NADPH Oxidase p22phox and Catalase Gene Variants Are Associated with Biomarkers of Oxidative Stress and Adverse Outcomes in Acute Renal Failure

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Reactive oxygen species are important mediators of injury in acute renal failure (ARF). Although polymorphisms that affect key pro- and antioxidant enzymes might alter the susceptibility to oxidative stress–mediated injury, the use of genetic epidemiology for the study of oxidative stress–related genes has received little attention in ARF. The relationship of single-nucleotide polymorphisms in the coding region (C to T substitution at position +242) of the pro-oxidant enzyme NADPH oxidase p22phox subunit gene and in the promoter region (C to T substitution at position −262) of the antioxidant enzyme catalase gene to adverse clinical outcomes was evaluated prospectively in a cohort of 200 hospitalized patients with established ARF of mixed cause and severity. Genomic DNA was extracted from peripheral blood leukocytes and analyzed with a restriction fragment length polymorphism PCR method. Genotype–phenotype associations were characterized by measuring circulating nitrotyrosine and catalase activity. Observed and expected genotype frequencies were not significantly different, and overall baseline characteristics were not significantly different according to the various genotype groups. A genotype–phenotype association was demonstrable between the NADPH oxidase p22phox genotypes and plasma nitrotyrosine level ($P = 0.06$), as well as between the catalase genotypes and whole-blood catalase activity ($P < 0.001$). Compared with the NADPH oxidase p22phox CC genotype group, the T-allele group had a higher cumulative probability of remaining hospitalized ($P = 0.03$). Compared with the NADPH oxidase p22phox CC genotype, the T-allele carrier state was associated with 2.1-fold higher odds for dialysis requirement or hospital death ($P = 0.01$). This association persisted with 2.0- to 2.2-fold higher odds for this composite outcome after adjustment for race; gender; age; and the Acute Physiology and Chronic Health Evaluation II score ($P = 0.03$), the Multiple Organ Failure score ($P = 0.01$), or presence of sepsis ($P = 0.02$). The polymorphism in the gene that encodes the NADPH oxidase p22phox subunit at position +242 is associated with dialysis requirement or hospital death among patients with ARF. Larger studies are needed to confirm these relationships.

J Am Soc Nephrol 18: 255–263, 2007. doi: 10.1681/ASN.2006070806

Under normal physiologic conditions, a homeostatic balance exists between the formation of reactive oxygen species (ROS) and their removal by endogenous antioxidant compounds (1). Oxidative stress occurs when this balance is disrupted by excessive production of ROS such as superoxide and/or by inadequate antioxidant defenses, such as suboptimal catalase level and/or activity (2). Both of these imbalances can occur in acute renal failure (ARF). Indeed, in recent years, considerable evidence has incriminated ROS in the pathogenesis of both ischemic and nephrotoxic kidney injury (3–7).

NADPH oxidase is a membrane-associated enzyme that catalyzes the production of superoxide and is highly expressed in neutrophils and endothelial cells (8). NADPH oxidase has several subunits, including p22phox and gp91phox, which are electron-transfer proteins (9). Whereas the first line of defense against the generation of superoxide is achieved by the enzyme superoxide dismutase resulting in the formation of hydrogen peroxide, catalase, an antioxidant enzyme, further metabolizes this molecule, thereby limiting oxidative stress–mediated injury.

In ARF, there are several potential sources of ROS, including the mitochondrial respiratory electron transport chain, xanthine oxidase activation as a result of ischemia/reperfusion, and endothelial- and neutrophil-associated respiratory burst through activation of NADPH oxidase (2,10). Genetic variation in the expression of these two key pro- and antioxidant enzymes might account partly for the interindividual variability that is observed in the manifestation of acute organ injury including ARF (10,11). A polymorphism in the gene that encodes the NADPH oxidase p22phox subunit at position +242, which consists of a C to T single-nucleotide substitution, has been well described and is associated

Received July 30, 2006. Accepted October 9, 2006.

Published online ahead of print. Publication date available at www.jasn.org.

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ISSN: 1046-6673/1801-0255

with variations in gene expression (12–15). Similarly, a functional polymorphism in the human catalase promoter, consisting of a C to T substitution at position −262, confers variation in basal expression and enzyme activity (16–18). In this study, we examined (*1*) whether polymorphisms in the NADPH oxidase (p22phox +242 C to T) and catalase (−262 C to T) genes are associated with circulating biomarkers of oxidative stress in a large cohort of patients with ARF, as measured by plasma nitrotyrosine (a by-product of superoxide and nitric oxide generation) (19), and whole-blood catalase activity, and (*2*) whether these polymorphisms are associated with adverse clinical outcomes.

