Diabetes mellitus, superoxide dismutase and peroxisome proliferator activated receptor gamma polymorphisms modify the outcome of end-stage renal disease patients of Han Chinese origin

Running Title: Superoxide dismutase, peroxisome proliferator activated receptor gamma and survival

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Abstract

Background: Increased oxidative stress significantly modifies the outcome of patients with diabetes mellitus (DM) and end-stage renal disease (ESRD), and is counteracted by anti-oxidative capacity. However, whether anti-oxidant single nucleotide polymorphisms (SNPs) influence the outcome of ESRD individuals with or without DM has not been tested before.

Methods: We prospectively enrolled multi-center ESRD patients of Han Chinese origin betwen 2002 and 2003, recording their anti-oxidant (superoxide dismutase [SOD2], glutathione peroxidase [GPX1]) and peroxisome proliferator activated receptor-γ (PPAR-γ) genotyping results, and stratified based on DM. They were followed up until 2008, with risk factors for mortality analyzed by Cox proportional hazard regression.

Results: We discovered that diabetic ESRD carriers of CC genotype of SOD2 exon 2 had an increased risk of mortality compared to non-diabetic ones with other genotypes (hazard ratio [HR] 4.04, p = 0.04), while GPX1 SNPs had no influence. Interactions between SOD2 and PPAR-γ SNPs regarding the mortality influence were also detected (for SOD2 CC genotype *x* PPAR-γexon 6 CT genotype, HR 3.19, p = 0.008), suggesting the importance of considering a combination panel of SNPs

on patient survival.

Conclusion: This might be the largest study focusing on the relationship between anti-oxidant SNPs and the outcomes of diabetic ESRD patients of Han Chinese origin. More studies are needed to validate our findings.

Introduction

Diabetes mellitus (DM) is characterized by a state of increased oxidative stress, resulting from the hyperglycemia-induced formation of advanced glycation endproduct (AGE) and other glycated molecules, as well as an impairment in anti-oxidative capacity.[1] Increased oxidative stress, elevated inflammation severity, and metabolic perturbation in patients with DM potentially lead to endothelial injury, coagulation abnormality, and the development of multiple diabetic complications, including nephropathy and microvascular damages.[1,2] Monitoring redox status using reactive oxygen species (ROS) markers (malondialdehyde, advanced oxidation protein products, or urine isoprostane) or anti-oxidants levels (erythrocyte glutathione levels or superoxide dismutase [SOD] activities) has also been found to correlate with disease activity and therapeutic responses among patients with DM.[3,4] Patients with chronic kidney disease and end-stage renal disease (ESRD) also exhibit a pro-inflammatory status and have higher levels of oxidative stress, leading to an increased risk of developing cardiovascular diseases.[5]

Judging from the importance of oxidative stress and anti-oxidants in the pathogenesis of DM and ESRD, it is likely that polymorphisms of anti-oxidant genes might affect the risk of developing DM, progression to ESRD, and the outcomes of diabetic patients with or without ESRD. Several reports have suggested that single nucleotide polymorphisms (SNPs) of SOD2 and glutathione peroxidase 1 (GPX1) were associated with altered risk of diabetic nephropathy, neuropathy, and coronary artery disease among patients with DM.[6,7] Certain SNP of the GPX1 gene is also found to increase the risk of renal events among patients with type 1 DM, and correlates with higher plasma ROS markers.[8] Previously, we also discovered that TT genotype of exon 2 of the SOD2 gene (rs4880) conferred a significantly lower risk of presenting ESRD among patients without DM (p = 0.014).[9] On the other hand, very few studies address the effect of anti-oxidant SNPs on the survival of ESRD patients. Anecdotal reports suggest that SOD1 allelic variations is associated with higher risk of mortality, especially of cardiovascular causes, among patients with DM, and increased oxidative stress has been attributed as the main culprit.[10] Subgroup analysis from a cohort of patients with myocardial infarction and chronic kidney disease (CKD) revealed that a SNP of paraoxonase 1, a lipoprotein antioxidant, also increased their mortality.[11] However, none of the existing studies focus on the effect of SNPs on the mortality of ESRD patients of Han Chinese population; furthermore, study addressing the interaction between DM, anti-oxidant SNPs, and SNPs of metabolism-related genes such as peroxisome proliferator activated receptor-γ (PPAR-γ) is still unavailable. In addition, identifying SNPs with outcome influence in ESRD patients can help uncover important pathophysiologic mechanism with treatment potential. Consequently, using a prospectively collected cohort, we investigated the effect of different anti-oxidant SNPs, PPAR-γ SNPs, DM, and their

interactions on the survival of ESRD patients of Han Chinese origin.

