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Polymorphisms in the myeloperoxidase gene locus are associated with acute kidney injury-related outcomes

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Abstract

Myeloperoxidase (MPO) is a lysosomal enzyme that may be involved in oxidative stress-mediated kidney injury. Using a 2-step approach, we measured the association of 4 polymorphisms across the length of the MPO gene with systemic markers of oxidative stress; plasma MPO and urinary 15-F_{2t}-isoprostane levels. Adverse outcomes were measured in a primary cohort of 262 adults hospitalized with acute kidney injury, and a secondary cohort of 277 adults undergoing cardiac surgery with cardiopulmonary bypass and at-risk for postoperative acute kidney injury. Dominant and haplotype multivariable logistic regression analyses found a genotype-phenotype association in the primary cohort between rs2243828, rs7208693, rs2071409, and rs2759 MPO polymorphisms and both markers of oxidative stress. In adjusted analyses, all 4 polymorphic allele groups had 2-3-fold higher odds for composite outcomes of dialysis or in-hospital death or a composite of dialysis, assisted mechanical ventilation or inhospital death. The MPO T-G-A-T haplotype copy-number was associated with lower plasma MPO levels and lower adjusted odds for the composite outcomes. Significant but less consistent associations were found in the secondary cohort. In summary, our 2-step genetic association study identified several polymorphisms spanning the entire MPO gene locus and a common haplotype marker for patients at-risk for acute kidney injury.

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DISCLOSURE None.

Keywords

acute kidney injury; cardiac surgery; dialysis; gene polymorphism; haplotype; mechanical ventilation; mortality; *MPO*

INTRODUCTION

Acute kidney injury (AKI) is a common occurrence in the hospital setting, and confers an increased risk of mortality, as well as heightened resource consumption including, prolonged hospital length of stay and increased costs^{1,2}. Although many factors have been identified as predictors of development and evolution of AKI³⁻⁹, there remains significant unexplained variability in the prediction of adverse kidney-related outcomes^{10,11}. In recent years, polymorphisms in several candidate genes have been explored as determinants of disease severity and adverse outcomes in patients with AKI¹²⁻¹⁹.

Myeloperoxidase (MPO) is a heme-containing enzyme belonging to the XPO subfamily of peroxidases. It is abundant in neutrophils and catalyzes the hydrogen peroxide dependent formation of hypochlorous acid, a potent oxidant²⁰. Neutrophils store MPO in cytoplasmic azurophilic granules²¹. Upon cellular activation and degranulation, MPO is delivered into phagolysosomes where it is required for killing of phagocytosed bacteria²². Activated neutrophils also release granule contents in the extracellular space. Elevated plasma MPO levels have been associated with a variety of clinical conditions including ischemia-reperfusion injury, acute pancreatitis, vasculitis, atherosclerosis, cancer and neurodegenerative disorders²³⁻²⁵.

The human *MPO* gene is located on chromosome 17q23.1²⁶. Several polymorphisms have been described in the *MPO* gene locus, some of which have been linked to systemic markers of oxidative stress in patients with chronic kidney disease^{27,28}. Genetic variation in the expression of this key enzyme might thus account for the inter-individual variability observed in the severity and outcome of AKI²⁹. In the present report, we first examine the association of 4 polymorphisms and haplotypes in the *MPO* gene locus with systemic markers of oxidative stress, measures of disease severity, and adverse clinical outcomes in hospitalized adults with an established diagnosis of AKI (*primary cohort*). Next, we examine the association of the same polymorphisms with adverse outcomes in a second cohort of adults at-risk for AKI undergoing cardiac surgery with cardiopulmonary bypass (*secondary cohort*).

RESULTS

Characteristics of Participants in the Primary and Secondary Cohorts

In the *primary cohort*, between November 2003 and February 2010, a total of 264 participants were enrolled of whom 2 were excluded due to study ineligibility and lack of genomic DNA sampling. The present analysis includes 262 participants who underwent genotyping analyses. At enrollment, mean age was 66 years, 91% were white, and 53% were men. Mean APACHE II score was 20, and 73% were in the intensive care unit. 137 (53%) subjects had stage-3 AKI, as defined by the AKI network criteria. 24% required mechanical

ventilation, 39% required dialysis, 22% died in-hospital, and 47% experienced the composite outcome of dialysis requirement or in-hospital death.

In the *secondary cohort*, between January 2004 year and August 2011, a total of 277 study participants were enrolled. In brief, mean age was 68 years, 95% were white, 72% were men, 37% had a history of heart failure, and preoperative mean serum creatinine was 1.1 mg/dL. Thirty-one percent underwent elective cardiac surgery with 51% requiring valve replacement. Mean aortic cross-clamp and cardiopulmonary bypass (CPB) perfusion time were 91 and 118 minutes, respectively. On post-operative day 1, mean APACHE II score was 13, initial median mechanical ventilation was 9 hours, and stage-2 or 3 AKI developed in 7 subjects (2.5%).

Characteristics of the Primary and Secondary Cohorts Stratified by *MPO* Gene Polymorphisms

In both the *primary* and *secondary cohorts*, tests for Hardy-Weinberg equilibrium showed no deviation from expected frequencies (Table 1). All 4 *MPO* gene polymorphisms had minor allele frequencies of greater than 5%.

The characteristics of the *primary cohort* stratified by the 4 *MPO* gene polymorphisms are shown in Table 2. In brief, demographic characteristics, co-morbidities and disease severity measures did not significantly differ across genotypes, with a few notable exceptions. The *MPO* rs2243828 minor C-allele (TC/CC genotype) group had a higher mean APACHE II score ($P=0.002$) and lower mean urine output ($P=0.05$) compared with the TT genotype group; the *MPO* rs7208693 minor T-allele group (GT/TT genotype) had a higher prevalence of cirrhosis ($P=0.02$) and more advanced stages of AKI ($P=0.02$), and a higher mean APACHE II score ($P<0.001$) compared with the GG genotype group; the *MPO* rs2071409 minor C-allele group (AC/CC genotype) had a higher prevalence of sepsis ($P=0.01$) and lower mean urine output ($P=0.02$) compared with the AA genotype group; the *MPO* rs2759 minor C-allele group (TC/CC genotype) had a lower prevalence of diabetes mellitus ($P=0.02$), higher prevalence of cirrhosis ($P=0.02$), sepsis ($P=0.03$), and shock ($P=0.04$), and a higher mean APACHE II score ($P<0.001$) and lower mean urine output ($P<0.001$) compared with the TT genotype group.