Materials and Methods

Study Design

This was a prospective cohort study of hospitalized patients with ARF, which was conducted between November 2003 and January 2006 at two tertiary care hospitals located in Boston, MA. All consecutive hospitalized adult patients with ARF, for whom nephrology consultation was requested, were eligible for enrollment. ARF was defined as an incremental increase in serum creatinine by 0.5, 1.0, or 1.5 mg/dl from a baseline level of ≤1.9, 2.0 to 4.9, and ≥5.0 mg/dl, respectively, as described previously (20,21). Exclusion criteria were age <18 yr, pregnancy, chronic dialysis therapy, receipt of an organ transplant within the previous year, and presence of acute obstructive uropathy. Written informed consent was obtained from all participants or their next of kin. The institutional review board of each participating center approved the study protocol.

Data Collection

Medical records of study participants were reviewed prospectively to retrieve hospitalization data, including baseline demographic characteristics, coexisting conditions, and renal variables including serial serum creatinine values and presence of oliguria (as defined by urine output <400 ml/d). The prevalence of advanced chronic kidney disease (CKD) was assessed on the basis of a premorbid GFR of <30 ml/min per 1.73 m^2, as calculated by the Modification of Diet in Renal Disease (MDRD) study equation (22). This more specific definition was adopted for the following reason. Patients with markers of kidney damage such as proteinuria or abnormalities on imaging studies or on kidney biopsy have CKD regardless of the GFR estimate. However, there is some uncertainty as to whether patients who do not have markers of kidney damage and have a GFR estimate just below 60 ml/min per 1.73 m^2 in fact have CKD. Some of these patients may have a measured GFR >60 ml/min per 1.73 m^2 and therefore would not be considered to have CKD (23). Therefore, using a threshold of 30 ml/min per 1.73 m^2 ensured the specificity of a CKD designation. At the time of enrollment, two severity-of-illness scores were calculated: The Acute Physiology and Chronic Health Evaluation II (APACHE II) score (24) and the Multiple Organ Failure (MOF) score (25). The presence of sepsis was ascertained using the systemic inflammatory response syndrome criteria (26). Outcomes of interest were hospital length of stay, dialysis requirement, hospital death, and a composite outcome of dialysis requirement or hospital death.

Blood Sampling and DNA Extraction

At the time of enrollment, 15 ml of EDTA-anticoagulated whole blood was collected, plasma was separated, and the rest of the whole-blood sample was aliquotted and stored at −80°C for subsequent DNA extraction. Genomic DNA was extracted using a spin column method according to the manufacturer's instructions (Qiagen, Valencia, CA). Final DNA concentrations were set at 50 to 200 ng/ml and determined by minigel electrophoresis.

NADPH Oxidase p22phox +242 (C to T) Genotyping

The C to T substitution of the +242 polymorphism produces an RsaI digestion site. In brief, for this RsaI restriction fragment length polymorphism, a DNA fragment that contained the C242T polymorphic site of the gene that encodes for the p22phox subunit was amplified from genomic DNA by PCR with a sense primer 5′-TGC TTG TGG GTA AAC CAA GGC CGG TG-3′ and an antisense primer 5′-AAC ACT GAG GTA AGT GGG GGT GGC TCC TGT-3′, using an initial denaturation at 96°C for 2 min, followed by 30 cycles of amplification in a three-step reaction that consisted of denaturation at 94°C for 30 s, annealing at 56°C for 60 s, and extension at 72°C for 60 s. The PCR product then was digested with RsaI at 37°C for 3 h. The RsaI digestive products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide, and the bands were visualized under ultraviolet light. Digested PCR products yielded 348-bp bands in CC homozygotes, 188- and 160-bp bands in TT homozygotes, and all three bands in heterozygotes (12). Figure 1 (left panel) displays a representative DNA gel of the various gene polymorphisms.

Catalase −262 (C to T) Genotyping

The C to T substitution of the −262 gene polymorphism abolishes the XmaI restriction enzyme digestion site. In brief, PCR was performed from genomic DNA using a touchdown protocol (GC genomic kit; Clontech, Palo Alto, CA). The sense primer 5′-TAA GAG CTG AGA AAG CAT AGC T-3′ was used together with the antisense primer 5′-AGA GCC TCG CCC CGC CGG ACC G-3′ to incorporate a mutation to abolish an existing XmaI site. The PCR amplification reaction was performed by initial denaturation at 94°C for 3 min, followed by 19 cycles of amplification in a three-step reaction, each consisting of denaturation at 94°C for 60 s, annealing at 92°C for 30 s (with −0.5°C per cycle), and extension at 70°C for 40 s. The PCR product subsequently was digested with XmaI for 2 h and analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide, and the bands were visualized under ultraviolet light. The −262 T variant yields an undigested product of 185 bp, relative to the digested −262 C variant, which yields a 155-bp fragment (16). For all of the genotyping, two blinded investigators who were unaware of the patients' clinical characteristics determined the genotypes. Figure 1 (right panel) displays a representative DNA gel of the different genotypes.

