Common genetic variants of the human uromodulin gene regulate transcription and predict plasma uric acid levels

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Uromodulin (UMOD) genetic variants cause familial juvenile hyperuricemic nephropathy, characterized by hyperuricemia with decreased renal excretion of UMOD and uric acid, suggesting a role for UMOD in the regulation of plasma uric acid. To determine this, we screened common variants across the UMOD locus in one community-based Chinese population of 1000 individuals and the other population from 642 American twins and siblings of European and Hispanic ancestry. Transcriptional activity of promoter variants was estimated in luciferase reporter plasmids transfected into HEK-293 cells and mIMCD3 cells. In the primary Chinese population, we found that carriers of the GCC haplotype had higher plasma uric acid, and three promoter variants were associated with plasma uric acid. UMOD promoter variants displayed reciprocal effects on urine uric acid excretion and plasma uric acid concentration, suggesting a primary effect on renal tubular handling of urate. These UMOD genetic marker-on-trait associations for uric acid were replicated in the independent American cohort. Site-directed mutagenesis at trait-associated UMOD promoter variants altered promoter activity in transfected luciferase reporter plasmids. Thus, UMOD promoter variants seem to initiate a cascade of transcriptional and biochemical changes influencing UMOD secretion, leading to altered plasma uric acid levels.

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Circulating uric acid concentration constitutes a risk factor for both renal and cardiovascular disease[1] and unusual mutations at the uromodulin (*UMOD*) locus exhibit disturbances in uric acid metabolism. UMOD is an ~80–90 kDa glycoprotein, consisting of 640 amino acids, including 48 cysteine residues, exclusively synthesized by the thick ascending limb (TAL) and early distal convoluted tubule in the kidney.[2] UMOD is initially trafficked into the endoplasmic reticulum, then shuttled to the apical membrane of cells as a GPI (glycosyl-phosphatidylinositol)-linked molecule, and finally released into the urine by proteolytic cleavage.[3–5] Healthy individuals typically excrete as much as 20–70 mg of UMOD per day, rendering it the most abundant protein in human urine.[6]

UMOD mutations have been identified in patients with familial juvenile hyperuricemia nephropathy (OMIM 162000), medullary cystic kidney disease 2 (OMIM 603860), and glomerulocystic kidney disease (OMIM 609886),[7–9] which are characterized by hyperuricemia and progressive kidney disease. Thus, UMOD is a candidate regulator of renal uric acid excretion. In a recent genome-wide association study, common genetic variant rs4293393 in the *UMOD* gene promoter was associated with serum uric acid.[10]

UMOD may contribute to regulation of sodium reabsorption through the TAL:[11] decreased UMOD secretion might lead to a reduction of sodium reabsorption by the TAL,[12] perhaps compensated through increased reabsorption of sodium by the proximal tubule. As sodium reabsorption is coupled to increased urate reabsorption in the proximal tubule,[13] plausible mechanisms linking UMOD to serum uric acid are apparent.

In this study, we typed common genetic variants spanning the *UMOD* locus, to establish associations between the *UMOD* gene and plasma uric acid in different populations, and also explored whether the effect of *UMOD* on plasma uric acid was mediated by regulation of renal uric acid or sodium excretion.

RESULTS

Primary studies in a community-based cohort of Beijing

Association of UMOD haplotypes and common variants with plasma uric acid. We genotyped seven common variants (Supplementary Tables S1 and S2 online) across the *UMOD* locus. We also used seven common variants to construct haplotype blocks in Haploview[14] for all subjects in the Chinese population (Table 1). The analysis revealed three blocks across the UMOD genes, in which single nucleotide polymorphisms (SNPs) in UMOD promoter region, intron, and 3′ downstream were all highly correlated within each block (Supplementary Table S3 online). Common promoter haplotypes displayed associations with plasma uric acid levels: carriers of promter haplotype GCC showed higher plasma uric acid, whereas homozygotes of promoter haplotype ATT presented lower plasma uric acid levels (Table 2).

We then tested the association of each of the seven individual SNPs with plasma uric acid in the cohort. Three promoter SNPs, rs4293393 C/T, rs6497476 C/T, and rs13333226 G/A, each associated with plasma uric acid. rs13333226 G-allele carriers ($n = 135$) have higher plasma uric acid than A/A homozygotes ($n = 791$, 279.2 ± 7.0 vs. 263.4 ± 2.9 μmol/l, $P = 0.009$); rs6497476 C allele carriers ($n = 114$) have higher plasma uric acid than T/T homozygotes ($n = 781$, 281.2 ± 7.7 vs. 263.3 ± 2.9 μmol/l, $P = 0.005$); and finally rs4293393 C allele carriers ($n = 137$) have higher plasma uric acid compared with T/T homozygotes ($n = 792$) (279.3 ± 7.1 vs. 264.3 ± 2.9 μmol/l, $P = 0.032$) (Table 2).