Similarly, the characteristics of the *secondary cohort* stratified by the 4 *MPO* gene polymorphisms are shown in Table 3. In brief, the pre-operative, operative and post-operative variables did not significantly differ across genotypes, with a few exceptions. The *MPO* rs7208693 minor T-allele group (GT/TT genotype) had a higher prevalence of planned elective surgery ($P=0.03$) and valve replacement ($P=0.04$), a prolonged mean aortic cross clamp time ($P=0.01$) and CPB perfusion time ($P<0.001$), and a higher post-operative day-1 mean APACHE II score ($P<0.001$) compared with the GG genotype group; similarly, the *MPO* rs2071409 minor C-allele group (AC/CC genotype) had a higher prevalence of planned elective surgery ($P=0.002$), valve replacement ($P=0.02$), and dialysis requirement ($P=0.001$), and a higher median mechanical ventilation duration ($P=0.007$) and higher post-operative day-1 mean APACHE II score ($P=0.01$) compared with the AA genotype group; the *MPO* rs2759 minor C-allele group (TC/CC genotype) had a prolonged mean CPB perfusion time ($P=0.05$) compared with the TT genotype group.

Association of *MPO* Genotypes with Plasma *MPO* and Urinary 15-F_{2t}-Isoprostane Levels

In the *primary cohort*, there was a strong genotype-phenotype association (Figure 1), as evidenced by the presence of higher plasma *MPO* levels among the *MPO* rs2243828 ($P=0.02$), rs7208693 ($P=0.01$), rs2071409 ($P=0.003$), and rs2759 ($P=0.03$) minor-allele carriers. When the heterozygous and homozygous minor-allele genotypes were combined, the *MPO* rs2243825 ($P=0.04$), rs7208693 ($P=0.001$), rs2071409 ($P=0.02$), and rs2759 ($P=0.03$), minor-allele carriers remained significantly associated with higher plasma *MPO* levels (Table 2). To a lesser degree, there was a similar but less robust association between higher urinary 15-F_{2t}-isoprostane levels and the *MPO* rs2243828 ($P=0.03$), rs7208693 ($P=0.09$), and rs2759 ($P=0.02$) minor-allele genotypes (Table 2).

In the *secondary cohort*, there was a similar but less global genotype-phenotype association (Table 3), as evidenced by higher pre-operative plasma *MPO* levels among minor allele genotype carriers for 2 of the 4 the *MPO* polymorphisms, rs7208693 ($P<0.001$) and rs2071409 ($P<0.001$).

Association of *MPO* Gene Polymorphisms and Adverse Outcomes

For the *primary cohort*, the results of the multivariable genetic dominant models are displayed in Table 4, showing a robust association between the individual *MPO* polymorphic minor alleles and the outcome of dialysis requirement or in-hospital death, after adjustment for sex, race, and APACHE II score. Compared with the TT genotype group, the *MPO* rs2243828 C-allele group had an adjusted OR of 2.19 (95% CI 1.25, 3.82) for this composite outcome. A similar association was observed for the *MPO* rs7208693 T-allele group compared with the GG genotype group (adjusted OR 2.73; 95% CI 1.38, 5.38); the *MPO* rs2071409 C-allele group compared with the AA genotype (adjusted OR 2.09; 95% CI 1.18, 3.69); and the *MPO* rs2759 C-allele group compared with the TT genotype group (adjusted OR 3.15; 95% CI 1.22, 8.12). In all the models, plasma *MPO* level was independently associated with the 2 composite outcomes (Table 5), and after adding this covariate to the models (model 3), the associations of the *MPO* gene polymorphisms with the composite outcome persisted but were attenuated (Table 4). Of note, in the *primary cohort*, there was no association between urinary 15-F_{2t}-isoprostane level and adverse outcomes (data not shown). The associations between the individual *MPO* polymorphic minor alleles and the composite of dialysis requirement, assisted mechanical ventilation or in-hospital death were less global, displaying significance on adjusted analyses for 2 of the 4 (rs2243828 and rs7208693) polymorphisms (Table 4).

For the *secondary cohort*, the results of the multivariable genetic dominant models are displayed in Table 6, showing a strong association between 2 of the 4 *MPO* polymorphic minor alleles and the composite outcome of stage-2 AKI, dialysis requirement, prolonged mechanical ventilation or in-hospital death, after adjustment for sex, race, age, heart failure, surgery status, and CPB time. Indeed, compared with the AA genotype group, the *MPO* rs2071409 C-allele group had an adjusted OR of 2.82 (95% CI 1.22, 6.50) for this composite outcome. A similar association was observed for the *MPO* rs2759 C-allele group compared with the TT genotype group (adjusted OR 3.56; 95% CI 1.44, 8.82). Of note, in the

secondary cohort, there was no association between plasma MPO level and the composite outcome (OR 0.89; 95% CI 0.62, 1.27; P = 0.514).

Sensitivity Analyses

In sensitivity analyses performed in the *primary cohort* using additive genetic models (Table 4), each copy of the *MPO* rs2243828 C-allele, rs7208693 T-allele, and rs2071409 C-allele was associated with 2.13- (95% CI 1.35, 3.37), 2.72- (95% CI 1.39, 5.35) and 2.26- (95% CI 1.40, 3.64) fold higher adjusted odds for dialysis requirement or in-hospital death, respectively. Using additive genetic models in the *secondary cohort*, each copy of the *MPO* rs2071409 C-allele was associated with 1.91- (95% CI 1.04, 3.51) fold higher adjusted odds for the composite of stage-2 AKI, dialysis requirement, prolonged mechanical ventilation or in-hospital death (Table 6).

Sensitivity analyses restricted to white subjects did not affect any of the adjusted analyses in both the *primary* and *secondary cohort* (Supplemental Table 1).

Association of *MPO* Haplotypes and Adverse Outcomes

We next generated a Haploview plot on the 4 *MPO* polymorphisms. Figure 2 displays the linkage disequilibrium (LD) plot at the *MPO* gene locus with representation of the 4 variants analyzed in the *primary cohort*. The LD plot revealed substantial marker-on-marker correlation (as defined by the D' parameter) across the ~11 kbp human *MPO* locus, with the rs2243828 and rs2759 polymorphisms displaying the highest correlation, as evidenced by a D' parameter of 74, on a scale of 0 to 100.

We then conducted haplotype analyses. The most frequent haplotype identified in the *primary cohort* across the 4 *MPO* polymorphisms (rs2243828, rs7208693, rs2071409 and rs2759) was T-G-A-T, which was estimated on 308 of the 524 chromosomes with a frequency of 59%, followed by C-G-A-T (10%) and C-G-C-T (9%). As shown in Figure 3A, patients with a higher copy-number of the T-G-A-T haplotype had significantly lower plasma MPO levels (P=0.002). This association translated into a protective effect. Indeed, as shown in Table 4, the presence of one copy or 2 copies of the T-G-A-T haplotype was associated with a respective OR of 0.16 (95% CI 0.06, 0.49) and 0.13 (95% CI 0.04, 0.42) for the outcome of dialysis requirement on in-hospital, after adjustment for sex, race, and APACHE II score. A similar independent protective effect was observed for the composite of dialysis requirement, assisted mechanical ventilation, or in-hospital death (Table 4). In the *secondary cohort*, the most frequent haplotype identified across the 4 *MPO* polymorphisms was also T-G-A-T, which was estimated on 330 of the 554 chromosomes with a similar frequency of 60%, followed by C-G-A-T (13%) and C-G-C-T (5%). A higher copy-number of the T-G-A-T haplotype was also associated with lower plasma MPO levels (P<0.001; Figure 3B), and a similar but less global protective effect of the T-G-A-T haplotype was observed (Table 6).