Measurement of Nitrotyrosine

Nitrotyrosine was measured in plasma using a sandwich ELISA (Oxis International, Portland, OR). In this procedure, nitrotyrosine was

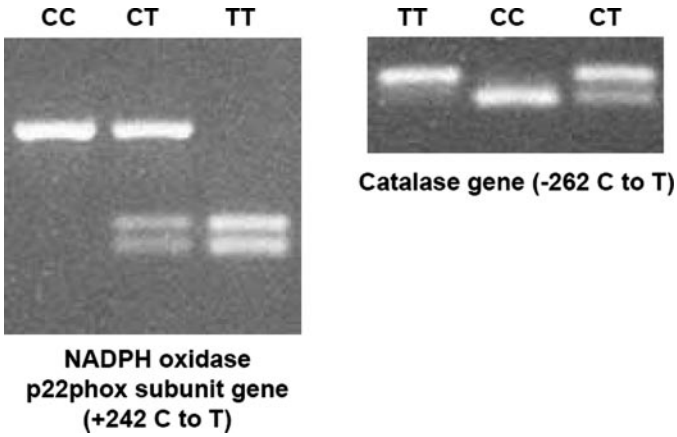

Figure 1. Representative DNA gel of the various NADPH oxidase p22phox subunit +242 C to T (left) and catalase −262 C to T (right) gene polymorphisms.

captured by a solid-phase mAb and detected with a biotin-labeled goat polyclonal anti-nitrotyrosine. After a wash to remove unbound material, a streptavidin peroxidase conjugate was added to bind to the biotinylated antibody. After washing away unbound conjugate, a substrate, tetramethylbenzidine, was added to allow color development. Color development was stopped, and the intensity of the color (optical density) was measured by a spectrophotometer (Beckman Coulter, Fullerton, CA) set at 450 nm. Results are expressed in nM and are adjusted for the white blood cell count. The assay's lower limit of detection is 2 nM. The intra-assay coefficient of variation is 2.3%, and the interassay coefficient of variation is 11.2%.

Measurement of Catalase Activity

Catalase activity was measured in whole blood using a commercially available chromophore-based reagent spectrophotometric assay (Oxis International). In this two-step procedure, the rate of dismutation of hydrogen peroxide (H_2O_2) to water and molecular oxygen is proportional to the concentration of catalase. In brief, the whole-blood samples were diluted at 1:1000 and incubated for 1 min in the presence of a known concentration of H_2O_2. The reaction then was quenched with sodium azide. The remaining amount of H_2O_2 was determined by the oxidative coupling reaction of 4-aminophenazone and 3,5-dichloro-2-hydroxybenzenesulfonic acid, catalyzed by horseradish peroxidase (27). The resulting quinoneimine dye was measured by a spectrophotometer (Beckman Coulter) set at 520 nm. Results are expressed in U/ml and are adjusted for the hemoglobin level and white blood cell count. The lower limit of detection is 1.71 U/ml. The intra-assay coefficient of variation is 3.1%, and the interassay coefficient of variation is 3.4%.

Statistical Analyses

The genotype frequencies were tested for Hardy Weinberg equilibrium using a standard χ^2 test. Comparisons between genotype groups were made by the Kruskal-Wallis test and the two-tailed Mann Whitney test for continuous variables and by Fisher exact test for categorical variables.

A general linear model was used to estimate the mean plasma nitrotyrosine level and whole-blood catalase activity in each genotype category, adjusting for white blood cell count and/or hemoglobin level. For this analysis, the results are displayed as means ± SE.

Kaplan-Meier curves were used to compare hospital length of stay within the NADPH oxidase p22phox and catalase genotype groups, respectively. In this analysis, death was censored, and the log-rank statistic was used to test differences between groups. Robust regression analysis also was used to explore the relationship of the NADPH oxidase p22phox and catalase genotypes with hospital length of stay (28).

Logistic regression analysis was used to examine the association of the NADPH oxidase p22phox and catalase genotypes with the composite outcome of dialysis requirement or hospital death. This composite end point was chosen because it takes into consideration survival bias for dialysis requirement. The models were adjusted for several baseline covariates, including race, gender, age, APACHE II score, MOF score, and presence of sepsis.

