eight elective coronary artery bypass grafting procedure patients were enrolled in the study. Angiotensin-converting enzyme (ACE) II, ID, and DD, apolipoprotein E (APO E), and angiotensin II type 1 receptor (AGTR1) A1166C genotypes were detected by polymerase chain reaction. Plasma levels of ACE were analyzed by enzyme-linked immunosorbent assay. Acute kidney injury after cardiac surgery was graded according to the RIFLE (risk, injury, failure, loss, and end-stage kidney disease) classification.

**Results.** In our study, 21.8% of patients had acute renal impairment after cardiac surgery. Among the 54 patients with acute kidney injury, ACE D allele frequency was 0.620. The plasma levels of ACE were significantly higher in the D allele carriers ($P = .018$). Three of the 54 patients with acute kidney injury were APO E ε4 allele carriers ($P = .002$). AGTR1 C allele carriers constituted 46% of all patients with postoperative acute kidney injury. There was no statistically significant difference between A allele homozygotes and C allele carriers with respect to postoperative renal dysfunction ($P > .05$).

**Conclusions.** The present findings support the hypothesis that ACE I/D and APO E gene polymorphisms may play a role in the development of acute kidney injury after cardiac surgery. However, AGTR1 does not have a unique association with postoperative renal impairment.

*Received May 21, 2007; received in revised form June 29, 2007; accepted July 2, 2007.*

*Correspondence: Selim Isbir, MD, Assoc. Prof. of Cardiovascular Surgery, Marmara University Hospital, Tophanelioglu Cad. No: 13-15 Altunizade, Istanbul, Turkey 34664; 90-216-327-10-10; fax: 90-216-651-00-40 (e-mail: isbir@yahoo.com).*

## INTRODUCTION

Acute kidney injury (AKI) after cardiac surgery is a devastating complication associated with increased mortality and morbidity [Lassnigg 2005]. Mortality is almost 60% in patients requiring hemodialysis after cardiac surgery. Several patient risk factors have been identified for the development of postoperative AKI. These include, age, diabetes mellitus, severe arteriosclerosis of the aorta, and hypertension. Besides these, although not widely accepted, genetic polymorphisms are also thought to be a considerable risk factor for this disorder [Stafford-Smith 2005].

The renin-angiotensin system (RAS) is a well known enzymatic cascade that has an important role in cardiovascular homeostasis. The genetic variants of this system such as angiotensin II type 1 receptor (AGTR1) and angiotensin-converting enzyme (ACE) are vasoconstrictor polymorphisms and are shown to be associated with renal functions. ACE gene, which is located at chromosome 17q23, regulates the plasma levels of ACE in 20% to 50% of subjects [Tokgozoglu 1997; Bautista 2004]. There are 3 genotypes of ACE I/D polymorphism; II, ID, and DD. DD genotype is suggested to be important and an independent risk factor of many clinical conditions such as coronary and carotid artery disease, myocardial infarction, hypertension, and heart failure [Leatham 1994; Losito 2000; Schut 2004; Ilhan 2005; Sayed-Tabatabei 2005]. AGTR1 gene is also shown to be a determinant of renal hemodynamic functions, especially in diabetics. A polymorphism of the AGTR1 gene is described by whether there is either adenine (A) or cytosine (C) base at region 1166 of the gene. It has been suggested that C allele is an independent risk factor of cardiovascular diseases [Benetos 1996].

Apolipoprotein E (APO E) is a cardinal protein in lipid metabolism and it is associated with atherosclerosis. It also has an influence on renal function after cardiac surgery [MacKensen 2004]. Common APO E gene isoforms are ε2, ε3, and ε4, which are located at 19q13.2. APO E4 is defined as the sum of ε2/4, ε3/4, and ε4/4, whereas APO non-E4 is defined as the sum of ε2/2, ε2/3, and ε3/3. APO E4 is known to increase cholesterol levels in humans [MacKensen 2004].