Using the SNP Spectral Decomposition (SNPSpD) method to account for multiple testing, the experiment-wide significance threshold required to maintain a type-I error rate at less than 5% was 0.013, reflecting linkage disequilibrium within this block. In the *primary*

cohort, this threshold ($P < 0.013$) was exceeded by markers rs2243828 and rs7208693 for the outcome of dialysis requirement or in-hospital death in the adjusted dominant-model analyses (model 3). The threshold was also exceeded by markers rs2243828, rs7208693, and rs2071409 in the adjusted additive-model analyses. Using this method, the T-G-A-T haplotype also remained associated with dialysis requirement or in-hospital death. The association between the 4 MPO SNPs and the composite outcome of dialysis requirement, assisted mechanical ventilation or in-hospital death became less robust after applying this method. Similarly, in the *secondary cohort*, the only marker that remained associated with the composite outcome was rs2759 in the adjusted dominant-model analyses.

DISCUSSION

In the present 2-step genetic study, we first examined the relationship of 4 polymorphisms in the *MPO* gene locus located in the promoter (rs2243828), coding (rs7208693 and rs2759), and non-coding (rs2071409) regions, with plasma MPO and urinary 15-F_{2t}-isoprostane levels, disease severity measures, and adverse clinical outcomes in a large cohort of hospitalized adults with a diagnosis of AKI, which constituted our *primary cohort*. The observed and expected genotype frequencies were not significantly different, and fulfilled Hardy-Weinberg equilibrium. Minor allele frequencies ranged from 7 to 31% for all 4 polymorphisms. The overall population was 91% white, and there were no race, sex, or age differences within genotype groups. Strong genotype-phenotype associations were observed between the 4 *MPO* gene polymorphisms and the two selected markers of oxidative stress, plasma MPO (all 4 SNPs) and urinary 15-F_{2t}-isoprostane (SNPs rs2243828 and rs2759) levels. All 4 polymorphisms were independently associated with 2- to 3-fold higher odds for the composite outcome of dialysis requirement or in-hospital death. A similar but less robust association was observed for the composite outcome of dialysis requirement, assisted mechanical ventilation or in-hospital death. Finally, we identified a common haplotype (T-G-A-T) whereby the number of copies was associated with lower plasma MPO level and lower odds for adverse outcomes. The results remained robust across several sensitivity analyses including the use of additive models, restriction to white subjects only, and correction for multiple testing using the SNPSpD method. We next examined similar associations in a *secondary cohort* consisting of adults at-risk for AKI undergoing cardiac surgery with cardiopulmonary bypass, and observed a significant though less global genotype-phenotype association between 2 of the 4 *MPO* gene polymorphisms and the outcome of stage-2 AKI, dialysis requirement, prolonged mechanical ventilation or in-hospital death.

Myeloperoxidase is an essential enzyme involved in oxidative responses to a variety of stimuli. Following release from neutrophils and monocytes through degranulation, MPO reacts with hydrogen peroxide formed by the respiratory burst to form a complex that can oxidize various compounds including chloride, which is oxidized to hypochlorous acid leading to the formation of chlorine and chloramine. These end-products are powerful oxidants that can exert biological effects in the kidney³⁰. Indeed, *in situ* MPO activity has been used as an index of neutrophil kidney parenchymal infiltration in experimental models of ischemia-reperfusion injury³¹⁻³⁵ and nephrotoxicity^{36,37}.

Given the importance of MPO in mediating oxidative cellular injury in response to ischemic and toxic kidney injury, one might anticipate that polymorphisms disrupting or enhancing the function or expression of MPO would alter oxidative stress-mediated cellular responses in AKI. Several polymorphisms in the *MPO* gene locus have been described and variably linked to measures of oxidative stress, including rs7208693 (Val53Phe in exon-2 and examined in our study) associated with higher MPO activity³⁸, and rs2333227 (located in the promoter region), associated with lower MPO transcriptional activation²³.

In the *primary cohort*, the potential mechanisms underlying the protective association of the common *MPO* T-G-A-T haplotype with the composite endpoint of dialysis requirement or in-hospital death are not completely understood, but we propose that the improved outcomes observed among carriers of this haplotype might in part be due to lower generation or release of MPO from neutrophils, resulting in less extensive organ injury. This hypothesis is supported by the observed lower plasma MPO levels with a higher copy-number of the T-G-A-T haplotype, which is driven by the major rather than minor alleles of the individual *MPO* SNPs.

To our knowledge, this is the first study to test the hypothesis of whether representative polymorphisms across the *MPO* gene locus associate with not only intermediate phenotypes, i.e., plasma and urinary markers of oxidative stress, but also adverse clinical outcomes in 2 separate cohorts of adults with a diagnosis of AKI (*primary cohort*) as well as adults at-risk for AKI (*secondary cohort*). We used overlapping composite outcome measures to allow meaningful comparisons between the 2 cohorts. The demonstrable *MPO* genotype-phenotype associations, whereby all 4 polymorphic alleles were associated with higher plasma MPO levels, strengthen the credibility of the observed association between the various polymorphic alleles and adverse outcomes. We also used clinically relevant covariates in the multivariable models. These include the APACHE II score in the *primary cohort*, which captures severity of the acute illness, and heart failure, surgery status, and CPB time in the *secondary cohort*, which capture known risk factors for post-cardiac surgery AKI. Our composite outcomes included in-hospital death, which accounted for survival bias when assessing endpoints of artificial organ support (i.e., dialysis requirement or assisted/prolonged mechanical ventilation). Finally, the results observed on the *secondary cohort* support and validate our primary findings.

There are several study limitations that should be noted. The heterogeneity of the *primary cohort* was offset by the selective inclusion of subjects with more advanced stages of AKI requiring nephrology consultation. Although the 2 cohorts were relatively small for genetic epidemiological analyses, there was no deviation from expected genotype frequencies, and the ~91-95% majority of white subjects likely minimized the potential influence of race on genotype frequencies. Although the observed genetic associations might reflect some degree of genetic admixture, the association of higher plasma MPO level with increased odds for adverse outcomes in the *primary cohort* argues for the suitability of this oxidative stress marker as a downstream effector of polymorphisms in the *MPO* gene locus. The attenuation of the point estimates in the genetic markers after adding plasma MPO levels to the multivariable models supports this hypothesis. Multiple testing, which is an important limitation of the analysis, was addressed by applying the SNPSpD method to derive stricter

P values to define significance. Finally, within the associated MPO haplotype block, several potentially functional genetic variants lie, but the biological mechanism by which MPO haplotypes might influence renal traits remains uncertain.