Results are expressed as means (±SD) or percentages. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using SAS (version 9.1; SAS Institute, Cary, NC).

Results

Distribution of NADPH Oxidase p22phox and Catalase Genotypes

Table 1 displays the NADPH oxidase p22phox +242 and catalase −262 genotype distributions in the study population. Observed and expected genotype frequencies were not significantly different, and the allele frequencies for each polymorphism met the Hardy Weinberg equilibrium ($P > 0.05$).

Association of NADPH Oxidase p22phox and Catalase Genotypes with Circulating Pro- and Antioxidant Biomarkers

As shown in Figure 2, patients with the NADPH oxidase p22phox TT genotype had higher plasma nitrotyrosine levels as compared with those with the CC genotype ($P = 0.06$). Similarly, as shown in Figure 3, patients with the catalase CT and TT genotypes had lower whole-blood catalase activity as compared with those with the CC genotype ($P < 0.01$).

Baseline Characteristics of the Cohort Stratified by the NADPH Oxidase p22phox and Catalase Genotypes

Table 2 displays the baseline characteristics of the cohort according to the various NADPH oxidase p22phox genotypes. In brief, no significant age, gender, and race differences were observed among the genotype groups. In addition, the remaining baseline characteristics did not differ significantly among the genotype groups.

Table 3 displays the baseline characteristics of the cohort according to the various catalase genotypes. No significant differences in the baseline characteristics were demonstrable among the catalase genotype groups.

Table 1. Observed and expected genotype distribution

Polymorphism	Genotype Distribution			χ^2 Statistic	*P*
	CC	CT	TT		
NADPH oxidase p22phox +242 (C to T)					
observed	85 (42%)	82 (41%)	33 (17%)	2.40	0.12
expected	79 (39%)	93 (47%)	28 (14%)		
Catalase −262 (C to T)					
observed	112 (56%)	74 (37%)	14 (7%)	0.14	0.71
expected	111 (55%)	76 (38%)	13 (7%)		

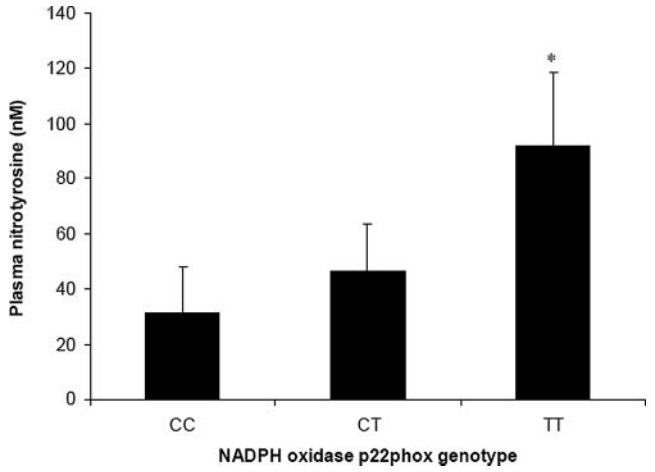

Figure 2. Plasma nitrotyrosine level stratified by the NADPH oxidase p22phox genotypes. The data are presented as mean (SE) values that are adjusted for the white blood cell count. *P = 0.06 *versus* CC genotype.

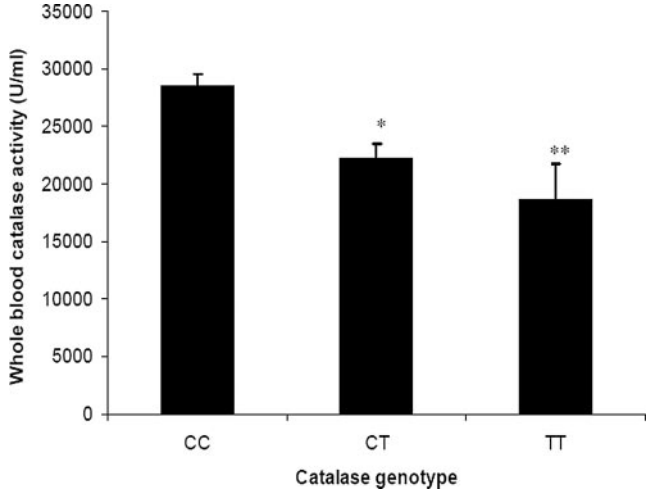

Figure 3. Whole-blood catalase activity stratified by the catalase genotypes. The data are presented as mean (SE) values that are adjusted for the white blood cell count and hemoglobin level. *P = 0.0004 *versus* CC genotype; **P = 0.003 *versus* CC genotype.

