UMOD Polymorphisms Associated With Kidney Function, Serum Uromodulin and Risk of Mortality Among Patients With Chronic Kidney Disease, Results From the C-STRIDE study

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Abstract

Background: Several genome-wide association studies have identified single nucleotide polymorphisms (SNPs) in *uromodulin (UMOD)* gene, such as 12917707, rs4293393, rs6497476 and rs13333226, as susceptibility genetic variants associated with chronic kidney disease (CKD) based on the case-control study design. We aimed to explore the associations of the variants with baseline phenotypes and prospective prognosis of CKD among 2731 Chinese patients with CKD stage 1-4. Polymorphisms of rs11864909, rs4293393, rs6497476 and rs13333226 were genotyped using the Sequenom MassARRAY iPLEX platform.

Results: rs13333226 and rs4293393 were in complete linkage disequilibrium. Based on the T dominant model, T allele of rs11864909 was associated with baseline levels of eGFR and serum uromodulin with linear regression coefficients of 2.68 (95% confidence interval [CI]: 0.61, 4.96) and -12.95(95%CI: -17.59, -7.98), respectively, after adjustment for cardiovascular and kidney specific risk factors. After a median follow-up of 4.94 years, both G allele of rs4293393/rs13333226 and C allele of rs6497476 were associated with reduced risk of all-cause mortality with multivariable adjusted hazard ratios of 0.341(95%CI: 0.105, 0.679) and 0.344(95%CI: 0.104, 0.671), respectively. However, no associations were found between the variants and eGFR slope in the linear mix effect model.

Conclusions: Variant of rs11864909 in the *UMOD* gene was associated with levels of eGFR and serum uromodulin, while those of rs4293393 and rs6497476 associated with all-cause mortality among patients with CKD.

Background

Uromodulin, also known as Tamm-Horsfall protein, is the most abundant protein in human urine, which is exclusively synthesized in the thick ascending limb (TAL) of the loop of Henle in the kidney. The protein has important roles in ion transport, maintenance of water and electrolyte balance, protection against urinary tract infection and kidney innate immunity(1, 2). Besides being excreted into tubular fluid through an intracellular route to the apical cell pole, uromodulin can be released into plasma by transport to the basolateral cell site via Golgi apparatus and cytoplasmic vesicles. As measurement of urinary uromodulin may be influenced by specific preanalytic conditions, such as centrifugation, vortexing and conditions and duration of storage, serum uromodulin may represent more stable concentration of the biomarker(3). A recently published prospective cohort study recruiting patients undergoing coronary angiography demonstrated higher level of serum uromodulin was associated with better metabolic profile and reduced risk of mortality after a median 9.9 years of follow-up(4). Consistently, our study team reported lower levels of serum uromodulin were independently associated with higher risk of incident end-stage kidney disease (ESKD) among patients with chronic kidney disease (CKD)(5).

Mutations in the encoding gene of uromodulin, the *UMOD* gene, have been found to be associated with hereditary autosomal-dominant tubulointerstitial diseases(6). In addition, several genome-wide association studies (GWAS) have identified common variants in the promoter region of *UMOD* gene relating to estimated glomerular filtration rate (eGFR), risk of CKD, and urinary/serum uromodulin levels, highlighting the role of uromodulin in the pathophysiology of CKD(7–9). However, most GWASs were conducted among participants of European or African American ancestry, with only one study, the Asian Genetic Epidemiology Network, providing evidence for population in east Asia, where rs11864909, replacing rs12917707, which has a low minor allele frequency among population of east Asia, was identified as the most significant genetic variant of the association with CKD-related traits(10).

Examination of associations between *UMOD* polymorphisms and CKD-related phenotypes and prognosis of the disease has implications in understanding pathophysiology of the disease. As east Asians were not well represented in the previous GWAS studies, further evidence among a cohort of Chinese population may inform consistency and/or difference of the association between ethnicities. Hence, we aimed to explore the association between the candidate single nucleotide polymorphisms (SNP) in promoter region of *UMOD* identified in previous GWAS and traits of CKD, including baseline eGFR, serum uromodulin, prognosis and complications of CKD, among participants in the Chinese Cohort Study of CKD (C-STRIDE).

Results

The flowchart of selecting study participants was shown in Fig. 1. The participants had a mean age of 48.94 years with 59.83% of male and the majority of them were Han ethnicity (93.00% vs. 3.24% of other ethnicities and 3.76% missing). The distribution of all four studied genetic variants complied with Hardy-Weinberg equilibrium (all *P*-values > 0.05). The number of participants with CC, TC and TT genotype of rs11864909 were 1988, 676 and 67, respectively. The measurements for the correlation and magnitude of linkage disequilibrium (LD) between the SNPs were listed in Supplementary table 1. rs13333226 and rs4293393 were in complete LD, so only rs4293393 was used in the following analyses. Patients had lower levels of serum uromodulin, but higher levels of eGFR through the CC, TC to TT genotypes of rs11864909 (both *P*-values < 0.05). Although the A allele of rs4293393 and T allele of rs6497476 were shown to be associated with higher level of both eGFR and serum uromodulin, the differences did not reach statistical significance due to the corresponding alleles accounting for the majority of the population (Fig. 2).