In conclusion, in the present 2-step genetic study, we provide evidence that 4 polymorphisms in the *MPO* gene locus and a common corresponding haplotype influence the risk of adverse outcomes in hospitalized adults with or at-risk for AKI. Although this effect seems to be mediated by modulation of the extracellular release of MPO, additional studies are needed to establish the mechanisms underlying the influence of the identified genotypes on disease traits.

METHODS

Study Design and Participants

The *primary cohort* was derived from a prospective cohort study conducted at two acute care hospitals where adults with AKI, in whom nephrology consultation was requested, were eligible for enrollment. Acute kidney injury was defined as a rise in serum creatinine by 0.5, 1.0, or 1.5 mg/dl from a baseline level of < 1.9, 2.0-4.9, or ≥ 5.0 mg/dL, respectively³⁹. Although this definition was adopted prior to the development of the AKI network consensus definition⁴⁰, we used this classification to categorize stages of AKI. Exclusion criteria were age < 18 years, pregnancy, chronic dialysis, organ transplantation within the prior year, and urinary obstruction.

The *secondary cohort* was derived from a prospective cohort study of patients at-risk for AKI following cardiac surgery, conducted at four acute care hospitals, where adults scheduled to undergo on-pump cardiac surgery were eligible for enrollment. Exclusion criteria were age < 18 years, off-pump surgery, pregnancy, acute or chronic dialysis dependence, and organ transplantation within the prior year.

Institutional review board approval was granted, and written informed consent was obtained from all participants or next of kin.

Data Collection

Data collection for the *primary cohort* included baseline demographic characteristics, comorbid conditions, 24-hour urine output at enrollment, and serial serum creatinine values. Sepsis was ascertained using the systemic inflammatory response syndrome criteria⁴¹, and two severity-of-illness scores were calculated, the Acute Physiology and Chronic Health Evaluation (APACHE) II score⁴², and the Multiple Organ Failure (MOF) score⁴³. Pre-existing chronic kidney disease was defined on the basis of a baseline estimated glomerular filtration rate of less than 60 ml/min/1.73 m², which was calculated using the Modification of Diet in Renal Disease (MDRD) study equation⁴⁴.

Data collection for the *secondary cohort*, included baseline demographic characteristics, pre-operative comorbid conditions and laboratory variables, intra-operative variables (surgery electivity and type, CPB perfusion time and aortic cross clamp time), and several

post-operative variables including, day-1 APACHE II score and urine output, initial hours of assisted mechanical ventilation, and serial serum creatinine values over the first 3 days.

Blood and Urine Sampling

At enrollment, EDTA-anticoagulated whole blood and urine were collected. Plasma was separated, and the remaining blood was aliquoted for subsequent DNA extraction. Urine samples were obtained from a fresh void or the access port of a Foley catheter, kept on ice, centrifuged (within 30 minutes) for 10 min at 3,000 rpm, treated with a protease inhibitor cocktail (Complete Mini, Roche Diagnostics, Mannheim, Germany), aliquoted in cryotubes and stored at -80°C .

Selection Criteria for Genetic Variants

To obtain comprehensive representation of the genetic variability in the *MPO* gene locus (reference sequence GeneBank accession number, NM 000250.1), we selected tag-SNPs using the HapMap genome browser for European populations (<http://hapmap.ncbi.nlm.nih.gov/>) with the tagger pair-wise method, a r^2 cutoff of 0.8, and a minor allele frequency of at least 10%. These genetic variants were also selected on the basis of published reports on investigated clinical and functional associations. Through this selection process, we identified 4 *MPO* polymorphisms of interest spanning the gene (5' to 3' direction) (Table 1), located in the promoter (-765 T to C, RefSNP rs2243828), exon 2 ($+157$ G to T, RefSNP rs7208693), intron 11 ($+9890$ A to C, RefSNP rs2071409), and exon 12 ($+2149$ T to C, Ile717Val, RefSNP rs2759).

TaqMan Genotyping and Haplotype Analyses

Genomic DNA was prepared from whole blood leukocytes using the QiAmp DNA blood kit (Qiagen, Valencia, CA). Using the Applied Biosystems 7900HT fast real-time PCR system (Applied Biosystems, Foster City, CA), DNA samples were genotyped by combining the TaqMan universal PCR master mix with TaqMan SNP genotyping assay into a 384-well plate according to a standardized protocol. Resulting sequences were analyzed by the SNP software (SDS version 2.2), which utilizes an advanced multi-component algorithm to calculate distinct allele/marker signal contributions from fluorescence measurements for each sample-well during the assay plate read with the SNP auto-caller. The SNP auto-caller automatically determines sample genotypes and generates a cluster plot to better visualize data across samples.

Pair-wise patterns of LD across the *MPO* gene locus were visualized by Haploview, plotting LD as the D' parameter (scaled from 0-100). Haplotypes were imputed from the *MPO* polymorphism diploid genotypes using PLINK version 1.07, a toolset for whole-genome association and population-based linkage analysis⁴⁵ (<http://pngu.mgh.harvard.edu/~purcell/plink/>). The most frequent haplotype (T-G-A-T) identified across the 4 (5' to 3' direction) *MPO* polymorphisms (rs2243828, rs7208693, rs2071409 and rs2759) was expressed as number of chromosomal copies (0, 1, or 2 haplotypes) per diploid individual.

Measurement of Markers of Oxidative Stress

To evaluate whether the *MPO* gene polymorphisms were associated with markers of oxidative stress, we measured plasma levels of MPO, the *MPO* gene product, and urinary levels of 15-F_{2t}-isoprostane, a marker of lipid peroxidation. Plasma MPO was measured by solid-phase ELISA (R&D Systems, Minneapolis, MN), results reported as ng/ml. The intra- and inter-assay coefficient of variation is 7.3% and 8.1%, respectively. Urinary 15-F_{2t}-isoprostane was measured by competitive ELISA (Northwest Life Science Specialties, LLC, Vancouver, WA), results were normalized to urinary creatinine and expressed as ng/mg of creatinine. The intra- and inter-assay coefficient of variation is 5% and 10%, respectively. All measurements were performed in duplicate.