Association of NADPH Oxidase p22phox and Catalase Genotypes with Hospital Length of Stay

As shown in Table 4, patients with the NADPH oxidase p22phox CT and TT genotypes had a nonsignificant trend toward prolonged hospital length of stay as compared with those with the CC genotype (P = 0.06 for trend). When combined, the NADPH oxidase p22phox T-allele group had a significantly more prolonged hospital length of stay as compared with the CC genotype group (17 *versus* 11 d; P = 0.02). As shown in Table 5, there was no association between the catalase genotypes and hospital length of stay.

Kaplan-Meier survival analysis demonstrated that compared with the NADPH oxidase p22phox CC genotype group, the T-allele group had a higher cumulative probability of remaining hospitalized (P = 0.03 by log rank test; Figure 4). However, the association between the NADPH oxidase p22phox T allele and hospital length of stay became nonsignificant after adjustment for race, gender, and APACHE II score (2.1 additional days; 95% confidence interval −0.8 to 5.0; P = 0.16). Of note, there was no association between plasma nitrotyrosine level and hospital length of stay (data not shown).

Association of NADPH Oxidase p22phox and Catalase Genotypes with Dialysis Requirement and the Composite Outcome of Dialysis Requirement or Hospital Death

As shown in Table 4, patients with the NADPH oxidase p22phox CT and TT genotypes had a nonsignificant trend toward higher dialysis requirement as compared with those with the CC genotype (P = 0.05 for trend), and when combined, the NADPH oxidase p22phox T-allele group had a significantly higher dialysis requirement compared with the CC genotype group (45 *versus* 31%; P = 0.04). Of note, plasma nitrotyrosine level was not significantly different among patients who required dialysis, as compared with those who did not (41.1 ± 11.6 *versus* 51.6 ± 16.4 nM; P = 0.18).

On univariate analysis (Table 6), compared with the NADPH oxidase p22phox CC genotype, the T-allele carrier state was associated with 2.1-fold higher odds for dialysis requirement or hospital death. This association persisted with 2.0- to 2.2-fold higher odds for this composite outcome after adjustment for race; gender; age; and the APACHE II score, the MOF score, or presence of sepsis.

Compared with the CC genotype group, there was no association between the catalase −262 T-allele group and dialysis requirement (Table 5) or the composite outcome of dialysis requirement or hospital death (Table 6). Of note, preexisting CKD was not associated with the composite outcome of dialysis requirement or hospital death (odds ratio 1.13; 95% confidence interval 0.61 to 2.08), and in the multivariate analysis, the forced inclusion of CKD in the model did not alter significantly the association of the NADPH oxidase p22phox or catalase genotypes with the composite outcome (Table 6).

Discussion

Our study evaluated the relationship of genetic variants in the coding region (at position +242) of the pro-oxidant enzyme NADPH oxidase p22phox subunit gene and the promoter region (at position −262) of the antioxidant enzyme catalase gene to adverse clinical outcomes in a large cohort of hospitalized patients with established ARF of mixed cause and severity. The observed and expected genotype frequencies were not significantly different, thereby fulfilling the Hardy Weinberg equilibrium. There were no race, gender, or age differences within genotype groups. The NADPH oxidase p22phox genotypes influenced baseline plasma nitrotyrosine levels, a byproduct of superoxide and nitric oxide generation and a footprint of peroxynitrite formation. Similarly, the catalase genotypes influenced baseline whole-blood catalase activity. The NADPH oxidase p22phox T-allele carrier state (CT and TT genotypes) also was associated with prolonged hospital length of stay and 2.0-fold higher odds for dialysis requirement or hospital death.

Table 2. Baseline characteristics of patients with ARF by NADPH oxidase p22phox genotypes[a]

Characteristic	NADPH Oxidase p22phox Genotypes			*P*
	CC (n = 85)	CT (n = 82)	TT (n = 33)	
Age (yr)	64 (16)	66 (13)	66 (17)	0.69
Male gender (%)	54	59	45	0.45
Race (%)				0.69
white	88	94	88	
black	6	4	9	
other	6	2	3	
Admission type (%)				0.77
medical	73	70	67	
surgical	27	30	33	
Contributing cause of ARF (%)				0.80
ischemic	26	29	18	
nephrotoxic	18	15	18	
ischemic and nephrotoxic	7	8	9	
septic	6	11	15	
multifactorial/other	43	37	40	
Coexisting conditions (%)				
diabetes	44	46	30	0.27
heart failure	11	17	15	0.49
cirrhosis	8	9	6	0.98
chronic lung disease	21	11	15	0.20
CKD	27	29	30	0.93
ICU setting (%)	67	76	79	0.30
APACHE II score	15 (6)	16 (6)	17 (7)	0.45
MOF score ≥2 (%)	25	29	21	0.66
Sepsis (%)	38	51	36	0.15
Serum creatinine (mg/dl)				
baseline value	1.6 (0.6)	1.6 (0.6)	1.5 (0.7)	0.56
enrollment value	3.8 (2.3)	3.6 (2.0)	3.6 (1.3)	0.57
peak value	4.4 (2.5)	4.8 (3.7)	3.9 (1.4)	0.80
discharge value	2.5 (1.5)	2.7 (2.1)	2.2 (1.3)	0.56
Oliguria (%)	20	21	18	0.95