Material and Methods

Study design and the enrollment of participants

This study addressed the follow-up results of ESRD Han Chinese patients with different anti-oxidant and PPAR-γ SNPs.[9,12,13] DM was treated as the dividing variables in this study to better characterize their impact on survival. All the ethical review boards of the participating institutes approved the study protocol (National Taiwan University Hospital), and participants provided written informed consent. The study protocol adheres to the Declaration of Helsinki. In brief, patients were prospectively recruited from medical centers, regional hospitals, and dialysis clinics from Taiwan between 2002 and 2003. All participants were interviewed and their demographic profiles (age, gender), dialysis duration, comorbidities (DM) at enrollment, and the causes of ESRD were recorded. DM was defined according to patients' history or laboratory findings of 2 episodes of fasting glucose levels higher than 126 mg/dL. Laboratory profiles, including hemogram and serum biochemistry panels, were collected on enrollment for subsequent analysis. Participants were prospectively followed up until death or the end of 2008, whichever occurred first.

Determination of SNPs among the ESRD participants

The genotyping process has also been explained in detail previously.[9,13] In essence, leukocyte-derived genomic DNA was used for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), to determine the SNP status of anti-oxidant genes including SOD2 (V16A or *rs*4880) and GPX1 (P200L or *rs*1050450), and also of PPAR-γ (P12A or *rs*1801282 and C161T or *rs*3856806). Primer pairs for each SNP fragments can be retrieved according to the existing literature.[9,13] For rs1801282, the primer pairs are 5'-GCCAATTCAAGCCCAGTC-3' and 5'-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3'. For rs3856806, the primer pairs are 5'-TTTGACTGAACCCCCTGTTG-3' and 5'-CAGAATAGTGCAACTGGAAGA-3'. For rs1050450, the primer pairs are 5'-GCTTCCAGACCATTGACATC-3' and 5'-TCCCAAATGACAATGACACAG-3'. For rs4880, the primer pairs are 5'-CTGACCGGGCTGTGCTTTCT-3' and 5'-CAACGCCTCCTGGTACTTCT-3'.

For rs1801282, restriction enzyme BstUI was used, generating restriction sites (CG/CG); afterwards, GG genotype was recognized if fragments of 227 bp and 43 bp were found on gel, while CG or CC genotype were recognized if fragments of 270 bp, 227 bp, 43 bp or a single 270 bp fragment were found. For rs3856806, restriction enzyme NlaIII was used, generating two fragments (245 bp and 101 bp) if CC genotype, four fragments (245 bp, 204 bp, 101 bp, and 41 bp) if CT genotype, or three segments (204 bp, 101 bp, and 41 bp) if TT genotype. For rs1050450, ApaI was used, generating two fragments (261 bp and 53 bp) if CC genotype, three fragments (314 bp, 261 bp, and 53 bp) if CT genotype, or a single fragment (314 bp) if TT genotype. For rs4880, BsaWI was used, generating two fragments (175 bp and 50 bp) if TT genotype, three fragments (225 bp, 175 bp, and 50 bp) if CT genotype,

or a single fragment (225 bp) if CC genotype.

Statistical analysis

We performed statistical analysis using the SAS, 9.1.3 software (SAS Institute Inc., Cary, NC, U.S.A.) and the R, 2.11.1 software (R Foundation for Statistical Computing, Vienna, Austria). We described continuous variables as mean ± standard deviation (SD), whereas categorical variables were described as frequencies with percentages. In order to investigate the effect of these SNPs and their interaction with DM on the outcomes of ESRD patients, we first compared the clinical features, laboratory results, and SNP statuses between ESRD patients with and without comorbid DM. Factors potentially influencing patient survival were evaluated first in univariate analysis using chi-square test, Fisher's exact test, two-sample *t* test, Wilcoxon rank-sum test, and log-rank test, as appropriate. Finally, Cox proportional hazards regression modeling was utilized to examine the effect of risk factors on the probability of ESRD patient survival, incorporating demographic profiles, DM or not, laboratory data, SNP statuses, and interaction variables, using stepwise variable selection methods. The interaction term sets considered in our regression were provided in the supplementary file. All the significant and non-significant covariates in univariate analysis and their interaction terms (or moderators) were put on the variable list to be selected.