Outcome Measures

In the *primary cohort*, the main outcomes of interest were the composite of dialysis requirement or in-hospital death as well as dialysis requirement, assisted mechanical ventilation, or in-hospital death. In the *secondary cohort*, the main outcome measure was the composite of stage-2 or 3 AKI according to the AKI network consensus definition (>2-fold increase in serum creatinine over the first 3 post-operative days)⁴⁰, dialysis requirement, prolonged (>24 hours) mechanical ventilation according to definition by the Society of Thoracic Surgeons⁴⁶, or inhospital death.

Statistical Analyses

The genotype frequencies were tested for Hardy-Weinberg equilibrium using a standard Chi-square test for any deviation of the observed frequencies. Comparisons between genotype groups were made by the *t*-test and the two-tailed Mann Whitney test for continuous variables, and by Chi square or Fisher exact test for categorical variables. Continuous variables are reported as mean (with standard deviation) or median (with 25th and 75th percentile) according to their distribution. Binary variables are reported as count (with percentage).

Multivariable logistic regression analyses were performed to examine the association of each *MPO* gene polymorphism with the afore-mentioned outcomes, using dominant genetic models (i.e., one or two copies of the minor allele). In the *primary cohort*, the models were adjusted for sex, race, and age (model 1); sex, race, and APACHE II score (model 2); or sex, race, APACHE II score, and log-transformed plasma MPO level (model 3). In the *secondary cohort*, the models were adjusted for sex, race, and age (model 1); or sex, race, age, heart failure, surgery status, and CPB time (model 2).

Similar multivariable logistic regression analyses were performed for the association of the *MPO* T-G-A-T, haplotype and the outcomes of interest. Several sensitivity analyses were performed, including the use of additive genetic models and restriction of the cohort to white subjects only. The SNPSpD method⁴⁷ was used to correct for multiple testing for SNPs that were in LD with each other, on the basis of the spectral decomposition of matrices of pairwise LD between SNPs displayed in the Haploview plot of the *MPO* gene locus (Figure 2). In brief, this method estimates within a haplotype block, the experiment-wide significance threshold required to maintain the type-1 error rate at 5% or less.

The results of the logistic regression analyses are displayed as odds ratios (OR) with 95% confidence interval (CI). All statistical analyses were performed using the SAS software (version 9.2, SAS Institute, Cary, NC). Differences were considered statistically significant at a P value of less than 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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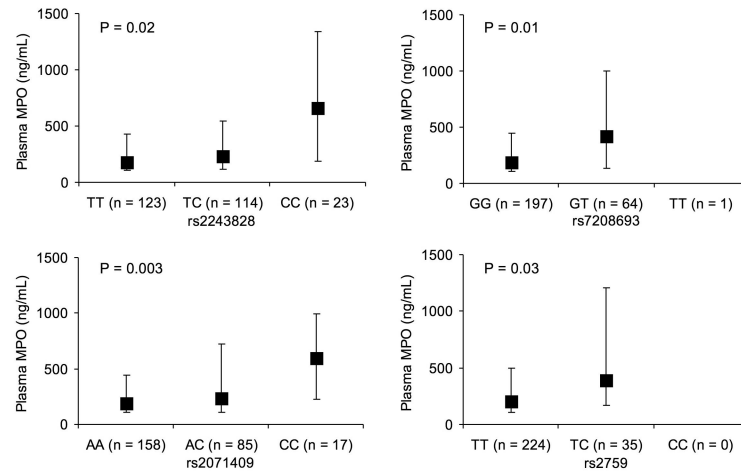


Figure 1. Plasma levels of myeloperoxidase (MPO) in the primary cohort stratified by the MPO genotypes

The data are represented as median (25th and 75th percentile) values. The *P* values are derived from by Wilcoxon signed-rank test.

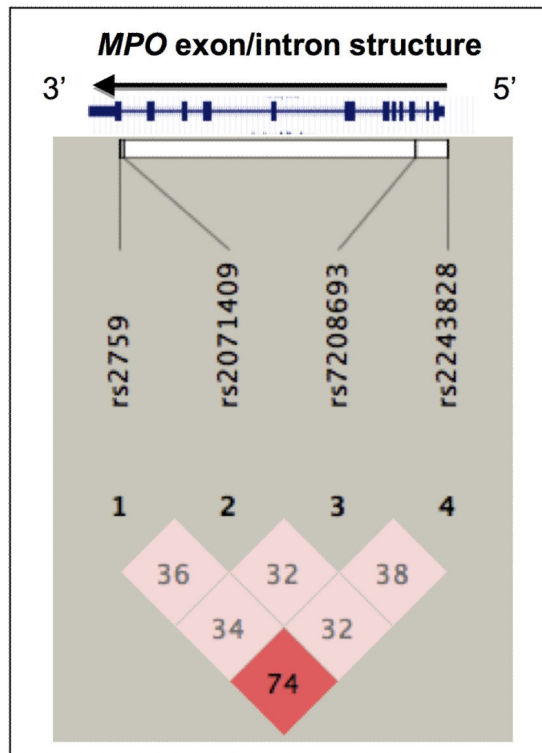


Figure 2. Haploview plot of the *MPO* gene locus in the primary cohort

The plot illustrates pair-wise linkage disequilibrium (LD) between the 4 *MPO* polymorphisms, based on the D' parameter value (scaled from 0-100). The darker a rhombus appears, the higher the LD is between the respective polymorphisms.

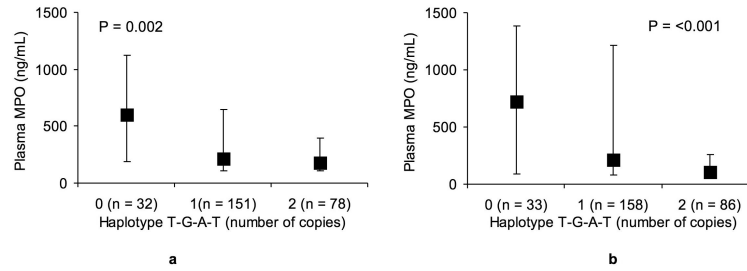


Figure 3. Plasma levels of myeloperoxidase (MPO) in the (3A) primary and (3B) secondary cohort stratified by the *MPO* haplotype T-G-A-T copy number (0, 1, 2 copies per diploid individual)

The data are represented as median (25th and 75th percentile) values. The *P* values are derived from by Wilcoxon signed-rank test.