[a]Data are means (SD) or percentages. APACHE II, Acute Physiology and Chronic Health Evaluation II; ARF, acute renal failure; CKD, chronic kidney disease; MOF, Multiple Organ Failure.

ROS are important mediators of cellular injury after organ ischemia and reperfusion (3–7). Although polymorphisms that affect key pro- and antioxidant enzymes might alter the susceptibility to oxidative stress–mediated injury, the use of genetic epidemiology for the study of oxidative stress–related genes has received little attention in ARF (10,11). Whereas a number of polymorphisms of oxidant stress–related genes might be functionally relevant in human disease (29), we chose to evaluate the genetic variants of two key leukocyte- and endothelium-associated enzymes: NADPH oxidase and catalase. Given its importance in generating superoxide during the respiratory burst of neutrophils, one might anticipate that polymorphisms that disrupt the function of NADPH oxidase would alter oxidative stress–mediated injury in conditions such as ARF. Several polymorphisms in the gene that encodes the NADPH oxidase p22phox subunit have been described. Among them, the C to T single-nucleotide substitution within exon 4 at position +242 results in an amino acid substitution at position 72 (histidine to tyrosine) and modulates enzyme activity through heme-binding affinity (12). The NADPH oxidase p22phox +242 T-allele carrier has been associated with reduced respiratory burst activity in human endothelial cells (13) and neutrophils (15). However, clinical studies that have examined the influence of this polymorphism on circulating biomarkers of lipid peroxidation have yielded conflicting results (30–32). In this study, the NADPH oxidase p22phox +242 T allele was associated unexpectedly with higher plasma nitrotyrosine levels. This contradictory finding might be due in part to the fact that nitrotyrosine is a circulating biomarker of both superoxide and nitric oxide generation. We can only speculate as to whether linkage disequilibrium also might exist between this NADPH oxidase p22phox polymorphism and the gene that

Table 3. Baseline characteristics of patients with ARF by catalase genotypes[a]

Characteristic	Catalase Genotypes			*P*
	CC (n = 112)	CT (n = 74)	TT (n = 14)	
Age (yr)	66 (14)	63 (17)	66 (12)	0.58
Male gender (%)	54	53	64	0.77
Race (%)				0.82
white	88	92	100	
black	7	4	0	
other	5	4	0	
Admission type (%)				0.60
medical	73	66	71	
surgical	27	34	29	
Contributing cause of ARF (%)				0.24
ischemic	27	26	22	
nephrotoxic	18	15	14	
ischemic and nephrotoxic	7	11	0	
septic	5	16	7	
multifactorial/other	43	32	57	
Coexisting conditions (%)				
diabetes	40	45	54	0.58
heart failure	17	9	15	0.30
cirrhosis	11	5	0	0.81
chronic lung disease	15	16	23	0.64
chronic kidney disease	29	27	31	0.88
ICU setting (%)	71	73	77	0.97
APACHE II score	16 (6)	16 (7)	15 (4)	0.70
MOF score ≥2 (%)	22	31	29	0.39
Sepsis (%)	42	43	50	0.83
Serum creatinine (mg/dl)				
baseline value	1.5 (0.6)	1.6 (0.6)	1.7 (0.8)	0.90
enrollment value	3.7 (2.2)	3.7 (1.9)	3.5 (1.9)	0.86
peak value	4.5 (3.4)	4.5 (2.3)	4.4 (2.2)	0.74
discharge value	2.5 (1.7)	2.6 (1.7)	2.7 (1.8)	0.84
Oliguria (%)	18	23	21	0.69

[a]Data are means (SD) or percentages.

