

Single-nucleotide polymorphism of the *UMOD* promoter is associated with the outcome of chronic kidney disease patients

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Abstract. Uromodulin (*UMOD*) is the most abundant protein secreted in urine and the mutated form of the *UMOD* gene is associated with *UMOD*-associated kidney disease (UAKD). Although *UMOD* accumulates in the kidney of UAKD patients, it is unclear whether this also occurred in the chronic kidney disease (CKD) patients. Therefore, the association of single-nucleotide polymorphisms (SNPs) in the promoter region of *UMOD* gene with the kidney survival time of CKD was investigated. The promoter region of the *UMOD* gene was sequenced for 111 CKD patients. The Kaplan-Meier method was used to identify the disease outcome associated with SNPs in the promoter region of the *UMOD* gene in CKD patients. The Cox proportional hazard model was used to identify risk factors for the kidney survival time of CKD. SNPs in reference to GenBank accession NG-000016 were detected at 23 sites of the 481-bp in the *UMOD* promoter region from the CKD patients and the healthy controls. The 6 SNPs with minor allele frequency >5% in the CKD patients or controls were used for disease risk and outcome analysis. The frequent allele rs13333226AA was associated with a shorter period of kidney survival time in CKD patients ($P=0.005$). The length of kidney survival time in CKD patients with the rs13333226AA genotype was significantly shorter than that of patients with the frequent allele rs13333226AG+GG (relative risk, 0.361; 95% confidence interval, 0.171-0.761; $P=0.007$). In conclusion, analysis of genetic polymorphisms in *UMOD* may help to identify the CKD patient subgroups at a high risk for a disease outcome, thereby helping to refine therapeutic decisions in CKD patients.

Introduction

Chronic kidney disease (CKD) constitutes a serious public health burden worldwide (1). In line with the global trend, the CKD prevalence is also increasing in developing countries (2,3). Recently, based on a report published in the Lancet in 2012, CKD prevalence increases with age and affects >10% of the Chinese adult population (4); the estimated prevalence of CKD in China is similar to that reported for developed countries, such as the USA (13%) (5) and Norway (10%) (6). CKD can progress to end-stage renal disease (ESRD) that requires dialysis or transplantation. In addition, CKD causes substantial morbidity and is an independent risk factor for cardiovascular disease (7) and over mortality (8).

The major known risk factors for CKD are hypertension and diabetes (9). However, only a few of the individuals exposed to these risk factors will develop CKD in their life time, suggesting that individual differences, including genetic susceptibility factors, may be one of the most critical agents in the incidence of CKD. Identification of genetic risk variants for CKD may provide novel insights into underlying biological mechanisms.

Uromodulin (*UMOD*) is the most abundant protein in human urine and has been known for >50 years (10). Since the discovery of *UMOD*, increasing research has been performed illustrating its novel roles. Previous studies have indicated that *UMOD* mutations contribute to familial juvenile hyperuricemia nephropathy, medullary cystic kidney disease 2 (11) and that promoter variants of the *UMOD* gene are associated with the estimated glomerular filtration rate (eGFR), blood pressure, plasma uric acid level and incidence of CKD (12-14). In the present study, the single-nucleotide polymorphisms (SNPs) were genotyped in the promoter region of the *UMOD* gene in CKD patients to assess its association with the kidney outcome of CKD.

Materials and methods

Tissue specimens and DNA extraction. The present study was a prospective trial. Blood samples were obtained from 111 CKD patients at The Fourth Hospital of Hebei Medical University (Hebei, China) between 2002 and 2008. The presence of CKD was defined in accordance with the 2002 National Kidney Foundation Kidney Disease Outcomes

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Quality Initiative classification (15) on the basis of an eGFR of <60 ml/min/1.73 m² for ≥90 days. A previous study in China reported an eGFR using the four-variable modified diet in the renal disease formula (16). The etiologies of CKD in these patients were all chronic glomerulonephritis, including membranous nephropathy (n=64, 57.7%) and non-membranous nephropathy (n=47, 42.3%). The survival period of the kidney was equivalent to the time from CKD diagnosis to ESRD, which was defined as starting dialysis treatment or receiving a renal transplant (17). Total DNA was extracted using a Wizard Genomic DNA extraction kit (Promega Corporation, Madison, WI, USA) and stored at -20°C. The study was approved by the Human Tissue Research Committee of The Fourth Hospital of Hebei Medical University. All the patients provided written informed consent for the collection of the samples and subsequent analysis.