Because of the limited sample size of the TT genotype, we combined TC and TT genotypes of rs11864909 together and compared characteristics of patients between CC versus TC&TT genotypes. Besides higher level eGFR and lower levels of serum uromodulin, patients with TC&TT genotypes of rs11864909 had more male and more carriers of AA genotype of rs4293393 and TT genotype of rs6497476 (all *P*-values < 0.05) (Table 1). The distributions of covariates stratified by genotypes of rs13333226/rs4293393 or rs6497476 were shown in Supplementary tables 2 and 3. We also compared characteristics between those included in and excluded from the analysis. The level of eGFR was comparable between the populations. However, participants included in the analysis were younger, more likely to smoke and have a cardiovascular disease (CVD) history, less likely to have an etiology of glomerulonephritis, had much higher level of urinary albumin-to-creatinine ratio (UACR), but lower level of systolic blood pressure (BP) than those excluded (all *P*-values < 0.05, in Supplementary table 4).

Table 1
Characteristics of study participants stratified by genotypes of rs11864909

Characteristics	Total	C/C	T/C & T/T	*P*-value
	n = 2731	n = 1988	n = 743	
Age, years	48.94 ± 13.81	49.13 ± 13.78	48.42 ± 13.88	0.23
Male, n(%)	1634(59.83%)	1160(58.35%)	474(63.80%)	**0.01**
High school and above, n(%)	1509(55.70%)	1090(55.16%)	419(57.16%)	0.35
Current and ever smoking, n(%)	1043(39.40%)	737(38.35%)	306(42.21%)	0.07
Body mass index, kg/m^2	24.50 ± 3.62	24.42 ± 3.57	24.71 ± 3.74	0.07
Systolic blood pressure, mmHg	129.58 ± 17.98	129.71 ± 17.74	129.25 ± 18.59	0.57
Diastolic blood pressure, mmHg	80.97 ± 10.95	81.08 ± 10.95	80.67 ± 10.94	0.42
Using anti-hypertensive medication, n(%)	1620(73.01%)	1169(73.06%)	451(72.86%)	0.92
Diabetes mellitus, n(%)	625(25.52%)	448(25.13%)	177(26.58%)	0.46
History of CVD, n(%)	277(10.14%)	193(9.71%)	84(11.31%)	0.22
Creatinine, μmol/L	143(100, 207)	145(102, 210)	137(93, 199)	**0.02**
eGFR, ml/min/1.73 m^2	51.54 ± 30.44	50.49 ± 29.98	54.37 ± 31.49	**0.003**
eGFR < 60 ml/min/1.73 m^2, n(%)	1858(68.03%)	1381(69.47%)	477(64.20%)	**0.009**
ACR, mg/g	435.59(114.00, 991.20)	434.85(116.82, 985.43)	437.38(108.20, 1018.61)	0.92
Albuminuria groups, n(%)				0.88
<30 mg/g	315(11.81%)	233(11.99%)	82(11.33%)	
30–299 mg/g	770(28.86%)	562(28.91%)	208(28.73%)	
≥300 mg/g	1583(59.33%)	1149(59.10%)	434(59.94%)	
Uromodulin, ng/mL	91.60 ± 61.37	94.84 ± 62.79	82.99 ± 56.57	**< 0.001**
Etiology of CKD				0.63
Diabetic nephropathy	392(14.77%)	286(14.83%)	106(14.60%)	
Glomerulonephritis	1605(60.47%)	1156(59.96%)	449(61.85%)	
Others	657(24.76%)	486(25.21%)	171(23.55%)	
Genotype of rs13333226				**< 0.001***
AA	2333(85.43%)	1668(83.90%)	665(89.50%)	
GA	389(14.24%)	313(15.74%)	76(10.23%)	
GG	9(0.33%)	7(0.35%)	2(0.27%)	
Genotype of rs4293393				**< 0.001***
AA	2333(85.43%)	1668(83.90%)	665(89.50%)	
GA	389(14.24%)	313(15.74%)	76(10.23%)	
GG	9(0.33%)	7(0.35%)	2(0.27%)	
Genotype of rs6497476				**< 0.001***
TT	2382(87.22%)	1693(85.16%)	689(92.73%)	
TC	342(12.52%)	288(14.49%)	54(7.27%)	
CC	7(0.26%)	7(0.35%)	0(0.00%)	

Note: Number of missing: education-22, smoking status-84, body mass index-263, systolic blood pressure – 379, diastolic blood pressure – 379, using anti-hypertensive medication-512, diabetes mellitus-282, etiology of CKD-77, ACR-63, uromodulin-327.

Abbreviation: CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; ACR, albumin creatinine ratio ; CKD, chronic kidney disease.

* Fisher's exact test was used.

The level of eGFR and serum uromodulin was tightly correlated with the Pearson correlation coefficient of 0.67 (*P*-value < 0.001). The TC&TT genotypes of rs11864909 was associated with increased level of eGFR, but reduced level of serum uromodulin with regression coefficients of 2.68 (95% confidence interval [CI]: 0.61, 4.96) and − 12.95(95%CI: -17.59, -7.98), respectively, in the fully adjusted regression model. The haplotype composed of rs11864909, rs4293393 and rs6497476 (TAT vs. other types) was positively associated with eGFR but negatively associated with serum uromodulin with an augmented effect size than those with respect to the single SNP of rs11864909 (Table 2). No significant association was found for either rs4293393 or rs6497476 with the two serum phenotypes (Supplementary table 5). Stratified analysis showed that the associations of rs11864909 or haplotype spanning rs11864909, rs4293393 and rs6497476 with either eGFR or serum uromodulin were stronger among patients aged < 65 years, without hypertension, with normal eGFR (≥ 60 ml/min/1.73 m^2), A3 stage of UACR (≥ 300 mg/g) and etiology of glomerulonephritis (Supplementary table 6). In addition, no indication of serious multiple collinearity was detected given variance inflation factors (VIFs) for all covariates were less than 5.