Table 4. Outcomes of patients with ARF by NADPH oxidase p22phox genotypes[a]

Outcome Variable	NADPH Oxidase p22phox Genotypes			*P*
	CC (n = 85)	CT (n = 82)	TT (n = 33)	
Dialysis requirement (%)	31	49	36	0.05
Dialysis dependence at hospital discharge (%)	8	16	15	0.42
Hospital length of stay (d; median [quartile])	11 (8 to 22)	17 (11 to 29)	17 (9 to 27)	0.06
Hospital death (%)	24	24	27	0.90
Composite end point (%)	36	59	45	0.02

[a]The composite end point is dialysis requirement or hospital death.

encodes for one of the three nitric oxide synthase isoforms, particularly inducible nitric oxide synthase, an enzyme that typically is induced by acute proinflammatory stimuli that occur in the context of ARF (10).

In our study, patients with prerenal azotemia were not excluded *a priori*. The inclusion criteria purposefully were liberal, to have a broad representation of ARF with mixed cause and severity. At the time of enrollment, 70% of patients had a blood

Table 5. Outcomes of patients with ARF by catalase genotypes[a]

Outcome variable	Catalase Genotypes			*P*
	CC (n = 112)	CT (n = 74)	TT (n = 14)	
Dialysis requirement (%)	36	45	36	0.48
Dialysis dependency at hospital discharge (%)	9	15	29	0.61
Hospital length of stay (d; median [quartile])	15 (9 to 27)	12 (8 to 26)	18 (11 to 23)	0.64
Hospital death (%)	26	24	14	0.70
Composite end point (%)	46	49	43	0.93

[a]The composite end point is dialysis requirement or hospital death.

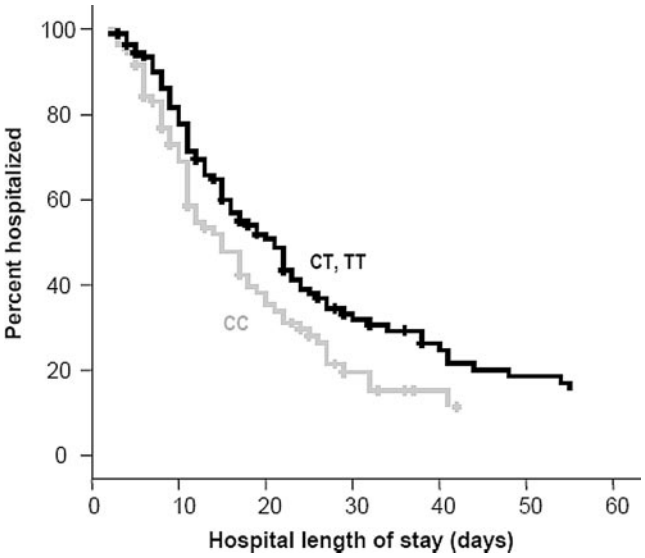

Figure 4. Kaplan-Meier analysis for the cumulative percentage of patients who had acute renal failure and remained hospitalized according to the NADPH oxidase p22phox genotype strata. CC genotype (gray line) *versus* T-allele or CT/TT genotypes (solid line). $P = 0.03$ by log rank test.

urea nitrogen–to–creatinine ratio of <20, 52% had a fractional excretion of sodium of >1%, and 75% had granular casts on the urine sediment, suggesting that the majority of patients had structural tubular disease. In addition, patients with preexisting CKD, as defined by GFR estimates of <30 ml/min per 1.73 m^2, also were included. By using the criteria that were developed originally by Hou *et al.* (20), we were able to capture a broader spectrum of disease including acute on chronic kidney disease. In the United States, patients who have ARF are older and have a large burden of comorbid diseases, with a 30% prevalence of CKD (33,34), arguing for the importance of this preexisting condition in the setting of ARF.

In our study, although a genotype–phenotype association was demonstrable between the catalase genotypes and whole-blood catalase activity, the lack of association between the catalase polymorphism and adverse outcomes is intriguing and merits further discussion. Very few studies have attempted to assign functional relevance of the catalase −262 gene polymorphism with the enzyme level or activity (16,18). Indeed, whereas one study observed that the T allele confers higher enzyme levels (16), a more recent report demonstrated that the T allele is associated with lower catalase activity (18). Although the reason for this discrepancy remains elusive and requires further study, immunologically reactive but enzymatically inactive catalase might account in part for this difference. Finally, genetic variants in other intracellular antioxidant enzymes such as glutathione peroxidase might be more important determinants of intracellular detoxification of ROS, thereby minimizing oxidative stress. Indeed, in mammalian cells, glutathione and the glutathione peroxidases constitute the principal intracellular antioxidant defense system, whereby this enzyme uses glutathione to reduce H_2O_2 to water and lipid peroxides to their respective alcohols. Catalase, by contrast, only degrades H_2O_2 to water. We can only speculate as to whether catalase is a less efficient antioxidant enzyme, as compared with glutathione peroxidase. Consequently, although a catalase genotype–phenotype association could be demonstrated in this study, this antioxidant enzyme gene might not be as relevant with respect to clinical outcomes in this cohort. This hypothesis warrants further testing by exploring associations between functional polymorphisms in the glutathione peroxidase gene with adverse clinical outcomes in patients with ARF. Experimental studies also have suggested that the role of catalase in antioxidant defense depends on the type of tissue and the model of oxidant-mediated injury. For example, mice that lack catalase develop normally but exhibit a retarded rate in decomposing H_2O_2 in the liver and lungs (35). Furthermore, experimental gene therapy that is aimed at overexpressing catalase does not protect against ischemia-reperfusion lung injury (36). These data argue that catalase might not play a critical role in combating oxidative stress.