Observed and expected distribution of *MPO* gene polymorphisms in the primary and replication cohort

Table 1

Locus	RefSNP number (dbSNP)	Single nucleotide polymorphism	Domain	Number of samples successfully genotyped		Allele frequencies		Hardy Weinberg equilibrium					
				Primary cohort	Secondary cohort	Primary cohort	Secondary cohort	Primary cohort	Secondary cohort				
<i>MPO</i>	rs2243828	-765 T to C	Promoter	260	277	T: 0.692	C: 0.308	T: 0.727	C: 0.273	χ^2 0.22	P value 0.64	χ^2 0.23	P value 0.63
<i>MPO</i>	rs7208693	157 G to T	Exon 2 (Val53Phe)	262	277	C: 0.874	A: 0.126	C: 0.884	A: 0.116	χ^2 3.14	P value 0.08	χ^2 2.51	P value 0.11
<i>MPO</i>	rs2071409	9890 A to C	Intron 11	260	275	A: 0.771	C: 0.229	A: 0.798	C: 0.202	χ^2 1.41	P value 0.23	χ^2 2.02	P value 0.16
<i>MPO</i>	rs2759	2149 T to C	Exon 12 (Ile717Val)	259	274	T: 0.932	C: 0.068	T: 0.923	C: 0.077	χ^2 1.36	P value 0.24	χ^2 0.27	P value 0.60

Table 2

Characteristics of the primary cohort according to the 4 *MPO* gene polymorphisms

Characteristic	rs2243828			rs7208693			rs2071409			rs2759		
	TT (n=123)	TC/CC (n=137)	P value	GG (n=197)	GT/TT (n=65)	P value	AA (n=158)	AC/CC (n=102)	P value	TT (n=224)	genotypes	P value
Age, years	67 (16)	65 (15)	0.13	66 (16)	66 (14)	0.97	66 (16)	66 (15)	0.96	66 (16)	65 (15)	0.62
Men, %	54	52	0.77	56	43	0.07	55	49	0.34	53	49	0.62
Race, %			0.48			0.86			0.89			0.12
White	93	88		90	91		90	91		91	86	
Black	5	9		7	8		8	6		6	14	
Other	2	3		3	1		2	3		3	0	
Contributing cause of AKI, %			0.57			0.21			0.79			0.20
Ischemic	32	26		27	32		29	27		26	43	
Nephrotoxic	15	17		16	14		17	14		17	9	
Septic	4	7		9	11		9	10		10	3	
Multifactorial/other	32	35		37	23		34	34		34	34	
Comorbid conditions, %												
Diabetes mellitus	44	45	0.92	46	39	0.28	48	39	0.19	47	26	0.02
Heart failure	16	16	0.96	17	12	0.35	17	16	0.87	17	14	0.74
Cirrhosis	5	10	0.16	5	14	0.02	6	10	0.22	6	17	0.02
Chronic lung disease	18	18	0.94	19	17	0.74	18	18	0.99	17	20	0.71
Chronic kidney disease	73	65	0.17	71	60	0.13	66	71	0.43	69	58	0.19
APACHE II score	18 (5)	21 (7)	0.002	19 (6)	23 (7)	<0.001	19 (6)	20 (6)	0.16	19 (6)	25 (7)	<0.001
Sepsis, %	38	47	0.17	40	51	0.13	37	53	0.01	40	60	0.03
Shock, %	25	31	0.33	26	32	0.36	27	30	0.50	25	43	0.03
Serum creatinine, mg/dl												
Baseline value	1.5 (0.6)	1.5 (0.8)	0.92	1.6 (0.7)	1.4 (0.6)	0.11	1.5 (0.6)	1.6 (0.9)	0.35	1.5 (0.7)	1.5 (0.6)	0.57
Enrollment value	3.5 (1.8)	3.6 (1.8)	0.89	3.6 (1.8)	3.5 (1.7)	0.88	3.5 (1.6)	3.7 (1.9)	0.40	3.5 (1.8)	3.7 (1.5)	0.64
Peak	4.3 (2.4)	4.4 (2.8)	0.63	4.2 (2.2)	4.7 (3.4)	0.24	4.2 (2.1)	4.6 (3.2)	0.22	4.4 (2.7)	4.2 (1.5)	0.77
Discharge	2.6 (1.9)	2.4 (1.4)	0.21	2.5 (1.8)	2.4 (1.4)	0.78	2.5 (1.8)	2.6 (1.5)	0.63	2.5 (1.8)	2.5 (1.2)	0.93
Urine output, L/day	1.4 (1.1)	1.1 (1.0)	0.05	1.3 (1.1)	1.1 (1.1)	0.10	1.4 (1.2)	1.1 (0.8)	0.02	1.4 (1.1)	0.7(0.7)	<0.001

Characteristic	rs2243828		P value	rs7208693		P value	rs2071409		P value	rs2759		P value
	TT (n=123)	TC/CC (n=137)		GG (n=197)	GT/TT (n=65)		AA (n=158)	AC/CC (n=102)		TT (n=224)	TC/CC (n=35)	
AKI stage at enrollment, %			0.23			0.02			0.68			0.06
Stage 1	49	38		28	45		41		46		26	
Stage 2	4	5		6	5		4		4		8	
Stage 3	47	57		66	50		55		50		66	
Oxidative stress markers												
Plasma nitrotyrosine, nM	12 (9, 22)	12 (10, 22)	0.31	12 (9, 23)	11 (9, 23)	0.79	12 (10, 23)	12 (11, 32)	12 (9, 23)	12 (11, 32)	12 (11, 32)	0.18
Myeloperoxidase (ng/ml)	176 (103,431)	248 (117,674)	0.04	191 (105,448)	188 (105,444)	0.001	301 (115,892)	390 (170,1208)	207 (105,501)	390 (170,1208)	390 (170,1208)	0.03
Urinary 15-F _{2t} -isoprostane, ng/mg	0.9 (0.5,1.7)	1.3 (0.6,2.7)	0.03	1.0 (0.6,2.0)	1.0 (0.5,2.1)	0.09	1.4 (0.7,2.3)	1.8 (1.0,2.8)	1.0 (0.5,2.1)	1.4 (0.7,2.3)	1.8 (1.0,2.8)	0.02

Abbreviations: AKI, acute kidney injury; APACHE, Acute Physiology and Chronic Health Evaluation.

Continuous variables are presented as mean (standard deviation) or median (25th, 75th percentile), and categorical variables as percentages.

