ORIGINAL ARTICLE

Common variants of the *UMOD* promoter associated with blood pressure in a community-based Chinese cohort

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Hypertension is an important risk factor for chronic kidney disease. The kidney, in turn, has an important role in the regulation of blood pressure (BP). On the basis of a genome-wide association study, single-nucleotide polymorphisms in the uromodulin (*UMOD*) promoter region had been considered to influence both renal sodium reabsorption and BP. In this study, we asked whether common variants across the *UMOD* gene influence BP in a community-based Chinese cohort. We screened seven common variants across the *UMOD* locus in a community-based population from Beijing, including 1000 individuals with 48% males and an average age of 63.7 ± 9.0 years. The urinary *UMOD* concentration was measured by enzyme-linked immunosorbent assay. We then analyzed the association of common variants of *UMOD* with BP. The *UMOD* promoter common variant rs13333226 G/A is associated with diastolic BP (DBP), and G allele carriers have higher DBP compared with A/A homozygotes ($P=0.035$). The variant rs6497476 C/T predicted the DBP level, with C homozygotes having a higher DBP compared with CT heterozygotes and T homozygotes ($P=0.025$). Urinary *UMOD* excretion was correlated with urinary sodium excretion ($R=0.239$, $P=0.656 \times 10^{-13}$). We determined that common variants of *UMOD* are associated with DBP level in a community-based Chinese cohort.

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INTRODUCTION

Hypertension (HTN) is an important risk factor for chronic kidney disease, and the kidney, in turn, has important roles in the regulation of blood pressure (BP).^{1,2} Enzymes, channels and receptors implicating sodium regulation within the kidney have been proven to be involved in the pathogenesis of HTN.^{3–6}

Uromodulin (*UMOD*) is the most abundant protein in human urine. Recently, it is found that *UMOD* has an effect on renal outer medullary potassium channels and thus regulate salt reabsorption along the thick ascending limb.⁷ Furthermore, in a genome-wide association study, one of the common variants of *UMOD*, rs13333226, is identified as being associated with HTN and cardiovascular risk in European cohorts.⁸

We therefore systematically genotyped common variants across the *UMOD* gene in a community-based cohort and explored whether common variants of the *UMOD* gene are associated with BP.

METHODS

Subjects

Individuals from a community-based cohort in an urban area of Beijing were included in this study. Briefly, 2310 individuals were evaluated in 2004,⁹ and 1504 of these individuals were re-evaluated in 2008.¹⁰ We were able to obtain consent and prepare genomic DNA from blood leukocytes obtained from 1000 of the participants in 2008, and this population was comparable to the original cohort with respect to the general baseline data, including age ($P=0.603$), gender ($P=0.718$), body mass index ($P=0.466$), systolic BP (SBP; $P=0.35$), diastolic BP (DBP; $P=0.237$), estimated glomerular filtration rate ($P=0.308$), high-density lipoprotein-cholesterol ($P=0.9$), triglycerides ($P=0.56$) and diagnosis of HTN ($P=0.836$). We obtained urine samples from 898 participants, and they were comparable to the 1000 subjects and to the 102 cases that gave blood, but not urine (Table 1).

BPs were measured according to the guidelines presented in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure.¹¹ Three readings were taken at 5-min intervals. The mean values of the three readings were calculated, unless the difference between the readings was >10 mmHg, in which case, the

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Table 1 Basic clinical features of the participants

Items	N1 = 1000	N2 = 898	N3 = 102	P-value (N1 and N2)	P-value (N2 and N3)
Male (%)	48	48	48	0.998	0.998
Age (years)	63.7 ± 9.0	63.5 ± 9.3	64.7 ± 9.7	0.773	0.286
BMI (kg m ⁻²)	25.3 ± 3.8	25.3 ± 3.8	25.4 ± 3.8	0.965	0.57
SBP (mmHg)	137.8 ± 19.9	138.1 ± 17.9	140.1 ± 17.8	0.671	0.304
DBP (mmHg)	81.0 ± 10.2	81.4 ± 10.3	81.2 ± 10.0	0.493	0.908
eGFR (ml min ⁻¹ per 1.73 m ²)	70.8 ± 14.9	70.9 ± 15.2	70.8 ± 14.9	0.899	0.899
HDL cholesterol (mmol l ⁻¹)	1.3 (1.1–1.6)	1.4 (1.2–1.7)	1.3 (1.1–1.6)	0.028	0.476
Triglycerides (mmol l ⁻¹)	1.3 (1.0–1.8)	1.3 (1.0–1.8)	1.2 (0.9–2.0)	0.918	0.351
Hypertension (%)	46.3	46.4	46.0	0.998	0.998
Anti-hypertension medication(%)	81.6	81.5	82.0	0.961	0.961