To ensure the quality of analysis results, model-fitting techniques for variable selection and regression diagnostics were used. All the univariate significant and non-significant covariates and their interaction variables were selected and the significance levels for entry (SLE) and for stay (SLS) were set to 0.15 or larger (up to 0.35). The best final model was identified by reducing the significance levels to 0.05.

Grønnesby-Borgan GOF test was used to assess the goodness-of-fit of the fitted Cox models. The generalized additive models (GAM) were applied to detect nonlinear effects of continuous covariates. In statistical testing, two-sided *p* value ≤ 0.05 was considered statistically significant.

Results

Clinical features of participants

A total of 671 ESRD patients of Han Chinese origin were enrolled from the participating institutes, with an average age of 58.9 ± 14.6 years and 48.7% male. ESRD patients with comorbid DM had significantly higher age ($p < 0.001$), shorter duration of dialysis ($p = 0.003$), and were more likely to have DMN-related ESRD ($p < 0.001$) (Table 1). ESRD patients with comorbid DM also had significantly lower serum albumin ($p = 0.002$), creatinine ($p < 0.001$), and higher ferritin levels ($p = 0.003$) than those without DM.

Genotyping results

Among the ESRD Han Chinese participants, TT genotype of rs4880, CC genotype of rs1050450, CC genotype of rs3856806, and CC genotype of rs1801282 were the most common SNPs observed (Table 1). There were no significant difference regarding the distribution of rs4880 (p = 0.205), rs1050450 (p = 0.301), and PPAR-γ (p = 0.452 for rs3856806 and 0.46 for rs1801282) genotypes between ESRD patients with and without DM (Table 1).

Follow-up results and risk factors influencing patient survival

During a mean 4.1 years of follow up, 209 (31.1%) patients died. ESRD patients who survived were significantly younger ($p < 0.001$), less likely to have DM ($p < 0.001$) and DMN-related ESRD ($p < 0.001$) than those who died (Table 2). Survivors had significantly higher hematocrit ($p = 0.009$), serum albumin ($p < 0.001$), cholesterol ($p = 0.019$), creatinine ($p < 0.001$), and potassium ($p = 0.049$) levels than non-survivors, but lower serum ferritin levels ($p = 0.004$). There was no significant difference in genotypes of rs4880, rs1050450, rs1801282, and rs3856806 between survivors and non-survivors (Table 2).

Among those with DM, survivors were also found to be significantly older ($p < 0.001$), had higher hematocrit ($p = 0.01$), serum albumin ($p < 0.001$), and creatinine ($p < 0.001$) than non-survivors (Table 3). Similarly, among those without DM, survivors were significantly older ($p < 0.001$), had higher serum albumin ($p < 0.001$), creatinine ($p < 0.001$), and lower serum ferritin ($p = 0.015$) than non-survivors (Table 4). In both groups, no significant difference was observed in tested SNPs (rs4880, rs1050450, rs1801282, and rs3856806) between survivors and non-survivors.

We next performed multiple Cox proportional hazards regression incorporating demographic profile, DM, and antioxidant gene SNP statuses, as well as their interaction variables. Considering rs4880 and rs1050450 only, we discovered that advanced age (hazard ratio [HR] 1.067 per year, 95% confidence interval [CI] 1.053-1.08), comorbid DM (HR 2.33, 95% CI 1.768-3.07), and the co-occurrence of DM and rs4880 CC genotype (HR 4.043, 95% CI 1.065-15.355) significantly increased the risk of mortality among ESRD patients of Han Chinese origin (Table 5). We further added the PPAR-γ SNPs-related covariates into the original Cox model, and found that the co-occurrence of DM and rs3856806 CC genotype (HR 2.783, 95% CI

2.029-3.816) or CT genotype (HR 2.099, 95% CI 1.43-3.081) also elevated the risk of mortality among these patients. Furthermore, we found that ESRD patients carrying both rs4880 CC genotype and rs3856806 CT genotype also had higher risk of mortality (HR 3.186, 95% CI 1.345-7.548), while those carrying both rs1801282 CG or GG genotypes and rs3856806 CT or TT genotypes had lower risk of mortality (HR 0.554, 95% CI 0.329-0.935) (Table 6).