Polymerase chain reaction (PCR) amplification and sequence analysis. The forward 5'-TTTCTCCATCCTGTCCCACT-3' (nucleotide 3501-3482) and reverse 5'-ATTCCAACCCAAACTCCC-3' (nucleotides 3021-3038) primers were used for amplification of a 481-bp product from the *UMOD* promoter region. PCR was performed according to the manufacturer's instructions of the PCR Master mix kit (Promega Corporation) and purified prior to sequencing. The PCR condition consisted of one incubation of 2 min at 95°C, followed by 35 cycles of a 30-sec denaturation at 95°C, a 30-sec annealing at 55°C and a 45-sec extension at 72°C, and a final extension at 72°C for 5 min. Cycle sequencing was performed with the Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystem, Foster City, CA, USA) and the products were separated on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystem). Polymorphisms were confirmed by repeated analyses from the two strands.

Statistical analysis. The kidney survival curve was calculated using the Kaplan-Meier method and compared with the log-rank test. Multivariate survival analysis was performed using a Cox proportional hazards model. All the statistical analyses were performed using the SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). For all the statistical tests, P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 111 CKD patients were enrolled in the present study. At baseline, the mean age of the 111 CKD patients was 57.1±16.0 years and 54.1% (n=60) were males. The mean eGFR was 82.3±11.6 ml/min/1.73 m², with 65.8% (n=73) of patients having an eGFR <60 ml/min/1.73 m². All the participants had an eGFR >30 ml/min/1.73 m² (Table I). A review was conducted every 6 months over a 5-year period. None of these patients were lost to follow-up in the 5 years. Data for the 5-year follow-up period were analyzed with clinical characteristics using the Kaplan-Meier method and were compared by the log-rank test. Age and body mass index were not statistically significant predictors of the length of kidney survival time in CKD patients, but gender, drinking, smoking, proteinuria and eGFR were correlated with kidney survival time in these patients (Table II). The kidney survival rates of

Table I. Baseline characteristics of the CKD participants.

Variables	All participants
Participants, no.	111
Male, %	54.1
Female, %	45.9
Median age, years	57.1±16.0
Drinking, %	
Yes	28.8
No	71.2
Current smoking, %	
Yes	43.2
No	56.8
BMI, kg/m ²	22.2±2.5
Hypertension, %	87.4
Systolic BP, mmHg	159.6±23.9
Diastolic BP, mmHg	87.4±15.7
MAP, mmHg	111.4±16.4
Etiologies of CKD, %	
Membranous nephropathy	57.7
Non-membranous nephropathy	42.3
eGFR, ml/min/1.73 m ²	82.3±11.6
<60, %	65.8

BMI, body mass index; MAP, mean arterial pressure; BP, blood pressure; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

female CKD patients were higher than those of male CKD patients; smoking habit showed an association with survival rates of the kidney in CKD patients when survival time of the kidney in smoking CKD patients was lower than those in non-smoking CKD patients. Consistent with the smoking result, the kidney survival time in drinking CKD patients was also lower than that in non-drinking CKD patients. The kidney survival rates of CKD patients with high proteinuria were also lower than those of the CKD patients with low proteinuria. The survival rates of the kidney in CKD patients with eGFR <60 ml/min/1.73 m² were lower than those of the kidneys in CKD patients with eGFR ≥60 ml/min/1.73 m². These data demonstrated that gender, drinking, smoking, proteinuria and eGFR of baseline were the risk factors for the kidney survival time of the kidney in the CKD patients.