There are several definitions of AKI in the literature, ranging from modest increase in serum creatinine to severe injury

Table 1. RIFLE Classification

| | GFR Criteria | Urine Output Criteria |
|---|---|---|
| Risk | Increased plasma creatinine × 1.5 | <0.5 mL/kg$^{-1}$/h$^{-1}$ × 6 hours |
| Injury | Increased plasma creatinine × 2 | <0.5 mL/kg$^{-1}$/h$^{-1}$ × 12 hours |
| Failure | Increased plasma creatinine × 3 or acute plasma creatinine ≥350 µmol/L or acute rise ≥44 µmol/L | <0.3 mL/kg$^{-1}$/h$^{-1}$ × 24 hours or anuria × 12 hours |
| Loss | Persistent acute renal failure = complete loss of kidney function >4 weeks | |
| End-stage kidney disease | End-stage kidney disease (>3 months) | |

requiring hemodialysis. However, there is no obvious marker of renal function for the postoperative period. Recently, the RIFLE (risk, injury, failure, loss, and end-stage kidney disease) classification was recommended based on the plasma creatinine and/or urine output levels [Bellomo 2004].

The aim of the present study is to evaluate the role of genetic variants of inflammatory and paracrine pathways that might be associated with AKI after cardiac surgery.

## MATERIALS AND METHODS

After institutional review board approval, 248 elective coronary artery bypass grafting procedure patients who were operated on between November 2004 and May 2006 were enrolled in the study. Patients who were on either hemodialysis or peritoneal dialysis were excluded. AKI was interpreted according to RIFLE classification [Bellomo 2004] (Table 1). According to this classification, patients were divided into 4 groups based on their plasma creatinine levels and/or urine output. The change in plasma creatinine level was determined as the difference between preoperative level and highest postoperative creatinine level measured during the time of intensive care unit stay. Serum creatinine levels were measured by a Cobas Integra 700 analyzer (Roche Diagnostics, Indianapolis, IN, USA).

### *Coronary Artery Bypass Grafting Procedure*

All operations were performed in a standardized approach with a Jostra HL-20 roller pump (Jostra, Hirrlingen, Germany), membrane oxygenator (Jostra), and a 40-µm arterial blood filter (Jostra). Mild to moderate (28-32°C) hypothermia and pulsatile flow of 2.2 to 2.4 L/m$^2$ were used. Myocardial protection was achieved with cold antegrade blood cardioplegia. Perfusion pressure was kept above 70 mmHg in all times. Ultrafiltration was used in patients with above normal creatinine levels. Aprotinin was not used in any of the patients. Blood samples for biochemical and genetic analysis were taken during induction (t1), during cardiopulmonary bypass (t2), and 12 hours after surgery (t3).

### *Isolation of DNA*

Blood specimens were collected in tubes containing ethylenediaminetetra-acetic acid (EDTA). DNA samples were extracted from whole blood by the salting-out procedure [Miller 1988].

### *Determination of the ACE I/D Genotype*

ACE I/D genotype was determined by polymerase chain reaction (PCR) using oligonucleotides (sense 5´ CTGGAGACCACTCCCATCCTTTCT 3´ and antisense 5´ GATGTGGCCATCACATTCGT CAGAT 3´). The PCR reaction mixture contained 100 ng DNA template, 0.5µM of each primer, 3 mM MgCl2, 0.5 mM of each dNTPs, and 1 U Taq DNA polymerase (MBI Fermentas, Glen Burnie, MD, USA). After denaturating the DNA for 5 minutes at 94°C, the reaction mixture was subjected to 30 cycles of denaturating for 1 minute at 94°C, 1 minute annealing at 58°C, and 2 minutes of extension at 72°C by the thermal cycler. The PCR products (190 and 490 bp) were electrophoresed on ethidium bromide–stained 2% agarose gel and visualized under an ultraviolet transilluminator. On electrophoresis, ACE genotype I/I showed a 490-bp band, genotype D/D showed a 190-bp band, and I/D genotype showed both 490-and 190-bp bands [Rigat 1992].

Each sample that had the DD genotypes was submitted to PCR amplification with a primer pair (5´ TGG GAC CAC AGC GCC CGC CAT TAC 3´, 5´ TCG CCA GCC CTC CCA TGC CCA TAA 3´) that recognizes an insertion-specific sequence. If the DNA was mistyped, a PCR product of 335 bp is present on ethidium bromide–stained 2% agarose gel [Shanmugam 1993].

### *Determination of Plasma Levels of ACE*

For the measurement of ACE activity, blood was collected in EDTA-coated polystyrene tubes and centrifuged immediately. The EDTA plasma was stored at –80°C until plasma ACE levels were determined by enzyme-linked immunosorbent assay (Cat No: ACE100; Chemicon International, Temecula, CA, USA).