Missing genotyping data on rs2243828 (n = 2), rs2071409 (n = 2), and rs2759 (n = 3)

Table 3

Characteristics of the *secondary cohort* according to the 4 *MPO* gene polymorphisms

Characteristic	rs2243828		P value	rs7208693		P value	rs2071409		P value	rs2759		P value
	TT (n=145)	TC/CC (n=132)		GG (n=214)	GT/TT (n=63)		AA (n=179)	AC/CC (n=96)		TT (n=233)	TC/CC (n=41)	
Preoperative variables												
Age, years	67 (12)	68 (11)	0.55	67 (11)	70 (12)	0.04	68 (12)	68 (11)	0.59	67 (12)	69 (11)	0.29
Men, %	77	67	0.09	73	70	0.63	73	71	0.68	74	61	0.08
White ethnicity, %	96	94	0.69	95	95	0.91	95	96	0.76	95	93	0.45
Hypertension, %	84	85	0.89	85	83	0.64	87	81	0.25	85	83	0.75
Diabetes mellitus, %	34	32	0.76	32	37	0.50	30	39	0.17	34	27	0.36
Heart failure, %	33	41	0.20	35	43	0.26	33	44	0.08	37	39	0.76
Chronic lung disease, %	28	33	0.36	30	30	0.99	28	34	0.29	28	44	0.16
Chronic kidney disease, %	18	27	0.15	21	32	0.22	21	25	0.53	22	27	0.59
Peripheral vascular disease, %	17	18	0.70	16	22	0.25	13	26	0.01	17	17	0.98
Cerebrovascular disease, %	11	11	0.93	11	11	0.98	11	12	0.94	12	7	0.38
3-vessel coronary artery disease, %	49	44	0.44	48	44	0.57	48	44	0.46	45	54	0.31
Left main coronary artery disease, %	21	29	0.11	26	21	0.43	24	25	0.86	23	30	0.37
Serum creatinine, mg/dL	1.1 (1.0)	1.1 (0.4)	0.45	1.1 (0.9)	1.1 (0.3)	0.50	1.1 (0.9)	1.1 (0.3)	0.95	1.1 (0.8)	1.1 (0.3)	0.62
Plasma myeloperoxidase, ng/mL	180 (87, 924)	180 (75, 949)	0.71	121 (74, 381)	1379 (802, 1721)	<0.001	115 (76, 527)	664 (124, 1513)	<0.001	179 (82, 834)	170 (67, 1213)	0.91
Operative variables												
Elective surgery, %	26	37	0.06	28	43	0.03	25	43	0.002	29	43	0.09
Valve replacement, %	45	57	0.05	47	62	0.04	46	60	0.02	50	55	0.53
Aortic cross clamp time, min	87 (40)	95 (43)	0.16	87 (37)	104 (52)	0.01	88 (39)	96 (45)	0.13	89 (41)	96 (45)	0.38
CPB perfusion time, min	113 (48)	124 (54)	0.07	111 (45)	142 (62)	<0.001	111 (49)	131 (54)	0.002	115 (51)	132 (53)	0.05
Postoperative variables												
Initial mechanical ventilation duration, hours	13 (23)	25 (98)	0.14	16 (47)	30 (117)	0.16	11 (12)	34 (116)	0.007	15 (45)	43 (146)	0.02
Day-1 APACHE II score	13 (7)	14 (7)	0.22	12 (7)	18 (6)	<0.001	13 (7)	15 (7)	0.01	13 (7)	14 (6)	0.54
Day-1 urine output, L/day	2.6 (1.0)	2.7 (1.0)	0.72	2.5 (1.0)	2.9 (1.0)	0.01	2.5 (1.0)	2.8 (1.1)	0.06	2.6 (1.0)	2.7 (1.3)	0.62
Stage-2 or 3 AKI*, %	2	3	0.58	3	0	0.51	1	5	0.07	2	7	0.05

Characteristic	rs2243828		P value	rs7208693		P value	rs2071409		P value	rs2759		P value
	genotypes	TC/CC (n=132)		TT (n=145)	GG (n=214)		GT/TT (n=63)	AA (n=179)		AC/CC (n=96)	TT (n=233)	
Dialysis requirement, %	2	2	0.99	2	3	0.53	0	6	0.001	2	5	0.20

CPB denotes cardiopulmonary bypass; APACHE, Acute Physiology and Chronic Health Evaluation; and AKI, acute kidney injury.

Continuous variables are presented as mean (standard deviation) or median (25th, 75th percentile), and categorical variables as percentages.

* > 2-fold increase in serum creatinine over the first 3 postoperative days. Missing genotyping data on rs2071409 (n = 2), and rs2759 (n = 3)

Table 4
Association of the 4 *MPO* gene polymorphisms with adverse outcomes in the primary cohort

<i>MPO</i> gene polymorphism	Dialysis requirement or in-hospital death			Dialysis requirement, mechanical ventilation, or in-hospital death		
	OR	95% CI	P value	OR	95% CI	P value
Dominant model						
rs2243828 (C-allele vs. T/T)						
- Unadjusted	2.53	1.53, 4.19	<0.001	2.31	1.40, 3.80	0.001
- Model 1	2.65	1.59, 4.41	<0.001	2.37	1.43, 3.92	0.001
- Model 2	2.19	1.25, 3.82	0.006	2.01	1.13, 3.57	0.017
- Model 3	2.12	1.20, 3.75	0.010	1.98	1.10, 3.57	0.023
rs7208693 (T-allele vs. G/G)						
- Unadjusted	3.77	2.06, 6.92	<0.001	3.79	2.02, 7.13	<0.001
- Model 1	4.04	2.18, 7.50	<0.001	3.91	2.07, 7.38	<0.001
- Model 2	2.73	1.38, 5.38	0.004	2.52	1.22, 5.18	0.012
- Model 3	2.66	1.31, 5.41	0.007	2.54	1.19, 5.41	0.016
rs2071409 (C-allele vs. A/A)						
- Unadjusted	2.07	1.25, 3.43	0.005	1.65	0.99, 2.73	0.053
- Model 1	2.11	1.27, 3.51	0.004	1.65	0.99, 2.73	0.054
- Model 2	2.09	1.18, 3.69	0.012	1.52	0.84, 2.73	0.164
- Model 3	1.88	1.05, 3.38	0.035	1.34	0.73, 2.46	0.342
rs2759 (C-allele vs. T/T)						
- Unadjusted	5.63	2.36, 13.45	<0.001	5.48	2.19, 13.71	<0.001
- Model 1	6.02	2.49, 14.55	<0.001	5.71	2.26, 14.40	<0.001
- Model 2	3.15	1.22, 8.12	0.018	2.66	0.96, 7.37	0.059
- Model 3	3.13	1.17, 8.40	0.024	2.68	0.93, 7.76	0.069
Additive model						
rs2243828 (per C-allele copy increase)						
- Unadjusted	2.46	1.63, 3.72	<0.001	2.22	1.48, 3.35	<0.001
- Model 1	2.54	1.67, 3.86	<0.001	2.25	1.49, 3.41	<0.001
- Model 2	2.13	1.35, 3.37	0.001	1.92	1.20, 3.09	0.007
- Model 3	2.07	1.29, 3.33	0.003	1.89	1.15, 3.09	0.012