Table 2
Genotypes of rs11864909 and haplotypes involving rs11864909, rs4293393 and rs6497476 and eGFR or uromodulin

Association model	β (95%CI) for eGFR	β (95%CI) for uromodulin*
rs11864909 (TT&TC vs. CC)		
Model 1	3.88(1.25, 6.62)	-11.78(-16.79, -6.12)
Model 2	3.36(0.94, 6.03)	-12.10(-17.27, -6.68)
Model 3	2.68(0.61, 4.96)	-12.95(-17.59, -7.98)
Model 4	5.65(4.02, 7.67)	-18.24(-22.27, -14.12)
Haplotype composed of rs11864909, rs4293393 and rs6497476 (TAT vs. other types)		
Model 1	7.85(3.17, 13.31)	-19.78(-28.46, -9.80)
Model 2	6.80(2.74, 12.17)	-20.62(-29.38, -11.09)
Model 3	5.71(2.01, 10.12)	-22.22(-29.87, -13.20)
Model 4	10.90(7.63, 14.77)	-32.73(-39.52, -25.44)
Note: The results listed were gained by using bootstrap method after 500 times of sampling with replacement. Model 1 was unadjusted. Model 2 was adjusted for age and gender. Model 3 was additionally adjusted for smoking, body mass index, systolic blood pressure, using anti-hypertensive medication, diabetes mellitus, etiology of CKD, and logrithm transformed urinary albumin creatinine ratio. Model 4 was additionally adjusted for uromodulin or eGFR, as appropriate.		
Abbreviation: eGFR, estimated glomerular filtration rate.		
* There are 379 missing values for uromodulin.		

In the survival analysis, the GA and GG genotypes of rs4293393 and TC and TT genotypes of rs6497476 were combined together to make balanced sample size between genotype groups. The follow-up time for ESKD, CVD events and all-cause mortality were 4.68(interquartile range: 3.87–5.59), 4.79(4.07–5.92) and 4.94(4.13–5.97) years, respectively. We found no significant difference for the incidence of ESKD, CVD events, and CVD specific mortality between the genotype groups of all the studied SNPs (all *P*-values of log-rank test > 0.05). However, there was a significantly higher incidence of all-cause mortality among patients with the AA genotype of rs4293393 or TT genotype of rs6497476 (both *P*-values of log-rank test < 0.05) (Table 3). In the Cox regression analysis, GG&GA vs. AA genotype of rs4293393, CC&TC vs. TT genotype of rs6497476 and CGC vs. other phases of haplotype spanning rs11864909, rs4293393 and rs6497476 were associated with reduced risk of all-cause mortality in the multivariable-adjusted model, with HRs of 0.341(95%CI: 0.105, 0.679), 0.344(95%CI: 0.104, 0.671) and 0.118(95%CI: 0.011, 0.446), respectively (Table 4). No violation of the proportional-hazards assumption was found for the genetic variants and covariates after assessment of the Schoenfeld residuals. The addition of serum uromodulin into the regression model with the other risk factors could enhance the magnitude of HRs. We detected a significant effect modification between 24-hour urinary sodium excretion and either genotypes of rs4293393 or those of rs6497476 (both *P*-values for interaction < 0.01) in the fully adjusted model, but not any significant interactions were detected between hypertension and the genetic variants. Levels of 24-hour urinary sodium excretion through genotypes of rs4293393 or rs6497476 were in Supplementary Fig. 1. We stratified the association by median level of 24-hour urinary sodium excretion (135 mmol/24 h). As there were very few cases of all-cause mortality occurred in the GA&GG genotypes of rs4293393 or in the CT&CC genotypes of rs6497476, making the regression analysis impossible, we only listed the incidence rates of the outcomes in the stratified analysis. The difference for the incidence of all-cause mortality was only prominent among those with higher levels of 24-hour urinary sodium excretion (≥ 135 mmol/24 h) (Supplementary table 7).

Table 3
Incidence rates for outcomes among all study participants and stratified by genotypes of variants

Genetic variant	ESKD			CVD events			All-cause mortality			CVD specific mort
	No. of Events (%)	Rate/100 patient-years	*P*-value for log-rank test	No. of Events (%)	Rate/100 patient-years	*P*-value for log-rank test	No. of Events (%)	Rate/100 patient-years	*P*-value for log-rank test	No. of Events (%)
All patients	444(16.26%)	3.66		218(7.98%)	1.70		122(4.47%)	0.91		48(1.76%)
rs11864909			0.97			0.74			0.50	
TT&TC	121(16.29%)	3.67		57(7.67%)	1.63		30(4.04%)	0.82		13(1.75%)
CC	323(16.25%)	3.66		161(8.1%)	1.72		92(4.63%)	0.95		35(1.76%)
rs4293393			0.68			0.57			**0.01**	
GA&GG	68(17.09%)	3.82		29(7.29%)	1.53		8(2.01%)	0.41		4(1.01%)
AA	376(16.12%)	3.63		189(8.1%)	1.73		114(4.89%)	1.00		44(1.89%)
rs6497476			0.84			0.31			**0.02**	
CT&CC	56(16.05%)	3.56		23(6.59%)	1.39		7(2.01%)	0.41		3(0.86%)
TT	388(16.29%)	3.67		195(8.19%)	1.75		115(4.83%)	0.99		45(1.89%)

Abbreviation: ESKD, end-stage kidney disease; CVD, cardiovascular disease.