Abbreviations: BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, inter-quartile range; SBP, systolic blood pressure.

eGFR was estimated with the equation developed from the CKD-EPI¹². Data presented as mean ± s.d. for normally distributed traits, or median IQR for not normally distributed variables. Anti-hypertension medication (%) calculated by numbers of taking anti-hypertension medication over numbers of hypertension.

mean of the two closest measurements was used. HTN was defined as SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or the use of anti-hypertensive medications. The estimated glomerular filtration rate was estimated with the equation developed from the Chronic Kidney Disease Epidemiology Collaboration.¹² In individuals undergoing antihypertensive therapies, the BP was imputed by adding 15 and 10 mm Hg to the SBP and DBP, respectively,¹³ during analyses.

Genomics

Single-nucleotide polymorphisms (SNPs) were obtained from public SNP databases (<http://www.ncbi.nlm.nih.gov/projects/SNP/>; Figure 1). The genomic DNA was typed using a matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry system developed by Sequenom according to a published protocol,¹⁴ or by a Taqman assay with supplied probes and primers on an ABI PRISM Sequence Detection System 7500 (Applied Biosystems, Foster City, CA, USA). The reproducibility of the genotyping was verified with 50 blinded replicate samples. The SNPs evaluated in this study had minor allele frequencies of ≥ 4%, and all were in Hardy–Weinberg equilibrium ($P > 0.05$). Although we genotyped 1000 cases, only 910 individuals were successfully genotyped for SNP rs13333226, and 879 cases for rs6497476. To prove that the missing data were randomly deleted, we compared clinical features between subgroups N1 (the whole population group), N2 (the successfully genotyped group) and N3 (the genotype failed group). No differences were identified (Supplementary Tables 1 and 2).

Biochemical assays

Urinary UMOD, sodium and creatinine were measured from stored diet spot urine specimens among a subset of 898 participants, balanced by genotype. The urinary creatinine and sodium were measured by the same means as the serum creatinine and sodium. Because of genotyping failure, only 853 cases yielded both genotyping data and urinary information for rs13333226, and 824 cases for rs6497476. These data were randomly missing without any inclusion or exclusion criteria. To prove the randomization, we compared clinical features between subgroups N1 (the whole population group), N2 (the successfully genotyped group) and N3 (the genotype failed group). No differences were identified (Supplementary Tables 3 and 4).

UMOD measurement

The measurement of urinary UMOD was adjusted according to Lau's protocol.¹⁵ First, 96-well microtiter plates were coated with polyclonal mouse anti-human UMOD antibodies (Biomedical Technologies Inc., Stoughton, MA, USA; BT-590). Urinary samples and UMOD standards (Biomedical Technologies) were then diluted and added to wells in duplicate. After 2 h of incubation at 37 °C, the wells were washed and monoclonal rabbit anti-human UMOD antibody (Biomedical Technologies) was added. After 1 h of incubation at 37 °C, goat anti-rabbit immunoglobulin

Haplotype blocks at the *UMOD* locus in the Chinese population

Figure 1 Haplotype blocks at the *UMOD* locus in the Chinese population. We used seven common variants (minor allele frequency > 5%) to construct haplotype blocks in Haploview¹⁶ for all subjects. A full color version of this figure is available at the *Hypertension Research* journal online.

horseradish peroxidase (CEDARLANE CL1032AP, Canada) was added. Color was developed by the addition of TMB (3,3',5,5'-tetramethylbenzidine) substrate. The reaction was stopped by adding 2N H₂SO₄, followed by reading immediately at optical density 490 and 570. The urinary UMOD concentration was determined by referring to a standard curve.

Statistical analyses

The pairwise linkage disequilibrium between each common SNP pair across the *UMOD* locus was quantified as D' or r^2 with Haploview software.¹⁶ Haplotype homozygosities were confirmed with plink software (URL: <http://pngu.mgh.harvard.edu/purcell/plink/>).¹⁷

The baseline characteristics were computed as the mean ± s.d. (or mean ± s.e.m.) for normally distributed traits and median (inter-quartile range) for

non-normally distributed variables. The analyses were conducted with SPSS (Statistical Package for the Social Sciences) software (SPSS v13.0; Chicago, IL, USA). If traits were not normally distributed, then their values were tested by non-parametric methods (Kruskal–Wallis or median tests). Univariate analyses were performed to evaluate the effects of particular SNPs on continuous traits (such as BP) and dichotomous traits (such as HTN status), and were adjusted by age and sex (as covariates).