Discussion

In the current study, we discovered that anti-oxidant gene SNPs interacted with DM in modifying the survival of ESRD patients of Han Chinese origin. Specifically, ESRD carriers of rs4880 CC genotypes and rs3856806 CT genotypes, and those with DM and carrying rs3856806 CC/CT genotypes had significantly higher risk of mortality, while rs1050450 did not influence their risk of mortality among ESRD patients. On the other hand, ESRD patients with rs1801282 and rs3856806 at the same time had lower risk of mortality compared to others without such SNPs. A complex interaction of biologic effects between DM, PPAR-γ SNPs, and SOD2 SNPs can thus be demonstrated among patients with ESRD.

Disturbance of oxidant-antioxidant balance in diabetic patients is reportedly associated with higher risk of adverse events including micro- and macro-vascular complications; although these complications predispose patients with DM to increased mortality, few studies specifically focus on the relationship between SNPs and the survival of diabetic patients. Among a large group of Caucasian patients, Neves *et al*. reported that the presence of 3 variants of the SOD1 gene was associated with higher cardiovascular mortality during follow-up among diabetic participants.[10] Our findings further add to the literature by showing that the presence

of DM in ESRD patients carrying CC genotype of rs4880 was associated with higher risk of mortality (Table 3). However, the fold change of risk increase differs between diabetic patients with and without ESRD and between those of different ethnic origin. For Caucasians without ESRD, the HR was 1.7 to 1.8, while for Han Chinese with ESRD, the HR rose to 4 (Table 5).[10] This discrepancy in the size of SNP influences suggests that ethnic differences and the comorbidities might be an important modifier for the effect of SNPs on patient outcomes.

It is interesting to find that the risk allele of anti-oxidant SNPs is consistent for the risk of ESRD and for the survival of ESRD patients. TT genotype of rs4880 was found to lower the risk of ESRD among non-diabetic patients previously[9]; we observed that CC genotype of rs4880 increased the risk of mortality among ESRD patients with DM (Table 5). This raises the possibility that C allele can be risk factor for developing ESRD and mortality for ESRD patients, while this association is modified by the presence or absence of DM, at least among Han Chinese patients. Others have derived findings that among Caucasian or Chinese patients, carrying C allele predicts poorer survival among those with different cancers[14,15], although a study on Mexican population suggested that diabetic patients with T allele of rs4880 had a significantly higher risk of macroalbuminuria than C allele carriers.[16] Ethnicity-specific SOD2 effect on patient outcomes should then be factored into consideration when interpreting our results.

Interactions between SNPs of different biological pathway-related genes have been found to affect patient outcomes. A large community-based study showed that significant interactions regarding the effect on patient outcomes were noted between SNPs of inflammation and metabolism related genes, including interferon-γ, C-reactive protein, endothelial nitric oxide synthase, and PPAR-γ.[17] Another study also disclosed an interaction between SNPs of PPAR-γ and those of epoxide hydrolase regarding the risk of precipitating chronic obstructive pulmonary disease.[18] We previously reported that PPAR-γ SNPs interacted significantly with anti-oxidant SNPs regarding their influences on the risk of ESRD among Han Chinese patients.[9] In the current study, we further demonstrated that interactions between PPAR-γ and anti-oxidant SNPs also affect the mortality of ESRD patients (Table 6). Since increased ROS levels frequently co-exist with inflammation, insulin resistance, and metabolic derangement in animal models and diabetic individuals[19,20], the SNP interactions we found might assist in estimating the outcomes of ESRD patients with DM. Determining SOD2 and PPAR-γ SNPs could be an important component during the prediction of survival in Han Chinese ESRD population, and presumably anti-oxidants might be prognostically beneficial for those with specific SOD2 and PPAR-γ genotypes.

The mechanisms underlying the relationship between SOD2, PPAR-γSNPs and ESRD patient survival might be explained by several putative reasons. Hyperglycemia among diabetic patients leads to the formation of AGE and increased oxidative stress, impairing nitric oxide release and preventing vasodilatation.[1,21] ROS levels are known to be increased among ESRD patients, and rise further with carriers of specific SOD2 SNPs and with hyperglycemia.[22,23] The resultant vasculopathy might precipitate cardiovascular events, increase diabetic

complications, and potentially link with higher mortality. Indeed, several studies revealed that exon 2 SNPs of SOD2 were associated with higher risk of diabetic retinopathy and nephropathy, both risk factors for mortality among ESRD patients.[16,24,25] On the other hand, PPAR-γ exon B and exon 6 SNPs are reportedly associated with higher inflammatory severity and an increased risk of coronary artery disease, diabetic nephropathy among patients with DM and ESRD.[13,26,27] Finally, these SNPs might be surrogate markers for other risk-bearing genetic traits through linkage disequilibrium. The above reasons might underlie the susceptibility to adverse outcomes among ESRD patients carrying specific variants of the PPAR-γgene.