Table 4
Association between genotypes of rs4293393 and rs6497476 and haplotypes involving the variants and all-cause mortality

Association model	Hazard ratio (95%CI)
rs4293393 (GG&GA vs. AA)	
Model 1	0.388(0.141, 0.752)
Model 2	0.399(0.143, 0.772)
Model 3	0.370(0.131, 0.697)
Model 4	0.341(0.105, 0.679)
rs6497476 (CC&TC vs. TT)	
Model 1	0.410(0.156, 0.748)
Model 2	0.420(0.159, 0.755)
Model 3	0.375(0.147, 0.699)
Model 4	0.344(0.104, 0.671)
Haplotype composed of rs11864909, rs4293393 and rs6497476 (CGC vs. other types)	
Model 1	0.168(0.025, 0.548)
Model 2	0.176(0.026, 0.556)
Model 3	0.141(0.022, 0.474)
Model 4	0.118(0.011, 0.446)

Note: The results listed were gained by using bootstrap method after 500 times of sampling with replacement. Model 1 was unadjusted. Model 2 was adjusted for age and gender. Model 3 was additionally adjusted for smoking, body mass index, systolic blood pressure, using anti-hypertensive medication, diabetes mellitus, etiology of chronic kidney disease, logarithm transformed urinary albumin creatinine ratio, and estimated glomerular filtration rate. Model 4 was additionally adjusted for uromodulin.

Among the participants with longitudinal measurement of eGFR (two or more times lasting for more than one year, n = 1337), there is a lower decline rate of eGFR among those with CC genotype of rs11864909, GA&GG genotypes of rs4293393, CT&CC genotypes of rs6497476 or haplotype phases of TAT spanning the SNPs. However, the differences of the eGFR slopes between the genotypes or haplotype phases were not statistically significant (all *P*-values for interaction between the genetic variants and time > 0.05) (Table 5). Sensitivity analysis included participants with three or more times of measurements of eGFR lasting for more than one year (n = 1046) yielded consistent results (Supplementary table 8).

Table 5
eGFR slope stratified by genotypes of rs11864909, rs4293393 and rs6497476 and haplotypes involving the variants

Genetic variant	eGFR slope (ml/min/1.73 m^2 per year)	*P*-value for interaction between genetic variant and time
rs11864909		0.58
TT&TC	-2.02	
CC	-1.76	
rs4293393		0.59
GA&GG	-1.56	
AA	-1.88	
rs6497476		0.48
CT&CC	-1.44	
TT	-1.89	
Haplotype		0.55
TAT	-1.76	
Others	-2.26	
Abbreviations: eGFR, estimated glomerular filtration rate.		
Abbreviation: eGFR, estimated glomerular filtration rate.		

Discussion

In this cohort study of patients with predialysis CKD of Chinese ethnicity, we found that the T allele of rs11864909 in the promotor of *UMOD* gene was associated with higher levels of eGFR and lower levels of serum uromodulin. The associations were consistent through different age, hypertension, eGFR, urinary ACR and causes of CKD groups. Furthermore, rs13333226, rs4293393 and rs6497476, also common variants of *UMOD* gene, were associated with the occurrence of all-cause mortality. When stratified by levels of 24-hour urinary sodium excretion, the association was only present among participants with the elevated excretion level of the urinary electrolyte.

Several common variants located in the *UMOD* gene promotor, such as rs12917707, rs13333226, rs6497476 and rs4293393, have been detected significantly associated with CKD-related traits in the recently published GWAS among populations of European descent (7, 9). Regarding the east Asians, another polymorphism in the promotor region of *UMOD*, rs11864909, was reported as the most significant genome-wide association signal, taking the place of rs12917707, owing to a very low minor allele frequency (< 0.01) of which among east Asians(10). In the current study of patients with CKD, we used a candidate gene strategy to select the above SNPs and confirmed the previous findings that T allele of rs11864909 was associated with elevated level of eGFR. Although we also detected either A allele of rs4293393/rs13333226 or T allele of rs6497476 was associated with higher concentration of eGFR, the differences failed to reach significant threshold due to extremely high frequency of the alleles (99.7% for both A allele of rs4293393/ rs13333226 and T allele of rs6497476). Besides eGFR, we measured serum uromodulin in the current study, which was positively correlated with the level of eGFR in previous studies(11, 12). We demonstrated that T allele of rs11864909 was associated with lower levels of the trait, which was consistent with the findings by Graciela and colleagues, where rs12917707 was associated with serum uromodulin based on a GWAS among participants of the Ludwigshafen Risk and Cardiovascular Health Study (2826 of the 3316 population with genotyping data). Likewise, genotypes of rs4293393, rs13333226 and rs6497476 were not significantly associated with the level of serum uromodulin in their study(4).

Besides CKD-related phenotypes, a recent GWAS has also reported the association between common variants of *UMOD* gene and hypertension(13). Another study by Gudbjartsson et al. additionally found that variants at *UMOD* was more strongly associated with CKD among older adults and those with multiple comorbidities, such as hypertension, diabetes and CVD, highlighting the role of the risk variants involved in the mechanisms relating to adaptation to aging(9). In fact, a basic medicine study, conducted by Trudu and colleagues, provided compelling evidence regarding the mechanisms linking the variants at *UMOD* and the development of hypertension and kidney lesions. They found overexpression of uromodulin due to the presence of *UMOD* risk variants both in vitro and in vivo, which could cause over-activation of TAL sodium–potassium–chloride co-transporter, leading to salt-sensitive hypertension and age-dependent kidney lesions. Blocking the pathway by diuretics could result in drop of BP(14). In the current study, we did not detect a significant relationship between all the studied SNPs and BP, which was consistent with the findings by Gudbjartsson and colleagues(9). However, we found that associations between rs11864909 and eGFR or serum uromodulin were more prominent among participants with higher urinary ACR (≥ 300 mg/g), which represents advanced kidney injury and is highly correlated with long-term hypertension and diabetes. However, we do not find advanced age or hypertension could modify the effect of the variant.