To quantify the effect of a particular genetic variant on a BP trait and to normalize such values across traits, we estimated the coefficient of determination (adjusted R^2) in general linear models (analysis of variance or regression) in the presence or absence of the genotype. Thus, the difference in the adjusted R^2 (full model minus model without genotype) represents the contribution of the genotype to the trait, and multiplication by 100 yields the coefficient of determination as a percentage of the trait variance, where $\text{variance} = s.d.^2$

RESULTS

Linkage disequilibrium at the *UMOD* genetic locus in the cohort

The observed genotype frequencies of the seven polymorphisms were all in the Hardy–Weinberg equilibrium. We used seven common variants (minor allele frequency >5%) to construct haplotype blocks in Haploview¹⁶ for all subjects. The linkage disequilibrium analysis revealed three blocks across the *UMOD* genes, such that the SNPs in the *UMOD* promoter region, intron and 3' downstream region were all highly correlated within each block, and the r^2 reached at least 0.93 (Figure 1).

Association of common variants of the *UMOD* promoter with DBP

Because the SNPs were highly correlated within each block, we tested the associations of seven common variants with DBP and SBP directly. None of the seven SNPs was associated with SBP. The common variant rs13333226 G/A predicted the DBP level, with G allele carriers having a higher DBP than A homozygotes ($P=0.035$) (Figure 2a; Table 2). Another SNP, rs6497476 C/T, also predicted the DBP level, with C carriers having a higher DBP than T homozygotes ($P=0.025$) (Figure 2b). We also detected the effects of gender, age, body mass index and gene (rs13333226 and rs6497476) on DBP levels by linear regression; a model combining age, gender, body mass index and gene predicted the BP level ($P<0.001$), whereas rs13333226 and rs6497476 themselves accounted for approximately 0.4%–0.5% of the DBP variance (Figure 2a and b). Other common variants were not identified as being associated with DBP.

Correlation between urinary *UMOD* excretion and urinary sodium excretion in the cohort

Urinary *UMOD* and sodium excretion were calculated as the urinary *UMOD* (sodium)/creatinine ratio. We found that the urinary *UMOD*/creatinine ratio correlated with the urinary sodium/creatinine ratio (Spearman's $R=0.239$, $P=0.656 \times 10^{-13}$, Supplementary Figure 1).

Therefore, we explored whether *UMOD* haplotypes or variants that were associated with DBP also influenced urinary sodium excretion in the cohort. As indicated in Table 3, rs13333226 A homozygotes had a higher urinary sodium/creatinine ratio than G allele carriers, although the P -value was at the marginal level. We obtained similar results for rs6497476.

We also tested the association of *UMOD* haplotypes or variants with the urinary *UMOD*/creatinine ratio. However, none of the variants were identified as influencing the urinary *UMOD* excretion.

DISCUSSION

In this study, we showed that common variants in the *UMOD* promoter region are associated with the continuous trait of

DBP in a community-based Chinese population. Among the three common promoter variants, rs13333226 and rs6497476 are also associated with DBP. The G allele (minor) of rs13333226 and the C allele (minor) of rs6497476 are associated with higher DBP.

Recent advances in molecular biology have unveiled the mechanism of HTN in certain situations to be associated with the upregulation of sodium reabsorption in the distal nephron, with an accompanying expansion of the extracellular volume.^{18,19} Aviv *et al.*^{20,21} reviewed previously reported clinical studies that indicated that the thick ascending limb (TAL) had a pivotal role in the susceptibility of Blacks to HTN. A mechanism was proposed in which an increase in NKCC2 activity could lead to the expansion of the extracellular volume and thus be associated with HTN.²²

UMOD is synthesized by the tubular cells of the TAL and the early distal convoluted tubule of the kidney.²³ It has been considered that UMOD might influence the colloidal osmotic pressure through the TAL, and thus regulate their active transport along this segment.²⁴ Recently, it was found that UMOD had an effect on the renal outer medullary potassium channels (ROMK2), and thus increased salt reabsorption along the TAL.⁷ According to these findings, it appeared that UMOD excretion may induce changes in sodium reabsorption in the TAL, thus influencing sodium reabsorption in the proximal tubules as well. Because UMOD may be involved in the regulation of sodium reabsorption, it is reasonable to hypothesize that UMOD influences BP. The associations of the *UMOD* gene with HTN were also reported in different populations. One study from the European cohorts found that the minor G allele of rs13333226 was associated with a lower risk of HTN.^{8,25} However, in these cohorts, the minor G allele of rs13333226 is associated with lower urinary UMOD excretion and lower urinary sodium excretion. The association of lower BP and increased sodium reabsorption appeared counterintuitive.⁸ In our result, the minor G allele of rs13333226 was associated with higher DBP in a community-based Chinese population; this result is opposite to those found with other cohorts. We therefore tested whether the *UMOD* gene correlated with renal sodium excretion. Although the P -values were at the marginal level, the haplotype GCC, the G minor allele of rs13333226 and the T homozygotes of rs6497476 exhibited lower urinary sodium excretion, which maintained the expected correlation between lower sodium excretion and high BP to a certain extent. The effect of rs13333226 on BP appeared to contradict that found in the previous study. However, there are several possible explanations for this discrepancy. Our results showed that the *UMOD* genotype was associated with diastolic pressure, not with the risk for HTN. We did not control the sodium intake in this population. Because it appeared that UMOD was involved in the regulation of sodium reabsorption, UMOD might have more complicated effects on sodium regulation and BP. Finally, the different studies involved different populations in different places, so unknown factors may interact with the gene effects.