Table 2. Baseline and Perioperative Characteristics of Patients

| Clinical Characteristics | Renal Failure (n = 54) | Normal Renal Function (n = 194) | *P* |
|---|---|---|---|
| Age, y | 61.7 ± 11 | 60.3 ± 9.38 | .07 |
| Female, % | 26 | 25 | .87 |
| Body surface area, m$^2$ | 1.65 ± 0.23 | 1.78 ± 0.18 | .005* |
| Hypertension, % | 68 | 57 | .04* |
| Diabetes mellitus, % | 39 | 34 | .057 |
| Hyperlipidemia, % | 51 | 46 | .005* |
| Cardiopulmonary bypass time, min | 88.3 ± 39.08 | 85.5 ± 41.8 | .87 |
| Cross-clamp time, min | 51.2 ± 24.5 | 48.1 ± 23.8 | .065 |
| Ejection fraction, % | .53 | .55 | .83 |
| EuroSCORE | 3.8 ± 2.6 | 3.6 ± 2.5 | .88 |

*$P < .05$ statistically significant.

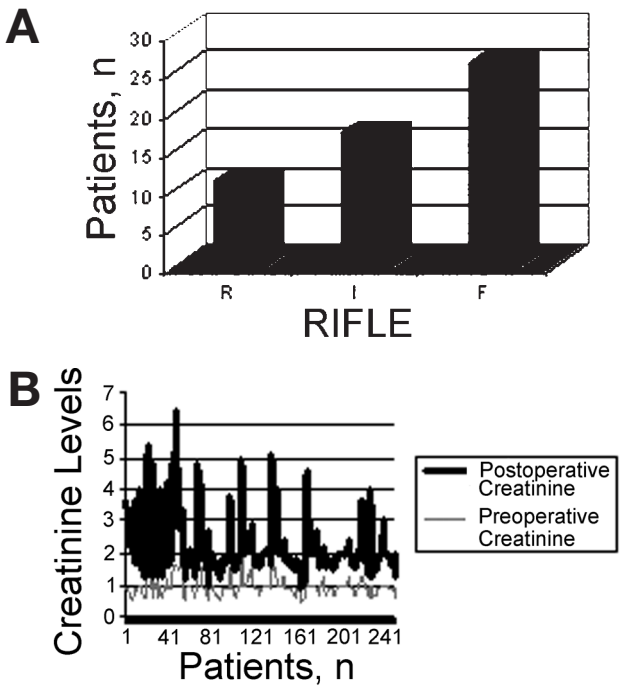


Figure 1. A, Distribution of patients with postoperative acute kidney injury (AKI) according to RIFLE classification. B, Figure showing the difference between preoperative and postoperative creatinine levels ($P = .000$).

### *APO E Genotyping*

Genotypes for APO E isoforms (ε2, ε3, ε4) were determined from leukocyte DNA by restriction fragment length polymorphism of amplified APO E sequences. Genomic DNAs (0.5-1.0 μg) were amplified using a forward primer E1, 5´-ATGGACGAGACCATGAAGGAG TTGAAG-3´ (codons 64-72), and a reverse primer E2, 5´ -ATGGACGAGACCAT GAAGGAG TTGAAG-3´ (codons 161-168). Amplification was achieved by 35 cycles of denaturation (1 minute at 96°C), annealing (2 minutes at 67°C), and extension (2 minutes at 72°C), followed by extension for 5 minutes at 72°C. The amplified 314-bp PCR product was directly digested with the restriction enzyme Hha I [Kontula 1990].

### *AGTR1 Genotyping*

Genomic DNAs were amplified using a forward primer (5´-AAT GCT TGT AGC CAA AGT CAC CT-3´, 5´-GGC TTT GCT TTG TCT TGT TG-3´). PCR-based protocols were used to identify the AGTR1 A1166C gene polymorphism as described previously [Tiret 1994].

### *Statistical Analysis*

Statistical analyses were performed using the SPSS version 11.00 for Windows (SPSS, Chicago, IL, USA). Discrete variables were expressed as counts or percentages and compared with chi-square or Fischer exact tests when appropriate. Continuous variables were expressed as mean ± SD and compared by Student *t* test or analysis of variance for repeated measures with Bonferroni corrections. Potential associations among clinical and biochemical variables, genotypes, and allele frequencies were first tested by univariate methods. All variables with $P < .1$ in univariate analysis were entered into multivariate analysis. To determine the relationship between different genotypes and acute renal injury, separate multivariable regression models were created for each of the 3 different genes. All $P < .05$ were considered significant.