Our study has several limitations. The ESRD patients we enrolled were of the same ethnic origin, and extrapolating our findings to ESRD patients of other geographic regions might not be feasible. We did not record the medication history nor serially document laboratory data at multiple time points during the follow-up period among these patients, rendering the calculation of time-averaged data difficult. Anti-oxidant SNPs are expected to influence the risk of cardiovascular events more prominently than that of infectious disease or cancer-related complications, but we did not have the causes of death in our cohort for analysis. In addition, it is possible that the levels of serum oxidative stress markers differ between ESRD patients with different SNPs of the tested anti-oxidant genes and PPAR-γ, although we did not have such data. Nonetheless, we believe that the size of our cohort and the use of regression analysis can partially offset these concerns. Most important of all, the relationship between anti-oxidant, PPAR-γ SNPs and ESRD patient mortality has not been addressed before, and our findings can contribute significantly to the existing literature. Further study is needed to confirm

and extend our results.

Conclusion

Diabetic patients frequently exhibit a status of increased oxidative stress, and those with progression to end-stage renal disease are at an even higher risk. For these patients, genetic determinants of anti-oxidant capacity can be an important defense mechanism against the adverse effect of oxidative stress; however, whether this relationship exists or not has not been tested before. Among a large cohort of Han Chinese patients, we discovered that diabetic ESRD carriers of CC genotype of rs4880 had an increased risk of mortality compared to non-diabetic ones with other genotypes, while GPX1 SNPs (rs1050450) had no influence on the mortality of ESRD patients. An interaction between SOD2 and PPAR-γ SNPs regarding the influence was also observed. This can be the largest study addressing the relationship between anti-oxidant SNPs and diabetic ESRD patient outcomes, and more studies might be needed to validate our findings.

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Competing Interest

The authors declare that they have no competing interest.

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Authors' contributions

CTC, JWH, CKC, YCC, CCF, CCC, and CJY conceived the idea, and collected the required data. CTC, JWH, CKC, YCC, FCH, CCF, CCC, and CJY carried out the statistical analyses, interpreted the data, and drafted the manuscript. All authors approved the submission of this manuscript.

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Table 1. Comparisons of demographic and clinical characteristics between the ESRD patients without and with DM

Variable	ESRD patients without DM (n = 418, 62.3%)	ESRD patients with DM (n = 253, 37.7%)	All patients (n = 671)	*p* value
Age (years)	56.4 ± 15.4	62.9 ± 12.0	58.9 ± 14.6	< 0.001
Gender				0.75
Female	212 (50.7%)	132 (52.2%)	344 (51.3%)	
Male	206 (49.3%)	121 (47.8%)	327 (48.7%)	
DM-caused ESRD	0 (0%)	179 (70.8%)	179 (26.7%)	< 0.001
Duration of dialysis (months)	72.6 ± 56.9	55.8 ± 50.2	66.2 ± 55.1	0.003
Laboratory profiles				
Hct	30.8 ± 4.7	30.6 ± 4.1	30.7 ± 4.5	0.971
Albumin (gm/dl)	3.9 ± 0.4	3.8 ± 0.5	3.9 ± 0.5	0.002
Cholesterol (mg/dl)	184.7 ± 46.1	181.6 ± 45.3	183.5 ± 45.8	0.483
Creatinine (mg/dl)	11.6 ± 2.4	10.6 ± 2.5	11.2 ± 2.5	< 0.001
Uric acid (mg/dl)	7.6 ± 1.5	7.7 ± 1.7	7.7 ± 1.6	0.978
K (meq/l)	4.6 ± 0.8	4.5 ± 0.9	4.6 ± 0.9	0.278
Tranferrin saturation	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.241
Ferritin (ng/ml)	511.7 ± 408.4	633.9 ± 703.2	557.4 ± 540.7	0.003
Genotyping results				
rs4880 genotype				0.205
CC	11 (2.6%)	6 (2.4%)	17 (2.5%)	
CT	134 (32.1%)	65 (25.7%)	199 (29.7%)	
TT	273 (65.3%)	182 (71.9%)	455 (67.8%)	
rs1050450 genotype				0.301
CC	378 (90.4%)	222 (87.7%)	600 (89.4%)	
TC	40 (9.6%)	31 (12.3%)	71 (10.6%)	
TT	0 (0%)	0 (0%)	0 (0%)	
rs3856806 genotype				0.452
CC	220 (52.6%)	146 (57.7%)	366 (54.5%)	
CT	165 (39.5%)	89 (35.2%)	254 (37.9%)	