The three SNPs (rs13333226, rs6497476 and rs4293393) that we genotyped here are common SNPs, changed from purine to pyrimidine, and were reported to be associated with serum creatinine, BP or plasma uric acid.^{8,26} The population genetic studies suggested that these SNPs may influence the transcriptional level and thus regulate UMOD expression and finally change UMOD excretion. However, further cell research must be conducted to confirm that they are functional SNPs.

Our study also has several limitations. Because our study was observational, the possibility of residual confounding by an

Figure 2 UMOD promoter common variants: effects on diastolic blood pressure in community based cohort of Chinese population. Diastolic blood pressure and result of multiple linear regression were shown. The rs13333226 G allele carriers have higher diastolic blood pressure compared with A homozygotes. Another SNP rs6497476 C allele carriers have higher diastolic blood pressure compared with T homozygotes. Data was presented as mean $\pm$ s.e.m. (a) rs13333226 and DBP: DBP was showed according to different genotypes: GG + GA 87.5 ± 1.1 mmHg; GA 84.9 ± 0.4 mmHg. (b): rs6497476 and DBP: DBP was showed according to different genotypes: CC 96.0 ± 3.9 mmHg; TC 86.5 ± 1.2 mmHg; TT 84.8 ± 0.4 mmHg.

unmeasured covariate exists. Additionally, the genotyping failure rate for rs6497476 is relatively high ($121/1000 = 12\%$). Although we performed genotyping for rs6497476 by matrix-assisted laser

desorption mass spectrometry, which is a good and reliable tool for SNP genotyping, the method still had certain limitations. In our study, the DNA quality was sufficient, as the failure rates of other

Table 2 Subject characteristics by genotypes of rs13333226

Items	Genotyping		P-value
	AA	GG+ GA	
N	777	133	
Male (%)	49	46	0.599
Age (years)	63.9±9.3	62.4±9.5	0.095
BMI (kg m ⁻²)	25.3±3.9	25.4±3.1	0.608
SBP (mmHg)	138.1±17.6	139.6±18.1	0.356
DBP (mmHg)	81.2±10.2	83.1±10.7	0.046
eGFR (ml min ⁻¹ per 1.73 m ²)	70.6±14.9	70.5±15.6	0.91
Triglycerides (mmol l ⁻¹)	1.3(0.9–1.7)	1.3(1.0–1.9)	0.07
HDL cholesterol (mmol l ⁻¹)	1.3(1.1–1.6)	1.3(1.1–1.5)	0.124

Abbreviations: BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, inter-quartile range; SBP, systolic blood pressure. eGFR was estimated with the equation developed from the CKD-EPI¹². Data presented as mean±s.d. for normally distributed traits, or median IQR for not normally distributed variables.

Table 3 UMOD common variants and urinary sodium/creatinine ratio

	Urinary sodium/creatinine (μmol mmol ⁻¹)			P-value
	AA (N=734)	GG+GA (N=119)		
rs13333226	22.97	21.37		0.068
rs6497476	CC (N=6)	CT(N=96)	TT(N=722)	0.047
	23.38	20.81	22.88	

Abbreviation: UMOD, uromodulin.

Figure 3 Uromodulin (UMOD): working hypothesis for pathophysiological consequences of common variants in the promoter.

SNPs were low. Therefore, the primers for these SNPs might be the major limitation resulting in genotyping failure.

In summary, we identified the association of common variants of UMOD with DBP in a Chinese cohort, suggesting that UMOD has important roles in the mechanisms of HTN. We described our hypothesis in Figure 3. The common variants of the UMOD promoter will influence the mRNA level and thus regulate the expression/secretion of UMOD in the renal TALs. The renal TALs have important roles in sodium reabsorption,^{20,21} and UMOD may regulate sodium reabsorption in this tissue²⁴ and thus regulate BP. One of our results appeared to be contradicted by previous studies, indicating that the

real mechanism of UMOD in the regulation of BP needs to be further explored.

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