## RESULTS

Patient demographics and operative data are shown in Table 2. Of the 248 patients, 54 (21.8%) had postoperative AKI based on RIFLE classification as combined RIFLE R, I, and F (Figure 1A). Figure 1B shows the difference between preoperative and postoperative creatinine levels. None of the patients in this study required renal replacement therapy. Because this study involves multiple parameters that affect the postoperative AKI, two different models were used. In the first model, preoperative and operative variables were used to analyze the postoperative AKI. There was no statistically significant difference between controls and patients with postoperative AKI in terms of operative variables. However, regarding hypertension and hyperlipidemia, there was a statistically significant difference between the patient groups ($P = .04$ and $P = .005$, respectively). In the second model, the effect of genetic variants tested to show whether there was any association between genotype and postoperative AKI. The distribution of ACE, APO E, and AGTR1 genotypes and allele frequencies in the study population is shown in Table 3. There was no operative or 30-day in-hospital mortality. Comparison of the preoperative ejection fraction and risk score (EuroSCORE) in controls and patients with postoperative AKI revealed no difference and were independent of genotype.

Among the 54 patients with postoperative AKI, ACE ID genotype was present in 21 patients, ACE II in 10, and ACE DD

Table 3. Genotypic Distribution and Allelic frequencies of ACE, APOE, and AGTR1 Genes*

| | Renal Failure, n (%) | Normal Renal Function, n (%) |
|---|---|---|
| ACE | | |
| II | 10 (18.5) | 68 (35) |
| ID | 21 (38.9) | 72 (37) |
| DD | 23 (42.6) | 54 (28) |
| APO E | | |
| APO E4 | 3 (5.6) | 47 (24) |
| APO non-E4 | 51 (94.4) | 147 (76) |
| AGTR1 (A1166C) | | |
| AA | 29 (54) | 100 (52) |
| AC | 21 (39) | 78 (40) |
| CC | 4 (7) | 16 (8) |

*ACE indicates angiotensin-converting enzyme; APO, apolipoprotein; AFTR1, angiotensin II type 1 receptor; A, adenine; C, cytosine.

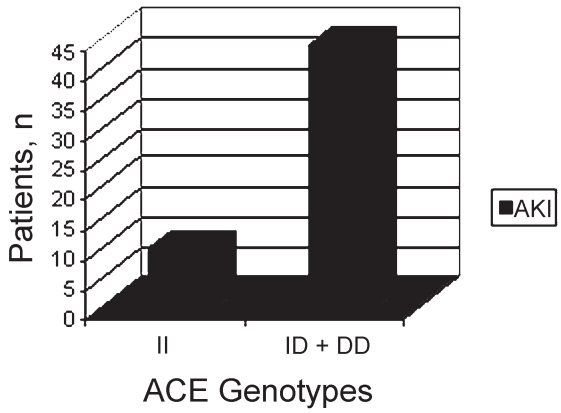


Figure 2. Patients carrying the angiotensin-converting enzyme (ACE) D allele were more prone to develop postoperative renal failure. *$P$ = .021.

in 23, thus the D allele frequency was 0.620. Postoperative AKI classified according to RIFLE was significantly associated with D allele carriage ($P$ = .021) (Figure 2). Figure 3A shows the plasma ACE levels that were measured at t1, t2, and t3 time intervals. The plasma ACE levels were significantly higher at the time intervals t2 and t3 ($P$ = .001 and $P$ = .008, respectively). When the plasma ACE levels and postoperative renal functions are concerned, elevated levels were detected in patients with AKI according to RIFLE classification postoperatively ($P$ = .034) (Figure 3B). Figure 4 shows the association between the plasma ACE levels and ACE genotypes where the plasma levels of ACE were found to be higher in carriers of D allele (t1 = 77.71 ± 16.63 pg/mL and t3 = 83.39 ± 17.09 pg/mL, $P$ = .018).

The APO E4 allele was present in 50 of 248 patients (20%). Three of the 54 patients with AKI were carriers of APO E ε4 allele. Compared with APO E4 patients, APO non-E4 patients showed an increased risk of AKI following surgery ($P$ = .002) (Figure 5).