TT	33 (7.9%)	18 (7.1%)	51 (7.6%)	
rs1801282 genotype				0.46
CC	382 (91.4%)	227 (89.7%)	609 (90.8%)	
CG	32 (7.7%)	25 (9.9%)	57 (8.5%)	
GG	4 (1.0%)	1 (0.4%)	5 (0.7%)	
Death	90 (21.5%)	119 (47.0%)	209 (31.1%)	< 0.001

The sample statistics presented in this table were mean ± standard deviation (SD) for continuous variables and frequency (percentage, %) for categorical variables. The listed *p*-values of statistical tests were calculated using the Wilcoxon rank-sum test for continuous variables and the Fisher's exact test for categorical variables.

Abbreviations: DM, diabetes mellitus; DMN, diabetic nephropathy; ESRD, end-stage renal disease; Hct, hematocrit

Table 2. Comparisons of demographic and clinical characteristics between the alive and dead ESRD patients

Variable	Alive (n = 462, 68.9%)	Dead (n = 209, 31.1%)	All (n = 671)	*p* value
Age (years)	54.9 ± 13.4	67.6 ± 13.2	58.9 ± 14.6	< 0.001
Gender				0.405
Female	242 (52.4%)	102 (48.8%)	344 (51.3%)	
Male	220 (47.6%)	107 (51.2%)	327 (48.7%)	
DM	134 (29.0%)	119 (56.9%)	253 (37.7%)	< 0.001
DM-caused ESRD	92 (19.9%)	87 (41.6%)	179 (26.7%)	< 0.001
Duration of dialysis (months)	68.7 ± 57.1	60.9 ± 50	66.2 ± 55.1	0.235
Laboratory profiles				
Hct	31.0 ± 4.3	30.1 ± 4.7	30.7 ± 4.5	0.009
Albumin (gm/dl)	4.0 ± 0.4	3.7 ± 0.5	3.9 ± 0.5	< 0.001
Cholesterol (mg/dl)	186.3 ± 44.9	177.3 ± 47.2	183.5 ± 45.8	0.019
Creatinine (mg/dl)	11.6 ± 2.4	10.3 ± 2.5	11.2 ± 2.5	< 0.001
Uric acid (mg/dl)	7.7 ± 1.6	7.5 ± 1.6	7.7 ± 1.6	0.267
K (meq/l)	4.6 ± 0.8	4.5 ± 1.0	4.6 ± 0.9	0.049
Tranferrin saturation	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.388
Ferritin (ng/ml)	505.3 ± 375.1	676.1 ± 787.4	557.4 ± 540.7	0.004
Genotyping results				
rs4880 genotype				0.245
CC	11 (2.4%)	6 (2.9%)	17 (2.5%)	
CT	146 (31.6%)	53 (25.4%)	199 (29.7%)	
TT	305 (66.0%)	150 (71.8%)	455 (67.8%)	
rs1050450 genotype				0.892
CC	412 (89.2%)	188 (90.0%)	600 (89.4%)	
TC	50 (10.8%)	21 (10.0%)	71 (10.6%)	
TT	0 (0%)	0 (0%)	0 (0%)	
rs3856806 genotype				0.3
CC	244 (52.8%)	122 (58.4%)	366 (54.5%)	
CT	179 (38.7%)	75 (35.9%)	254 (37.9%)	
TT	39 (8.4%)	12 (5.7%)	51 (7.6%)	
rs1801282 genotype				0.365

CC	416 (90.0%)	193 (92.3%)	609 (90.8%)
CG	41 (8.9%)	16 (7.7%)	57 (8.5%)
GG	5 (1.1%)	0 (0%)	5 (0.7%)

The sample statistics presented in this table were mean ± standard deviation (SD) for continuous variables and frequency (percentage, %) for categorical variables. The listed *p*-values of statistical tests were calculated using the Wilcoxon rank-sum test for continuous variables and the Fisher's exact test for categorical variables.