Regarding the relationship between variants of *UMOD* gene and adverse outcomes of CKD, the above-mentioned Ludwigshafen Risk and Cardiovascular Health Study also reported that T allele of rs12917707 was shown to be associated with reduced risk of mortality among those aged < 67 years after a median 9.9 years of follow-up(4). In our study, the majority of population was also aged < 67 years (89.53%) and we found consistent results that G allele of rs4293393/ rs13333226 and C allele of rs6497476 were associated with reduced risk of mortality. However, this association was in contrast to the serum uromodulin-lowering effect of the T allele of rs12917707, because the lower level of the serum biomarker was associated with increased risk of mortality as revealed in the above-mentioned study(4). In a previous publication, we also detected increased risk of ESKD associated with lower level of serum uromodulin

based on the same population with the current study(5). However, there is also evidence supporting the protective role of the serum uromodulin-lowering allele of the variants of *UMOD* gene regarding risk of CVD. Based on the large-scale Malmo Diet and Cancer study with the exclusion of prior CVD events at baseline and with a follow-up of 12 years (n = 26654), Sandosh et al. reported each copy of the G allele of rs13333226 was associated with a 7.7% reduction for risk of CVD after adjusting for age, sex and body mass index, suggesting protective effect on risk of CVD of the serum uromodulin-lowering allele of the variants of *UMOD* gene. Only a tiny abbreviation was observed when SBP and/or DBP was added into the regression model(13).

In our study, we also detected that the association between G allele of rs4293393/ rs13333226 or C allele of rs6497476 and reduced risk of mortality was only present in those with larger-than median level of 24 h-urinary sodium excretion (≥ 135 mmol/24 h). However, important confounding factors were not adjusted due to the limited number of events in certain genotype groups. Further studies with large sample size, especially with longer follow-up time and plenty of events, are needed to validate the findings.

The C-STRIDE study recruited a large sample of patients with CKD around mainland China and evaluated their clinical characteristics comprehensively. Despite the advantages, some limitations that should be admitted. Baseline eGFR and serum uromodulin were measured only once and may be subject to instability. Follow-up time for progression of CKD and occurrence of outcomes was comparatively short, which may limit the study power due to the limited number of events recorded.

Conclusions

We replicated the significant findings of an east Asians-based GWAS for the relationship between rs11864909 in promotor region of *UMOD* gene and phenotypes of eGFR and serum uromodulin among patients with CKD of Chinese ethnicity. In addition, the other three variants in *UMOD*, rs4293393, rs13333226 and rs6497476, were associated with risk of all-cause mortality in the population. The results provided further evidence regarding pathogenesis and prognosis of CKD related to *UMOD* gene.

Methods

Study Samples

C-STRIDE is an ongoing cohort study initiated in November 2011, which includes adult patients (18–74 years old) with CKD stage 1–4 in 39 clinical centers around China. The eGFR of patients should be between specific range according to different etiologies of CKD. For glomerulonephritis, eGFR should be ≥ 15 ml/min/1.73 m^2. For diabetic nephropathy, eGFR should be either between 15 ml/min/1.73 m^2 and 59 ml/min/1.73 m^2 or ≥ 60 ml/min/1.73 m^2 with 24-hour urinary protein ≥ 3.5 g or UACR ≥ 2000 mg/g or equivalent levels of other proteinuria measurements. For the etiology other than glomerulonephritis and diabetic nephropathy, eGFR should be between 15 ml/min/1.73 m^2 and 59 ml/min/1.73 m^2. The exclusion criteria included CKD caused by systemic inflammatory illness or autoimmune disease, isolated hematuria, hereditary kidney disease, kidney or other transplantation, treatment with immunosuppressive agents in the preceding 6 months to treat kidney or immune disease, HIV infection and/or diagnosis of AIDS, chronic heart failure with New York Heart Association Class III or IV, known diagnosis of cirrhosis, pregnancy or breast-feeding, malignancy treated with chemotherapy within last 2 years, and current participation in clinical trial(15). Totally, 3877 participants finished baseline examination between November 1, 2011 and December 31, 2017. Among the total population, 2754 participants had their blood sample shipped to the coordinating center. Of the 2754 participants, 2731 participants were successfully genotyped for the selected SNPs and included in the current analysis. The C-STRIDE study was conducted in accordance with the Declaration of Helsinki. The study has been approved by the ethics committee of Peking University First Hospital (No. 2011[363]). All participants provided informed consent.

Genotyping

Genomic DNAs of participants were isolated from blood leukocytes by the salting-out procedure. We genotyped 4 common variants in the promoter region of *UMOD* gene (rs11864909, rs4293393, rs6497476 and rs13333226). Genotyping of the SNPs was carried out using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Agena) according to the manufacturer's instructions(16). All the experiments were conducted by investigators who were blind to the phenotypes. Negative controls and duplicate samples were placed on each run to ensure correct genotyping. The genotyping call rates were 99.61% for rs11864909, 99.44% for rs4293393, 99.58% for rs6497476 and 99.58% for rs13333226.