AGTR1 C allele carriers constitute 46% of the patients with postoperative AKI. There was no statistically significant difference between A allele homozygotes and C allele carriers with respect to postoperative AKI ($P$ > .05).

## DISCUSSION

It is obvious that postoperative AKI has an independent effect on morbidity and mortality after cardiac surgery [Heringlake 2006]. The economic and clinical implications of postoperative AKI are considerable. Despite several clinical studies that have investigated the matter, the exact mechanism causing postoperative renal dysfunction is still unknown. There are different strategies to prevent this complication. However, it is not possible to apply all these methods to every single patient. With the ability to identify genetic predisposing factors, one can employ a goal-directed approach to treating high-risk patients and thereby improve mortality and morbidity after cardiac surgery.

The grading of renal dysfunction is another subject lacking consensus. Until now, many definitions of AKI have been used and Bellomo et al outlined the consensus criteria of renal injury and named it the RIFLE system [Bellomo 2004]. In this study, we performed an analysis of postoperative renal dysfunction according to the RIFLE system and our data represented an overall renal impairment rate of 21.8% that is similar to the literature [Bell 2005; Kuitunen 2006].

The present study demonstrates an association of the ACE I/D polymorphism and absence of APOE ε4 allele with the development of postoperative AKI. However, the A1166C variant in the AGTR1 gene did not show any likely association with renal dysfunction occurring after coronary artery bypass grafting.

ACE gene I/D polymorphism is one of the most studied polymorphisms in various clinical conditions. ACE D allele

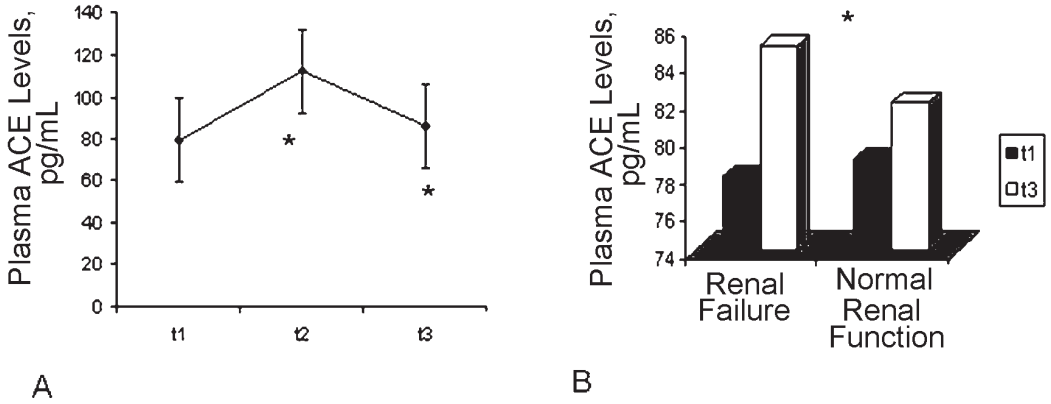


Figure 3. A, The plasma angiotensin-converting enzyme (ACE) levels were significantly higher during and after cardiopulmonary bypass (112.5 pg/mL and 86 pg/mL, respectively) compared to preoperative levels (78.7 pg/mL). *$P$ = .001 and $P$ = .008, respectively. t1 indicates during induction; t2, during cardiopulmonary bypass; t3, 12 hours postoperatively. B, Patients with postoperative acute renal failure showed significantly higher ACE plasma levels 12 hours postoperatively. *$P$ = .034.

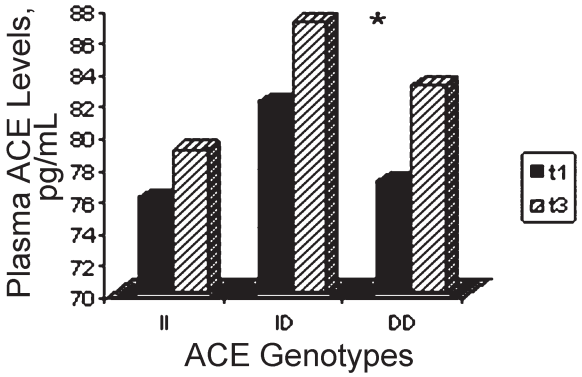


Figure 4. Angiotensin-converting enzyme (ACE) D allele carriers showed significantly higher plasma levels 12 hours postoperatively when compared with preoperative levels. *$P$ = .018. t1 indicates during induction; t3, 12 hours postoperatively.

influences many cardiovascular pathologies [Raynolds 1993; Volzke 2002]. We observed that D allele carriers were 49.7% of the patients in our study and the D allele frequency was 0.620 in the patients with postoperative AKI.