The effects of age, gender, estimated glomerular filtration rate (eGFR), body mass index, height, weight, waist, SBP (systolic blood pressure) and diastolic blood pressure (DBP) and *UMOD* gene (SNP) on plasma uric acid were tested by linear regression: a model combining age, gender, waist, eGFR, DBP and gene (SNP) predicted plasma uric acid ($P < 0.001$); rs13333226 itself accounted for ~0.4% of plasma uric acid variance ($P = 0.015$; Table 3), while rs6497476 itself accounted for ~0.5% of serum uric acid variance ($P = 0.013$; Table 3), and rs4293393 itself accounted for ~0.3% of serum uric acid variance ($P = 0.037$; Table 3).

We used estimation of the false discovery rate (FDR), in order to minimize false-negative results while maximizing true-positive results, using the Excel calculator of FDRs from *P*-values, at <http://www.rowett.ac.uk/~gwh/fdr.html>. All the FDR *P*-values for SNPs and haplotypes are listed in Table 2. Three promoter SNP effects were also confirmed by permutation (non-parametric) tests.

Association of UMOD variant with urinary uric acid excretion. We also investigated the association of *UMOD* SNPs with urinary uric acid excretion. rs6497476 C allele carriers ($n = 101$) have lower urinary uric acid to creatinine ratio than T/T homozygotes ($n = 712$, 282.7 ± 13.5 vs. 313.3 ± 6.0 μmol/mmol, $P = 0.023$; Figure 1), consistent with previous results that the C allele predicted higher plasma uric acid.

Association of a UMOD common haplotype with urinary UMOD excretion. We then investigated whether the common haplotypes or SNPs associated with urinary UMOD excretion. Neither single SNPs nor promoter haplotypes was identified to be associated with UMOD excretion. As a local region of high linkage disequilibrium at the UMOD locus region was identified in the HapMap CHB (China–Han–Beijing) subjects (Supplementary Figure S1 online), we also inferred longer 7-SNP haplotypes across the UMOD locus. The most common haplotype, ATTGGCA (spanning rs13333226, rs6497476, rs4293393, rs7198000, rs11859916, rs4383153, rs4632135) associated with urinary UMOD–creatinine ratio (Figure 2). Carriers of ATTGGCA displayed higher urinary UMOD excretion ($P = 0.016$).

Correlation between urinary UMOD, uric acid, and sodium excretions. Urinary uric acid-to-creatinine ratio, urinary UMOD-to-creatinine ratio, and urinary sodium-to-creatinine ratio were measured in spot urine in 898 cases. We found that plasma uric acid negatively correlated with urinary uric acid-to-creatinine ratio, as well as urinary UMOD-to-creatinine ratio. And we also found that the three biochemical traits, urinary uric acid-to-creatinine ratio, urinary UMOD-to-creatinine ratio, and urinary sodium-to-creatinine ratio, positively correlated with each other (Table 4).

Table 1 | Characteristics of the Chinese community–based cohort in this study

Variables	Participants ($n = 1000$)
Age (years)	63.7 ± 0.3
Male (%)	48.5%
BMI (kg/m^2)	25.3 ± 0.1
SBP (mm Hg)	137.8 ± 0.6
DBP (mm Hg)	81.0 ± 0.3
Plasma uric acid (μmol/l)	265.7 ± 2.6
Plasma creatinine (μmol/l)	138.33 ± 0.6
eGFR (ml/min per 1.73 m^2)	70.8 ± 0.5
Cholesterol (mmol/l)	5.3 (4.7–6.0)
Triglycerides (mmol/l)	1.3 (1.0–1.8)
Urinary ACR (median (IQR))	2.6 (1.3–4.9)
Hypertension (%)	46.3
Anti-hypertension medication (%)	81.6

Abbreviations: ACR: albumin to creatinine ratio in urine; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; IQR, interquartile range; SBP, systolic blood pressure.
Values shown are mean ± s.e.m., or median (IQR). Samples shown here were collected in 2008. eGFRs presented in this table were estimated from plasma creatinine using the equation developed from data on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI).[38]

Extension/replication studies in California twins and siblings

Of the seven SNPs typed at/near *UMOD* in the Beijing cohort, five were either genotyped or imputed in the San Diego subjects; each of these five variants was significantly ($P < 0.05$) associated with plasma uric acid: rs13333226 ($P = 0.0261$), rs4293393 ($P = 0.0261$), rs7198000 ($P = 0.0492$), rs11859916 ($P = 0.0492$), and rs4632135 ($P = 0.027$). Of note, rs4293393 was previously associated with serum uric acid.[10]

Heritability of plasma uric acid and uric acid excretion was also estimated in the twin cohort. h^2 was substantial for