Uromodulin Measurement

Uromodulin measurement was performed in the central laboratory of Peking University First Hospital, using fasting venous blood samples obtained at baseline study visit and stored at -80 °C until use. The detailed procedure for the measurement has been described in our previous publication(5). Briefly, we measured serum uromodulin by a commercially available enzyme-linked immunosorbent assay kit (Euroimmun AG, Lübeck, Germany) according to the manufacturer's instructions. At the mean concentration of 29.7 ng/mL, 102.0 ng/mL and 214.4 ng/mL, the intra-assay coefficient of variation was 3.2%, 2.2% and 1.8%, respectively. The lower detection limit of the assay was 2.0 ng/mL.

Measurement of Covariates

The trained staff in each clinical center conducted the questionnaire and physical examinations. Similar to uromodulin, other serum and urinary biomarkers for the current study was measured centrally at the Peking University First Hospital. The measurements of serum and urine creatinine were traceable to the isotope dilution mass spectrometry. eGFR was determined using the CKD-EPI creatinine Eq. (17). The UACR (mg/g creatinine) was calculated. The classification for the eGFR and ACR was determined according to the Kidney Disease Improving Global Outcomes guideline(18). 24-hour urine was collected and urinary sodium excretion was measured. Body mass index was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). BP

was measured three times at 5-minute intervals by a sphygmomanometer at the follow-up visit. The mean value of the three readings was calculated. Either abnormal BP (systolic BP ≥ 140 or diastolic BP ≥ 90) or using anti-hypertensive medications in the past two weeks was defined as hypertension. Diabetes was defined as either fasting blood glucose ≥ 7.0 mmol/L or a self-reported history of diabetes. History of CVD included a self-reported history or reviewing of medical records at baseline for myocardial infarction, serious cardiac arrhythmia, peripheral arterial disease, cerebrovascular events or hospitalization for congestive heart failure.

Outcomes

ESKD, CVD events and all-cause and CVD-specific mortality

ESKD, CVD events and mortality were followed-up until December 31, 2017. ESKD is defined as the initiation of hemodialysis, peritoneal dialysis or kidney transplantation. CVD events include non-fatal acute myocardial infarction, unstable angina, hospitalization for congestive heart failure, arrhythmia (including resuscitated cardiac arrest, ventricular fibrillation, sustained ventricular tachycardia, paroxysmal ventricular tachycardia, an initial episode of atrial fibrillation or flutter, severe bradycardia or heart block), cerebrovascular events (including intraparenchymal hemorrhage, subarachnoid hemorrhage, and cerebral infarction), and peripheral vascular diseases. Outcomes were investigated at a three to six-month interval through phone calls or routine clinical visits. Medical records were used to verify suspected outcomes of ESKD and CVD. Causes of death in ICD-10 codes I00-I99 were classified as CVD. An independent committee consisting of specialist physicians in Peking University First Hospital adjudicated the outcomes. If several CVD events occurred, the first event was used as the index event. We censored CVD events at occurrence of ESKD, death or end of follow-up (December 31, 2017), ESKD at death or the end of follow-up, all-cause mortality at the end of follow-up, while CVD-specific mortality at death from other reasons or the end of follow-up.

eGFR Slope

A subgroup of patients, who had repeated measures of eGFR (≥ 2 times) with the first and last measures spanning ≥ 1 year, were used to estimate eGFR slope, representing rate of eGFR change. A sensitivity analysis was conducted for those with ≥ 3 times of repeated measures of eGFR. Consistently, the time period between the first and last measures should be ≥ 1 year.

Statistical Analysis

Continuous data were presented as mean ± standard deviation or median (interquartile range), while categorical data were expressed as counts (percentage). One-way ANOVA was used to compare means of continuous variables conforming to normal distribution, while Kruskal-Wallis test was used in case of skewed distribution. Chi-square test was employed to compare proportions of categorical variables and to test Hardy-Weinberg equilibrium. Lewontin's D', logarithm of the odds score and r^2 were calculated to estimate the correlation and magnitude of LD between SNPs. In order to incorporate haplotypes spanning the studied SNPs into the regression analysis, we used expectation-maximization algorithm based HAPLOTYPE procedure in SAS software to generate maximum likelihood estimates of haplotype frequencies given the genotypes of the selected SNPs. Whereby, probabilities of possessing different haplotypes were assigned to each individual. The associations between genotypes of a single SNP or haplotype spanning multiple loci and concentrations of eGFR or serum uromodulin were examined by general linear regression model. VIFs were calculated for the covariates included in the multivariable model to detect potential multi-collinearity. If significant associations were detected, we further conducted analysis stratified by groups of age, hypertension, eGFR, ACR and etiologies of CKD to reflect heterogeneity of the association. Incidence rates of ESKD, CVD events, all-cause and CVD-specific mortality were calculated and compared through genotypes of SNPs by log-rank test. If significant differences were detected in the log-rank test, Cox proportional hazards regression model was used to quantify the association between genotype or haplotype and the outcomes. Proportional hazards assumption was tested by Schoenfeld residuals. As inspired by previous publications, demonstrating hypertension and urinary sodium excretion to be of potential effect modification on the association between *UMOD* gene and risk of mortality(13, 14), we included interaction terms between the studied SNPs and hypertension or 24-hour urinary sodium excretion into the Cox regression model. In addition, in order to test the influence of the SNPs on the progression of CKD, linear mixed effects model was used to calculate eGFR slopes between the genotypes or haplotypes of the SNPs. Follow-up time, genetic variants, interaction term between follow-up time and genetic variants were included as fixed effect items with an unstructured variance-covariance matrix, random intercept and random follow-up time.