***UMOD* promoter SNPs.** SNPs in reference to GenBank accession NG-000016 were detected at 23 sites of the 481-bp in the *UMOD* promoter region from the CKD patients and the healthy controls. SNPs with a minor allele frequency of <5% were excluded; thus, a remaining 6 SNPs (rs13332873, rs13332898, rs13332923, rs13333144, rs13333226 and rs13333292) were used for further analysis. At first, the distribution of germline SNPs at each site were compared between CKD patients and healthy controls to identify any link between an SNP and CKD risk; no association with CKD disease risk was detected

Table II. Univariate analysis of the clinical characteristics associated with the kidney survival time in chronic kidney disease patients.

Characteristics	Cases, no.	5-year survival rate of kidney, %	χ^2 test	P-value
Age, years				
≤55	49	24.5	0.234	0.629
>55	62	30.6		
Gender				
Male	60	20.0	7.000	0.008
Female	51	37.3		
Drinking				
Yes	32	15.6	8.695	0.003
No	79	32.9		
Smoking				
Yes	48	18.8	4.327	0.038
No	63	34.9		
BMI, kg/m ²				
<20	38	34.2	0.646	0.421
≥20	73	24.7		
MAP, mmHg				
≤105	36	31.9	0.015	0.899
>105	75	23.4		
Etiologies of CKD				
Membranous nephrology	57	26.4	1.245	0.262
Non-membranous nephrology	54	28.3		
Proteinuria				
High, ≥1 g/24 h	71	16.9	11.138	0.001
Low, <1 g/24 h	40	47.5		
eGFR, ml/min/1.73 m ²				
<60	73	14.5	29.420	<0.001
≥60	38	78.3		
<i>UMOD</i> (rs13333226)				
AA	59	20.3	15.331	<0.001
AG+GG	52	36.5		

BMI, body mass index; MAP, mean arterial pressure; CKD, chronic kidney disease; *UMOD*, uromodulin.

in any SNP in the *UMOD* gene, with no P<0.05 levels (data not shown).

Association of UMOD genotype and kidney survival time.

The association between the *UMOD* genotype and survival was compared subsequently. The kidney survival curve was plotted using the Kaplan-Meier method and compared with the log-rank test for all the CKD patients at these sites (rs13332873, rs13332898, rs13332923, rs13333144, rs13333226 and rs13333292). The result indicated that none of them are associated with the kidney survival time, except rs13333226

Table III. Multivariate analysis of prognostic factors associated with the kidney survival time in chronic kidney disease patients by the Cox proportional hazards model.

Factors	Relative risk	95% CI	P-value
Gender	0.703	0.353-1.397	0.314
Drinking	0.823	0.434-1.561	0.551
Smoking	0.848	0.431-1.665	0.631
proteinuria	0.452	0.265-0.772	0.004
eGFR	0.089	0.035-0.234	<0.001
rs13333226 <i>UMOD</i> (AA/AG+GG)	0.361	0.171-0.761	0.007

CI, confidence interval; eGFR, estimated glomerular filtration rate; *UMOD*, uromodulin.

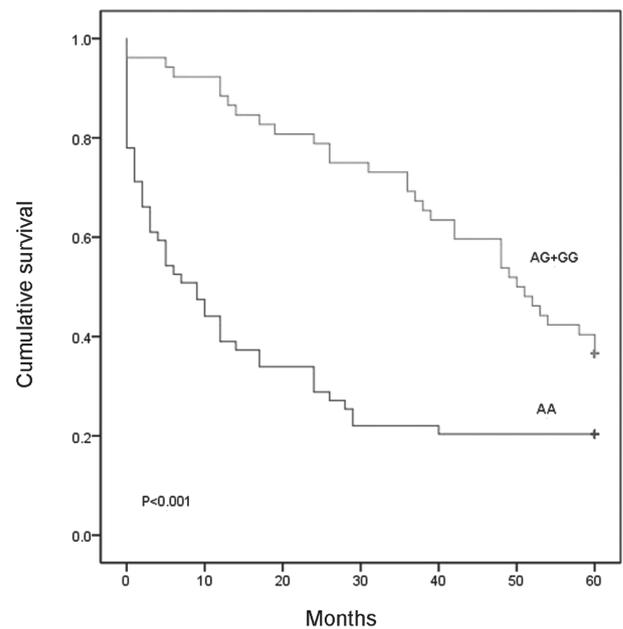


Figure 1. Genotype of *UMOD* rs13333226 SNPs and their association with the kidney survival of chronic kidney disease patients. *UMOD*, uromodulin; SNP, single-nucleotide polymorphism.