The plasma levels of ACE that were analyzed during cardiopulmonary bypass and 24 hours after the surgery were found to be higher in the ACE D allele carriers. This result is consistent with the literature since many studies reported elevated plasma and tissue levels of ACE in DD homozygotes [Nakai 1994; Danser 1995]. The mechanism by which the ACE I/D polymorphism may affect renal dysfunction after coronary artery bypass grafting is unclear. It is probably related to increased angiotensin II formation in kidneys by RAS.

Our results indicate that APO E ε4 allele is protective against postoperative renal dysfunction and APO ε4 allele carriage was inversely related to postoperative renal dysfunction. MacKensen et al reported that preoperative analysis of APO E ε4 allele may be helpful for risk stratification of renal injury after surgery [MacKensen 2004]. In fact, APO E ε4 allele is associated with the development of arteriosclerosis and one should think that APO E ε4 allele carriers might have increased postoperative AKI. However, this is not the case [Chew 2000]. The underlying effect of this mechanism is suggested to be APO E allele–related differences, especially in renal vulnerability to embolic injury due to detachment of the atheroma plaques during aortic manipulations [MacKensen 2004].

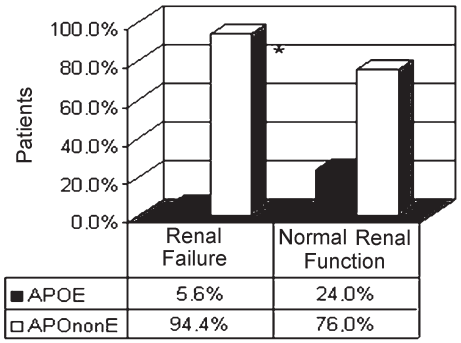


Figure 5. Apolipoprotein (APO) non-E4 patients developed postoperative renal failure more than the APO E4 patients. *$P$ = .002.

The gene encoding for AGTR1 is localized on chromosome 3. As a component of RAS, the binding of angiotensin II to AGTR1 results in vasoconstriction, cell growth, and proliferation, and especially in cardiac hypertrophy. Thus it is not surprising that the polymorphism of the AGTR1 gene is associated with cardiovascular diseases. Although Stafford-Smith et al reported a pairwise allele interaction of eNOS and AGTR1 with renal injury [Stafford-Smith 2005], our results did not show a unique association of AGTR1 A1166C polymorphism with postoperative AKI.

In summary, postoperative AKI is a serious complication of cardiac surgery. The mechanism causing AKI after coronary artery bypass grafting is still unknown; however, cardiopulmonary bypass and systemic inflammatory reaction syndrome occurring after cardiopulmonary bypass are supposed to be involved in this complex mechanism. Although some studies delineate the fact that genetic polymorphisms are associated with renal dysfunction after coronary artery bypass grafting, these reports are not conclusive. Our study provides evidence for the use of the ACE I/D genotype and APO E ε4 allele as a predictor of AKI after cardiac surgery; however, studies with a larger number of patients will help to identify high-risk populations and better define the effects of these polymorphisms.

## REFERENCES

Bautista LE, Ardila ME, Gamarra G, et al. 2004. Angiotensin-converting enzyme gene polymorphism and risk of myocardial infarction in Colombia. Med Sci Monit 10:473-9.

Bell M, Liljestam E, Granath F, et al. 2005. Optimal follow-up after continuous renal replacement therapy in actual renal failure patients stratified with the RIFLE criteria. Nephrol Dial Transplant 20:354-60.

Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P; Acute Dialysis Quality Initiative Workgroup. 2004. Acute renal failure: definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 8:R204-12.

Benetos A, Gautier S, Ricard S, et al. 1996. Influence of angiotensin-converting enzyme and angiotensin II type 1 receptor gene polymorphisms on aortic stiffness in normotensive and hypertensive patients. Circulation 94:698-703.

