Genome-wide studies reveal factors associated with circulating uromodulin and its relations with complex diseases

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Graphical abstract

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Genome-wide studies reveal factors associated with circulating uromodulin and its relations with complex diseases

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Abstract

**UMOD** is a major risk gene for monogenic and complex forms of kidney disease. The encoded kidney-specific protein uromodulin is highly abundant in urine and related to chronic kidney disease, hypertension, and pathogen defense. To gain insights into potential systemic roles, we performed genome-wide screens of circulating uromodulin using complementary antibody-based (N=13,985) and aptamer-based (N=18,070) assays. We detected 3 and 10 distinct significant (p<5e-8) loci, respectively. Integration of antibody-based results at the **UMOD** locus with functional genomics data (RNA-seq, ATAC-seq, Hi-C) of primary human kidney tissue highlights an upstream variant with differential accessibility and transcription in uromodulin-synthesizing kidney cells as underlying the observed *cis* effect. Shared association patterns with complex traits, including chronic kidney disease and blood pressure, place the **PRKAG2** locus in the same pathway as **UMOD**. Experimental validation of the third antibody-based locus, **B4GALNT2**, shows that the p.Cys466Arg variant of the encoded N-acetylgalactosaminyltransferase has a loss-of-function effect leading to higher serum uromodulin levels. Aptamer-based results point to enzymes writing glycan marks present on uromodulin and to their receptors in the circulation, suggesting that this assay permits investigating uromodulin’s complex glycosylation rather than its quantitative levels. Overall, our study provides new insights into circulating uromodulin and its emerging functions.
Introduction

Chronic kidney disease (CKD) can progress to kidney failure, is a major risk factor for cardiovascular morbidity and mortality, and a leading cause of death (1-3). CKD affects approximately 10% of adults (1). Genome-wide association studies (GWAS) of kidney function, CKD, and CKD progression in population-based studies have consistently identified the largest effect for common variants at the \textit{UMOD} locus (4-7). The encoded protein uromodulin, previously named Tamm-Horsfall protein, is the most abundant protein in the urine of healthy individuals (8). It is exclusively synthesized in the kidney’s thick ascending limb (TAL) of the loop of Henle (LOH) and the distal convoluted tubule (DCT) (9). Urinary uromodulin has important roles in protecting against urinary tract infections (10). Glycosylation accounts for approximately 30% of the mature protein’s molecular weight in urine and may be important for some of the protein’s functions, including an emerging immuno-modulatory role (8).

Common CKD risk variants in \textit{UMOD} are also associated with higher risk of hypertension, hyperuricemia and gout, and lower risk of kidney stone disease (4, 11-14). Their association with higher uromodulin transcript levels in kidney (7, 15) and higher uromodulin levels in urine (7, 16) directly implicates a pathophysiologic role of uromodulin. Rare mutations in \textit{UMOD} cause one of the most common monogenic kidney diseases, autosomal-dominant tubulo-interstitial kidney disease (17, 18).

\textit{UMOD} is hence a main driver of genetic kidney disease, and genetic studies of the kidney-specific protein uromodulin may yield insights not only into kidney disease but also into the protein’s other diverse functions and associated diseases. Such studies can also reveal regulators and interaction partners that can help to understand potential consequences of therapeutic manipulation and may reveal new entry points to do so, with the final goal to
reach pharmacological intervention (19). Previous studies of uromodulin have almost
exclusively focused on urine. The protein is, however, also released from the basolateral
membrane of renal TAL and DCT cells and reaches the blood, where its concentration is about
100-fold lower than in urine (8). In a previous study, urine and plasma uromodulin levels were
moderately correlated (20), although they are both associated with the kidney function
measure estimated glomerular filtration rate (eGFR). The mechanisms influencing circulating
uromodulin, whether circulating and urine uromodulin share association patterns with
complex diseases, and any factors related to the glycans carried by uromodulin are unknown.
Quantification of circulating uromodulin on a population scale has recently become feasible
(21-24). A small GWAS of serum uromodulin levels reported only an association with the
known CKD-associated UMOD variants in cis (23).

Here, we perform meta-analyses of GWAS of circulating uromodulin to obtain insights into
factors that may be relevant to CKD pathophysiology and into any systemic functions of this
kidney-specific protein. Using an antibody-based assay, we (i) identify an upstream variant at
the UMOD locus with differential accessibility and transcription in human uromodulin-
synthesizing kidney cell types and compartments that is strongly associated with circulating
and urine uromodulin, CKD and hypertension, (ii) place the PRKAG2 locus in the same
pathway as UMOD with respect to its disease associations, and (iii) show that p.Cys466Arg in
the uromodulin-glycosylating enzyme B4GALNT2 is a loss-of-function allele leading to higher
serum uromodulin levels. Using an aptamer-based assay, we identify non-overlapping loci
that point to enzymes writing glycan marks present on uromodulin and to their receptors in
the circulation. Together, our study based on human genetic evidence provides new insights
into circulating uromodulin and its emerging functions.
Results

GWAS meta-analysis identifies 13 genetic loci associated with circulating uromodulin

Characteristics of the 32,055 individuals from seven participating studies (ARIC, CHS, Fenland, GCKD, KORA, LURIC, ORIGIN), including distributions of age, sex, and the estimated glomerular filtration rate (eGFR), are shown Supplementary Table 1. There were 29,439 participants of European ancestry (EA), 400 African Americans (AA), and 2,216 Hispanics (HIS). GWAS of age-, sex-, and eGFR-adjusted and inverse normal rank transformed circulating uromodulin measurements were carried out in each of the seven studies using densely imputed genotypes (25, 26) (Supplementary Table 2) and combined via meta-analysis (Methods).