Bootstrap method with 500 times of sampling with replacement was conducted to generate 95% CI in the general linear regression and Cox regression model. As 95%CIs were very close between the bootstrap method and the theoretic method, we only presented results from the bootstrap method. *P* values less than 0.05 were considered as statistically significant. LD was estimated by using Haploview software(19). All other analyses were conducted by using SAS software (version 9.4, SAS Institute Inc, Cary, NC).

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. The study has been approved by the ethics committee of Peking University First Hospital (No. 2011[363]). All participants provided informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

J. W., L. L. and L. Z. designed the study; J. W., F. W., B. G., M.-H. Z. and L. Z. collected the data; J. W., L. L. and K. H. analyzed the data; J. W., K. H., F. W., B. G., M.-H. Z. and L. Z. drafted and revised the paper; all authors approved the final version of the manuscript.

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References

1. Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O. The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease. Kidney Int. 2011;80(4):338–47.
2. Serafini-Cessi F, Malagolini N, Cavallone D. Tamm-Horsfall glycoprotein: biology and clinical relevance. American journal of kidney diseases: the official journal of the National Kidney Foundation. 2003;42(4):658–76.
3. Youhanna S, Weber J, Beaujean V, Glaudemans B, Sobek J, Devuyst O. Determination of uromodulin in human urine: influence of storage and processing. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association -. European Renal Association. 2014;29(1):136–45.
4. Delgado GE, Kleber ME, Scharnagl H, Kramer BK, Marz W, Scherberich JE. Serum Uromodulin and Mortality Risk in Patients Undergoing Coronary Angiography. J Am Soc Nephrol. 2017;28(7):2201–10.
5. Lv L, Wang J, Gao B, Wu L, Wang F, Cui Z, et al. Serum uromodulin and progression of kidney disease in patients with chronic kidney disease. J Transl Med. 2018;16(1):316.
6. Hart TC, Gorry MC, Hart PS, Woodard AS, Shihabi Z, Sandhu J, et al. Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. J Med Genet. 2002;39(12):882–92.
7. Köttgen A, Glazer NL, Dehghan A, Hwang SJ, Katz R, Li M, et al. Multiple loci associated with indices of renal function and chronic kidney disease. Nat Genet. 2009;41(6):712–7.
8. Chambers JC, Zhang W, Lord GM, van der Harst P, Lawlor DA, Sehmi JS, et al. Genetic loci influencing kidney function and chronic kidney disease. Nat Genet. 2010;42(5):373–5.
9. Gudbjartsson DF, Holm H, Indridason OS, Thorleifsson G, Edvardsson V, Sulem P, et al. Association of variants at UMOD with chronic kidney disease and kidney stones-role of age and comorbid diseases. PLoS Genet. 2010;6(7):e1001039.
10. Okada Y, Sim X, Go MJ, Wu JY, Gu D, Takeuchi F, et al. Meta-analysis identifies multiple loci associated with kidney function-related traits in east Asian populations. Nat Genet. 2012;44(8):904–9.
11. Fedak D, Kuźniewski M, Fugiel A, Wieczorek-Surdacka E, Przepiórkowska-Hoyer B, Jasik P, et al. Serum uromodulin concentrations correlate with glomerular filtration rate in patients with chronic kidney disease. Pol Arch Med Wewn. 2016;126(12):995–1004.
12. Steubl D, Block M, Herbst V, Nockher WA, Schlumberger W, Satanovskij R, et al. Plasma Uromodulin Correlates With Kidney Function and Identifies Early Stages in Chronic Kidney Disease Patients. Medicine. 2016;95(10):e3011.
13. Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, et al. Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet. 2010;6(10):e1001177.
14. Trudu M, Janas S, Lanzani C, Debaix H, Schaeffer C, Ikehata M, et al. Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. Nature medicine. 2013;19(12):1655–60.
15. Gao B, Zhang L, Wang H, Zhao M. Chinese cohort study of chronic kidney disease: design and methods. Chin Med J. 2014;127(11):2180–5.
16. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Current protocols in human genetics. 2009;2:2.12.
17. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–12.
18. Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Ann Intern Med. 2013;158(11):825–30.

19. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21(2):263–5.