To our knowledge, this is the first study to test whether gene polymorphisms of pro-and antioxidant enzymes are associated with adverse clinical outcomes in patients with ARF. The heterogeneity of case mix and severity of illness in this cohort are offset by the selective inclusion of patients who had more severe ARF that required formal consultation of the clinical nephrology service. Although the sample size is substantial for a hospital-based study, it remains relatively small for genetic epidemiologic purposes. The study patient population was 90% white, reducing the diversity and dampening the potential impact of evaluating race and/or ethnic admixture on genotype prevalence. It is worth noting that although our study yielded no ethnic differences among genotype groups, when the analyses were confined to

Table 6. Association of NADPH oxidase p22phox and catalase genotypes with composite outcome of dialysis requirement or hospital death[a]

Predictor Variable	OR	95% CI	*P*
NADPH oxidase p22phox +242 T allele (*versus* CC genotype)			
unadjusted	2.11	1.19 to 3.75	0.01
adjusted for race, gender, and APACHE II score	2.12	1.10 to 4.10	0.03
adjusted for race, gender, age, and MOF score	2.20	1.17 to 4.11	0.01
adjusted for race, gender, age, and sepsis	2.04	1.11 to 3.74	0.02
adjusted for race, gender, age, APACHE II score, and CKD	2.17	1.12 to 4.22	0.02
Catalase −262 T allele (*versus* CC genotype)			
unadjusted	1.05	0.60 to 1.84	0.86
adjusted for race, gender, and APACHE II score	0.99	0.52 to 1.89	0.98
adjusted for race, gender, age, and MOF score	0.95	0.52 to 1.76	0.88
adjusted for race, gender, age, and sepsis	1.07	0.59 to 1.93	0.84
adjusted for race, gender, age, APACHE II score, and CKD	0.99	0.52 to 1.90	0.98

[a]CI confidence interval; OR, odds ratio.

white patients, the association between the NADPH oxidase p22phox T allele and the composite outcome did not alter the point estimates that were obtained in the original analysis (data not shown). Furthermore, the baseline covariates that were used in the analyses included the APACHE II score, the MOF score, and sepsis and incorporate several demographic, physiologic, and laboratory variables that are associated individually with the composite end point. The composite end point was chosen because it accounts for survival bias when dialysis requirement is being assessed as an outcome measure. The initiation of dialysis in ARF traditionally has been influenced by many factors, including physician preferences, thereby reflecting clinical practice biases and challenging this outcome measure when taken in isolation. Of note, the NADPH oxidase p22phox T allele was shown previously to confer protection against kidney failure in nondiabetic Japanese individuals (37). Finally, the demonstrable genotype–phenotype associations lend more credibility to the observed association between the NADPH oxidase p22phox genotypes and the composite end point of dialysis requirement or hospital death.

Conclusion

This study supports the hypothesis that oxidative stress–related gene polymorphisms predict adverse outcomes in hospitalized patients with ARF. Dialysis requirement or hospital mortality was significantly higher in patients with the +242 C to T polymorphism in the coding region of the NADPH oxidase p22phox subunit gene, which was associated with higher levels of plasma nitrotyrosine. These observations support previous knowledge that pro- and anti-inflammatory cytokine gene polymorphisms might play a pivotal role in the regulation of host inflammatory responses and contribute to greater morbidity and mortality in patients with ARF (38). Larger studies are needed to confirm these relationships, because there is considerable utility in the identification of oxidative stress–related genetic susceptibility markers that might help to stratify patients who have ARF and are at greater risk for adverse outcomes and help to determine those who might benefit from antioxidant therapy.

Acknowledgments

This study was supported by grants from the National Institutes of Health (DK065102 to B.L.J.) and the Natalie V. Zucker Research Center for Women Scholars at Tufts University School of Medicine (to M.C.P.).

Disclosures

None.

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