Chew ST, Newman MF, White WD, et al. 2000. Preliminary report on the association of apolipoprotein E polymorphisms, with postoperative peak serum creatinine concentrations in cardiac surgical patients. Anesthesiology 93:325-31.

Danser AH, Schalekamp MA, Bax WA, et al. 1995. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 92:1387-8.

Heringlake M, Knappe M, Vargas Hein O, et al. 2006. Renal dysfunction according to the ADQI-RIFLE system and clinical practice patterns after cardiac surgery in Germany. Minerva Anestesiol 72:645-54.

Ilhan N, Seckin D, Ilhan N, et al. 2005. Association of angiotensin-converting enzyme and angiotensinogen T174M gene polymorphisms with coronary artery disease. Adv Mol Med 1:181-8.

Kontula K, Aalto-Setala K, Kuusi T, et al. 1990. Apolipoprotein E polymorphism determined by restriction enzyme analysis of DNA amplified by polymerase chain reaction: convenient alternative to phenotyping isoelectric focusing. Clin Chem 36:2087-92.

Kuitunen A, Vento A, Suojaranta-Ylinen R, Pettila V. 2006. Acute renal failure after cardiac surgery: evaluation of the RIFLE classification. Ann Thorac Surg 81:542-6.

Lassnigg A, Schmidlin D, Mouhieddine M, et al. 2005. Minimal changes of serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study. J Am Soc Nephrol 16:195-200.

Leatham E, Barley J, Redwood S, et al. 1994. Angiotensin-1 converting enzyme (ACE) polymorphism in patients presenting with myocardial infarction or unstable angina. J Hum Hypertens 8:635-8.

Losito A, Selvi A, Jeffery S, et al. 2000. Angiotensin-converting enzyme gene I/D polymorphism and carotid artery disease in renovascular hypertension. Am J Hypertens 13:128-33.

MacKensen GB, Swaminathan M, Ti LK, et al; Perioperative Outcomes Research Group; Cardiothoracic Anesthesiology Research Endeavors (C.A.R.E.) Investigators of the Duke Heart Center. 2004. Preliminary report on the interaction of apolipoprotein E polymorphism with aortic atherosclerosis and acute nephropathy after CABG. Ann Thorac Surg 78:520-6.

Miller SA, Dykes DD, Polesky HF. 1988. Simples salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1215.

Nakai K, Itoh C, Miura Y, et al. 1994. Deletion polymorphism of the angiotensin I-converting enzyme gene is associated with serum ACE concentration and increased risk for CAD in the Japanese. Circulation 90:2199-2202.

Raynolds MV, Bristow MR, Bush EW, et al. 1993. Angiotensin-converting enzyme DD genotype in patients with ischaemic or idiopathic dilated cardiomyopathy. Lancet 342:1073-5.

Rigat B, Hubert C, Corvol P, Soubrier F. 1992. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene. Nucl Acids Res 20:1433.

Sayed-Tabatabaei FA, Schut AF, Vasquez AA, et al. 2005. Angiotensin converting enzyme gene polymorphism and cardiovascular morbidity and mortality: the Rotterdam study. J Med Genet 42:26-30.

Schut AF, Bleumink GS, Stricker BH, et al. 2004. Angiotensin converting enzyme insertion/deletion polymorphism and the risk of heart failure in hypertensive subjects. Eur Heart J 25:2143-8.

Shanmugam V, Sell KW, Saha BK. 1993. Mistyping of ACE heterozygotes. PCR Methods Appl 3:120-1.

Stafford-Smith M, Podgoreanu M, Swaminathan M, et al; Perioperative Genetics and Safety Outcomes Study (PEGASUS) Investigative Team. 2005. Association of genetic polymorphisms with risk of renal injury after coronary bypass graft surgery. Am J Kidney Dis 45:519-30.

Tiret L, Bonnardeaux A, Poirier O, et al. 1994. Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. Lancet 344:910-3.

Tokgozoglu SL, Alikasifoglu M, Atalar E, et al. 1997. ACE gene polymorphism and risk and extent of ischemic heart disease among Turkish patients. Coronary Artery Dis 8:137-41.

Volzke H, Engel J, Kleine V, et al. 2002. Angiotensin I- converting enzyme insertion/deletion polymorphism and cardiac mortality and morbidity after coronary artery bypass graft surgery. Chest 122:31-6.