Abbreviations: DM, diabetes mellitus; DMN, diabetic nephropathy; ESRD, end-stage renal disease; Hct, hematocrit

Table 3. Comparisons of demographic and clinical characteristics between the alive and dead ESRD patients with DM

Variable	Alive diabetic patients (n = 134, 53.0%)	Dead diabetic patients (n = 119, 47.0%)	All (n = 253)	*p* value
Age (years)	59.2 ± 10.6	67.2 ± 12.1	62.9 ± 12	< 0.001
Gender				0.705
Female	68 (50.7%)	64 (53.8%)	132 (52.2%)	
Male	66 (49.3%)	55 (46.2%)	121 (47.8%)	
DM-caused ESRD	92 (68.7%)	87 (73.1%)	179 (70.8%)	0.49
Duration of dialysis (months)	61 ± 61	58.3 ± 44.5	59.7 ± 53.8	0.452
Laboratory profiles				
Hct	31.2 ± 3.9	29.9 ± 4.2	30.6 ± 4.1	0.01
Albumin (gm/dl)	3.9 ± 0.4	3.7 ± 0.5	3.8 ± 0.5	< 0.001
Cholesterol (mg/dl)	184.9 ± 44	177.8 ± 46.6	181.6 ± 45.3	0.246
Creatinine (mg/dl)	11.2 ± 2.5	9.8 ± 2.3	10.6 ± 2.5	< 0.001
Uric acid (mg/dl)	7.8 ± 1.7	7.5 ± 1.7	7.7 ± 1.7	0.133
K (meq/l)	4.6 ± 0.8	4.5 ± 1.0	4.5 ± 0.9	0.225
Tranferrin saturation	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.087
Ferritin (ng/ml)	557.3 ± 343.6	721.5.1 ± 956.9	633.9 ± 703.2	0.494
Genotyping results				
rs4880 genotype				0.2
CC	2 (1.5%)	4 (3.4%)	6 (2.4%)	
CT	40 (30%)	25 (21%)	65 (25.7%)	
TT	92 (68.6%)	90 (75.6%)	182 (71.9%)	
rs1050450 genotype				0.344
CC	115 (85.8%)	107 (89.9%)	222 (87.7%)	
TC	19 (14.2%)	12 (10.1%)	31 (12.3%)	
TT	0 (0%)	0 (0%)	0 (0%)	
rs3856806 genotype				0.304
CC	72 (53.7%)	74 (62.2%)	146 (57.7%)	
CT	50 (37.3%)	39 (32.8%)	89 (35.2%)	
TT	12 (9%)	6 (5%)	18 (7.1%)	
rs1801282 genotype				0.912
CC	119 (88.8%)	108 (90.8%)	227 (89.7%)	
CG	14 (10.4%)	11 (9.2%)	25 (9.9%)	

GG	1 (0.7%)	0 (0%)	1 (0.4%)

The sample statistics presented in this table were mean ± standard deviation (SD) for continuous variables and frequency (percentage, %) for categorical variables. The listed *p*-values of statistical tests were calculated using the Wilcoxon rank-sum test for continuous variables and the Fisher's exact test for categorical variables.

Abbreviations: DM, diabetes mellitus; DMN, diabetic nephropathy; ESRD, end-stage renal disease; Hct, hematocrit

Table 4. Comparisons of demographic and clinical characteristics between the alive and dead ESRD patients without DM