Figures

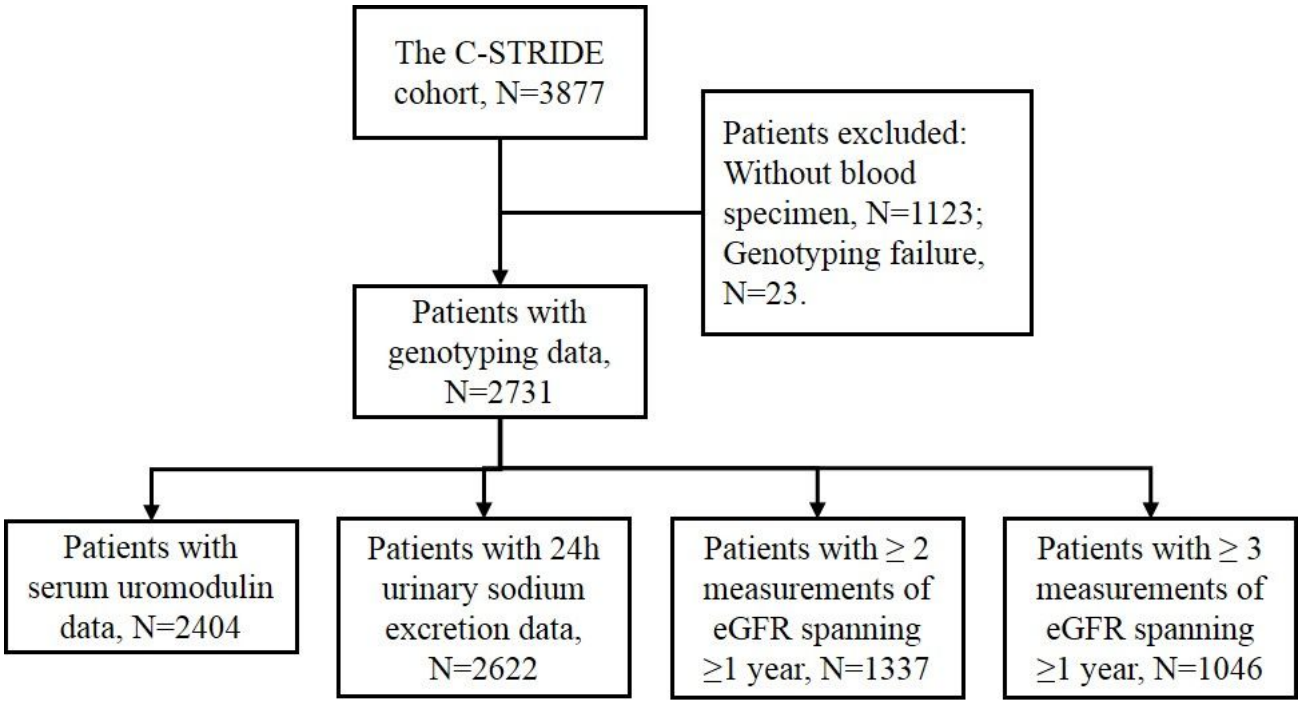

Figure 1

Flowchart of the participants selection. Abbreviation: eGFR, estimated glomerular filtration rate.

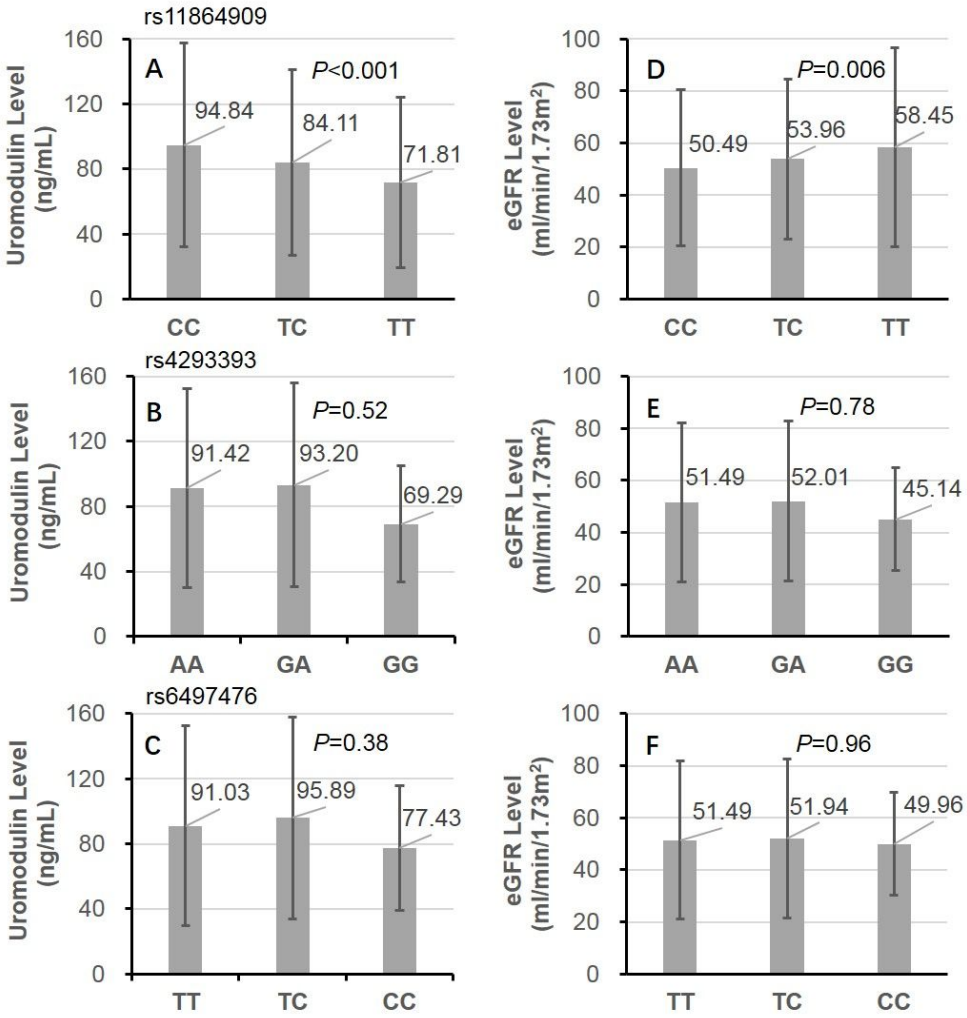

Figure 2

The distribution of serum uromodulin and eGFR between genotypes of rs11864909, rs4293393 and rs6497476. A. uromodulin levels through genotypes of rs11864909; B. uromodulin levels through genotypes of rs4293393; C. uromodulin levels through genotypes of rs64974763; D. eGFR levels through genotypes of rs11864909; E. eGFR levels through genotypes of rs4293393; F. eGFR levels through genotypes of rs64974763.

Supplementary Files

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