(<http://www.ncbi.nlm.nih.gov/snp/>). As shown in Fig. 1, the kidney survival time of CKD patients with rs13333226AA was shorter than that of the CKD patients with rs13333226AG+GG (P<0.001). Multivariate analysis was performed with Cox proportional hazards model, including the factors of gender, smoking, drinking, proteinuria, eGFR and rs13333226. As shown in Table III, the rs13333226 SNP was identified as independent predictors for the kidney survival time in CKD patients. The length of kidney survival time in CKD patients with the rs13333226AA genotype was significantly shorter than that of patients with the frequent allele rs13333226AG+GG [relative risk, 0.361; 95% confidence interval (CI), 0.171-0.761; P=0.007]. The high proteinuria and low eGFR of the baseline were also the risk factors for the kidney survival time of progressive kidney disease. Gender, smoking and drinking

were excluded in the multivariate analysis by the Cox proportional hazards model. These data demonstrated the strong prediction power of rs1333226 SNP on the kidney survival time of progressive kidney disease.

Discussion

UMOD is an 80-90 kDa glycoprotein expressed on the luminal surface of the apical membrane of renal tubular epithelial cells forming the thick ascending limb of Henle (18,19). *UMOD* may be a factor involved in the pathogenic process of kidney disease. Evidence from studies with humans and mice suggest that the role of *UMOD* is multifaceted (20-23). One study identified that the elevated urinary *UMOD* level was a risk factor for the future incidence of CKD (13). However, in diabetes type I it was shown that decreased *UMOD* excretion was a protective factor for renal failure and cardiovascular disease in adults (21). A study with *UMOD* knockout mice indicated that *UMOD* stabilized the outer medulla of the kidney in face of injury by decreasing inflammation (20). In the present study, 111 CKD patients were enrolled to determine the kidney survival-associated germline SNPs in the *UMOD* gene. The SNP rs1333226AA/AG+GG was identified for the association with kidney survival time in CKD patients at statistically significant levels by the log-rank test. Multivariate survival analysis identified the rs1333226 SNP to be an independent prediction marker for kidney survival time in CKD patients.

Therefore, we suggest that the SNP of *UMOD* rs1333226 may be a prognostic factor for kidney survival time in CKD patients. The survival time of kidneys in CKD patients with rs1333226AA genotype was significantly shorter than that of the kidney in CKD patients with rs1333226AG+GG (relative risk, 0.361; 95% CI, 0.171-0.761, $P=0.007$). Gender, drinking and smoking were excluded in the multivariate analysis by the Cox proportional hazards model. The SNP (rs1333226) that was genotyped in the present study is a common SNP that is associated with serum creatinine, blood pressure or plasma uric acid (12,24). The population genetic studies suggested that the SNP (rs1333226) may influence the transcriptional level and thus regulate *UMOD* expression and finally change *UMOD* excretion. However, further cell research should be conducted to confirm the role of the SNP at the rs1333226 site of the *UMOD* promoter region.

Consistent with earlier reports, the present data demonstrated that gender, smoking, drinking, proteinuria and eGFR were associated with the kidney survival time of CKD patients (25-28), which confirmed the validity of the data. The mechanism explained the association among smoking, drinking and CKD progression is that smoking and drinking all contribute to oxidative stress, which plays an important role in the development and progression of CKD (29). The association between gender and CKD progression suggests that sex hormones may be important determinants of the greater susceptibility of males to progressive kidney injury.

In conclusion, the association of common variants of *UMOD* with the kidney survival time in CKD patients was identified, suggesting that *UMOD* has important roles in the progression of CKD. The analysis of genetic polymorphisms in *UMOD* may help to identify CKD patient subgroups at high

risk for a disease outcome, thereby helping to refine therapeutic decisions in CKD patients.

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