Variable	Alive non-diabetic patients (n = 328, 78.5%)	Dead non-diabetic patients (n = 90, 21.5%)	All (n = 418)	*p* value
Age (years)	53.2 ± 14	68.2 ± 14.5	56.4 ± 15.4	< 0.001
Gender				0.075
Female	174 (53%)	38 (42.2%)	212 (50.7%)	
Male	154 (47%)	52 (57.8%)	206 (49.3%)	
Duration of dialysis (months)	78.7 ± 60.6	74.3 ± 62.7	77.7 ± 61	0.449
Laboratory profiles				
Hct	30.9 ± 4.5	30.4 ± 5.3	30.8 ± 4.7	0.16
Albumin (gm/dl)	4 ± 0.4	3.7 ± 0.4	3.9 ± 0.4	< 0.001
Cholesterol (mg/dl)	186.9 ± 45.4	176.6 ± 48.1	184.7 ± 46.1	0.047
Creatinine (mg/dl)	11.8 ± 2.4	10.9 ± 2.6	11.6 ± 2.4	0.002
Uric acid (mg/dl)	7.7 ± 1.5	7.6 ± 1.5	7.6 ± 1.5	0.882
K (meq/l)	4.6 ± 0.8	4.5 ± 0.9	4.6 ± 0.8	0.195
Tranferrin saturation	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.96
Ferritin (ng/ml)	484.4 ± 385.6	615.4 ± 473.6	511.7 ± 408.4	0.015
Genotyping results				
rs4880 genotype				0.97
CC	9 (2.7%)	2 (2.2%)	11 (2.6%)	
CT	106 (32.3%)	28 (31.1%)	134 (32.1%)	
TT	213 (64.9%)	60 (66.7%)	273 (65.3%)	
rs1050450 genotype				0.841
CC	297 (90.5%)	81 (90%)	378 (90.4%)	
TC	31 (9.5%)	9 (10%)	40 (9.6%)	
TT	0 (0%)	0 (0%)	0 (0%)	
rs3856806 genotype				0.933
CC	172 (52.4%)	48 (53.3%)	220 (52.6%)	
CT	129 (39.3%)	36 (40%)	165 (39.5%)	
TT	27 (8.2%)	6 (6.7%)	33 (7.9%)	
rs1801282 genotype				0.55
CC	297 (90.5%)	85 (94.4%)	382 (91.4%)	

CG	27 (8.2%)	5 (5.6%)	32 (7.7%)
GG	4 (1.2%)	0 (0%)	4 (1%)

The sample statistics presented in this table were mean ± standard deviation (SD) for continuous variables and frequency (percentage, %) for categorical variables. The listed *p*-values of statistical tests were calculated using the Wilcoxon rank-sum test for continuous variables and the Fisher's exact test for categorical variables.

Abbreviations: DM, diabetes mellitus; DMN, diabetic nephropathy; ESRD, end-stage renal disease; Hct, hematocrit

Table 5. Multivariate analysis of the predictors of time to death by fitting multiple Cox's proportional hazards model with the stepwise variable selection method

Covariate	Regression Coefficient	Standard Error	*z* Value	*p* Value	Hazard Ratio	Lower 95% Limit	Upper 95% Limit
Age (years)	0.064	0.006	9.892	< 0.001	1.067	1.053	1.080
DM	0.846	0.141	6.006	< 0.001	2.330	1.768	3.070
DM × rs1801282 CC genotype	1.397	0.681	2.052	0.04	4.043	1.065	15.355

* Cox's proportional hazards model: *n* = 671, number of events = 209, concordance = 0.751 (se = 0.021).

Abbreviations: DM, diabetes mellitus

Table 6. Multivariate analysis of the predictors of time to death by fitting multiple Cox's proportional hazards model with the stepwise variable selection method

Covariate	Regression Coefficient	Standard Error	*Z* Value	*p* Value	Hazard Ratio	Lower 95% Limit	Upper 95% Limit
Age (years)	0.066	0.006	10.212	< 0.001	1.069	1.055	1.082
DM × rs3856806 CC genotype	1.024	0.161	6.353	< 0.001	2.783	2.029	3.816
DM × rs3856806 CT genotype	0.741	0.196	3.787	< 0.001	2.099	1.430	3.081
DM × rs3856806 TT genotype	0.367	0.492	0.746	0.456	1.443	0.550	3.784
DM × rs4880 CC genotype	1.318	0.641	2.057	0.040	3.737	1.064	13.117
rs1801282 non-CC genotype × rs3856806 non-CC genotype	-0.590	0.266	-2.214	0.027	0.554	0.329	0.935
rs4880 CC genotype × rs3856806 CT genotype	1.159	0.440	2.633	0.008	3.186	1.345	7.548

* Cox's proportional hazards model: n = 671, number of events = 209, concordance = 0.7629 (se = 0.0207).

Other significant variables included rs1801282 GG genotype (hazard ratio < 1 x 10^6, p < 0.001) and rs4880 CC genotype × rs3856806 TT genotype (hazard ratio < 1 x 10^7, p < 0.001)

Abbreviations: DM, diabetes mellitus