MPO gene polymorphism	Dialysis requirement or in-hospital death		Dialysis requirement, mechanical ventilation, or in-hospital death		P value	
	OR	95% CI	OR	95% CI		
rs7208693(per T-allele copy increase)						
- Unadjusted	3.75	2.06, 6.85	<0.001	3.77	2.02, 7.05	<0.001
- Model 1	4.02	2.17, 7.42	<0.001	3.88	2.06, 7.31	<0.001
- Model 2	2.72	1.39, 5.35	0.004	2.51	1.22, 5.16	0.012
- Model 3	2.66	1.31, 5.39	0.007	2.53	1.19, 5.39	0.016
rs2071409 (per C-allele copy increase)						
- Unadjusted	2.26	1.48, 3.45	<0.001	1.87	1.23, 2.85	0.003
- Model 1	2.28	1.49, 3.50	<0.001	1.87	1.23, 2.85	0.004
- Model 2	2.26	1.40, 3.64	0.001	1.77	1.09, 2.86	0.021
- Model 3	2.07	1.27, 3.37	0.004	1.59	0.97, 2.62	0.067
T-G-A-T haplotype *						
- Unadjusted						
1 copy (vs. none)	0.15	0.05, 0.40	<0.001	0.18	0.07, 0.50	0.001
2 copies (vs. none)	0.10	0.04, 0.30	<0.001	0.14	0.05, 0.41	<0.001
- Model 1						
1 copy (vs. none)	0.15	0.05, 0.41	<0.001	0.18	0.07, 0.51	0.001
2 copies (vs. none)	0.10	0.03, 0.29	<0.001	0.14	0.05, 0.40	<0.001
- Model 2						
1 copy (vs. none)	0.16	0.06, 0.49	0.001	0.20	0.07, 0.62	0.005
2 copies (vs. none)	0.13	0.04, 0.42	0.001	0.20	0.06, 0.63	0.006
- Model 3						
1 copy (vs. none)	0.18	0.06, 0.55	0.003	0.22	0.07, 0.71	0.011
2 copies (vs. none)	0.15	0.05, 0.50	0.002	0.23	0.07, 0.77	0.017

95% CI denotes 95% confidence interval for the odds ratio; model 1 is adjusted for sex, race, and age; model 2 is adjusted for sex, race, and APACHE II score; model 3 is adjusted for sex, race, and APACHE II score, and plasma MPO level.

* The haplotype was generated from the MPO rs2243828, rs7208693, rs2071409 and rs2759 polymorphisms.

Table 5

Association of plasma MPO levels with adverse clinical outcomes in the primary cohort

Outcome variable	Plasma log-MPO level (per 1-SD increase)	P value	C statistic
<i>Dialysis requirement or in-hospital death</i>			
Unadjusted	1.96 (1.48, 2.58)	< 0.001	0.68
Adjusted for sex, race, APACHE II score, and rs2243828*	1.61 (1.18, 2.19)	0.002	0.79
Adjusted for sex, race, APACHE II score, and rs7208693*	1.61 (1.18, 2.20)	0.003	0.79
Adjusted for sex, race, APACHE II score, and rs2071409*	1.58 (1.16, 2.16)	0.004	0.79
Adjusted for sex, race, APACHE II score, and rs2759*	1.61 (1.18, 2.21)	0.003	0.79
Adjusted for sex, race, APACHE II score, and T-G-A-T haplotype	1.55 (1.13, 2.14)	0.007	0.80
<i>Mechanical ventilation, dialysis requirement or in-hospital death</i>			
Unadjusted	2.00 (1.51, 2.65)	< 0.001	0.68
Adjusted for sex, race, APACHE II score, and rs2243828*	1.62 (1.18, 2.23)	0.003	0.81
Adjusted for sex, race, APACHE II score, and rs7208693*	1.63 (1.17, 2.26)	0.004	0.82
Adjusted for sex, race, APACHE II score, and rs2071409*	1.60 (1.16, 2.22)	0.004	0.81
Adjusted for sex, race, APACHE II score, and rs2759*	1.67 (1.20, 2.31)	0.002	0.82
Adjusted for sex, race, APACHE II score, and T-G-A-T haplotype	1.57 (1.13, 2.19)	0.007	0.82

95% CI denotes the 95% confidence interval for the odds ratio (OR); MPO, myeloperoxidase; and SD, standard deviation

* the analysis refers to the dominant models.

Table 6

Association of the 4 *MPO* gene polymorphisms with > 2-fold increase in serum creatinine, dialysis requirement, prolonged initial mechanical ventilation (>24 hours) or in-hospital death in the secondary cohort

<i>MPO</i> gene polymorphism	Odds ratio	95% CI	P value
Dominant model			
rs2243828 (C-allele vs. T/T)			
- Unadjusted	1.38	0.68, 2.81	0.375
- Model 1	1.27	0.55, 2.91	0.571
- Model 2	1.20	0.52, 2.78	0.666
rs7208693 (T-allele vs. G/G)			
- Unadjusted	1.41	0.64, 3.12	0.395
- Model 1	1.05	0.42, 2.62	0.911
- Model 2	1.14	0.45, 2.87	0.780
rs2071409 (C-allele vs. A/A)			
- Unadjusted	2.90	1.41, 5.97	0.004
- Model 1	2.86	1.24, 6.57	0.013
- Model 2	2.82	1.22, 6.50	0.015
rs2759 (C-allele vs. T/T)			
- Unadjusted	3.29	1.46, 7.41	0.004
- Model 1	3.50	1.42, 8.58	0.006
- Model 2	3.56	1.44, 8.82	0.006
Additive model			
rs2243828 (per C-allele copy increase)			
- Unadjusted	1.62	0.94, 2.78	0.082
- Model 1	1.52	0.82, 2.81	0.179
- Model 2	1.47	0.79, 2.74	0.227
rs7208693(per T-allele copy increase)			
- Unadjusted	1.36	0.63, 2.94	0.438
- Model 1	1.04	0.42, 2.54	0.939
- Model 2	1.12	0.45, 2.80	0.801
rs2071409 (per C-allele copy increase)			
- Unadjusted	2.22	1.30, 3.78	0.003
- Model 1	1.94	1.06, 3.57	0.033
- Model 2	1.91	1.04, 3.51	0.037
T-G-A-T haplotype *			
- Unadjusted			
1 copy (vs. none)	0.37	0.15, 0.92	0.032
2 copies (vs. none)	0.24	0.08, 0.71	0.010
- Model 1			
1 copy (vs. none)	0.46	0.17, 1.27	0.134
2 copies (vs. none)	0.30	0.08, 1.16	0.080
- Model 2			

<i>MPO</i> gene polymorphism	Odds ratio	95% CI	P value
1 copy (vs. none)	0.49	0.18, 1.36	0.173
2 copies (vs. none)	0.32	0.08, 1.28	0.108

95% CI denotes 95% confidence interval for the odds ratio; model 1 is adjusted for age, sex, race and cardiopulmonary bypass time; model 2 is adjusted for age, sex, race, heart failure, surgery status, and cardiopulmonary bypass time.

* The haplotype was generated from the *MPO* rs2243828, rs7208693, rs2071409 and rs2759 polymorphisms.

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