Trans-ethnic meta-analysis of 10,735,251 genetic variants of minor allele frequency (MAF) >1% across five studies with antibody-based uromodulin quantification (CHS, GCKD, KORA, LURIC, ORIGIN; N=13,985) revealed three genomic loci with at least one significantly associated (p<5e-8) genetic variant (Figure 1A; Supplementary Table 3): UMOD/PDILT (index SNP rs77924615, p=6.4e-577), B4GALNT2 (rs7224888, p=1.8e-32), and PRKAG2 (rs55791829, p=2.9e-09). The genomic control parameter was 0.99, consistent with the absence of undetected population stratification (Supplementary Figure 1A). The estimated SNP-based heritability of uromodulin was 0.135 (95% confidence interval [CI] 0.010-0.259, Methods). Except for the UMOD locus, there was little heterogeneity of genetic effects in the five contributing studies (Supplementary Figure 2). The index variant rs77924615 at the locus with the strongest association, UMOD/PDILT, explained an estimated 18% of the serum uromodulin variance (Table 1, Methods).

The GWAS meta-analysis of 8,815,558 genetic variants across two studies with plasma aptamer uromodulin-readout (ARIC and Fenland; N=18,070) showed no evidence of inflation
(λ=0.99; Supplementary Figure 1B) and revealed 10 genome-wide significant loci (Figure 1B; Supplementary Table 3), with the statistically strongest association observed at rs34211178 upstream of ST3GAL6 on chromosome 3 (p=6.9e-442). ST3GAL6 encodes for ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase (27), an enzyme with alpha-2,3-sialyltransferase activity toward Gal-beta1,4-GlcNAc structures that are present on the glycoprotein uromodulin (28). The largest effect size was observed for a low frequency variant in ASGR2, with each minor allele associated with one standard deviation higher age- and sex-adjusted plasma aptamer uromodulin-readout. The estimated SNP-based heritability was 0.177 (95% CI -0.032-0.386). For each of the two assays, regional association plots for all 13 loci that achieved genome-wide significance are shown in Supplementary Figure 3, and association statistics in Supplementary Table 3.

The two meta-analyses of antibody- and aptamer-based uromodulin measurements identified different genetic loci. A cis-association between SNPs in the UMOD gene and levels of the encoded protein uromodulin were only observed with antibody quantification, supporting that this assay measures the amount of protein. Within each of the two meta-analyses, the association results showed consistent effect sizes and directions in all contributing studies, and both assays had low coefficients of variation (Methods). Moreover, genes identified with both assays can be connected to uromodulin through different sources of external evidence (see below). This indicates that the antibody- and aptamer-based assays for circulating uromodulin deliver reproducible measurements, but assess different properties of their respective targets such as protein amount and glycosylation pattern, respectively.

Secondary analyses, sex-specific effects, and association with urine uromodulin levels
Genome-wide discovery screens without adjustment for eGFR showed virtually identical results (Supplementary Figure 4A and B), indicating that kidney function did not confound genetic associations with uromodulin. A secondary analysis restricting to 11,369 EA participants with antibody-based measurements yielded very similar results as the primary trans-ethnic meta-analysis (Supplementary Table 3).

We next evaluated the presence of sex-specific genetic effects, motivated by the observation that women have higher serum uromodulin levels than men (22, 29, 30). Higher circulating uromodulin in women as compared to men was observed for both the antibody- (mean of the mean uromodulin 103.62 ng/ml in women vs. 92.76 ng/ml in men) and the aptamer-based assay (10329 vs. 9813 relative fluorescence units). Sex-specific analyses identified several genome-wide significant loci for both assays (Supplementary Figure 5), all of which were also identified in the primary combined analyses. The index SNPs at the 13 significant loci did not show evidence for sex-specific differences (Supplementary Figure 6), nor did GWAS of the X-chromosome or a genome-wide test for differences of SNP effects on uromodulin between men and women yield significant findings (Supplementary Table 4).

Given that a previous GWAS meta-analysis of urine uromodulin reported significant associations at the UMOD locus (16), we queried the association between the 13 index SNPs identified in this study and urine uromodulin levels among 29,262 EA individuals (Methods). Except for rs77924615 at UMOD/PDILT (p=5.3e-97), which explained 1.4% of the urine uromodulin variance, none of the other SNPs showed significant (p<3.8e-03 = 0.05/13) associations (Supplementary Table 5).

Prioritization of causal variants in uromodulin-associated loci
Statistical fine-mapping was carried out to identify the most likely causal variants in uromodulin-associated loci (Methods). Conditional analyses supported the presence of more than one independent signal at \textit{UMOD/PDILT} \((n=2)\), \textit{B4GALNT2} \((n=3)\), \textit{ST3GAL6} \((n=4)\) and \textit{ASGR1/ASGR2} \((n=2; \textbf{Supplementary Table 6})\). For each of 20 independent, uromodulin-associated signals within the 13 identified loci, we calculated a SNP set that contains the variant driving the respective association signal with 99% posterior probability. There were 12 sets with <20 variants, five of which had <5 variants \textbf{(Supplementary Table 6)}.

Credible set variant annotation showed several noteworthy findings \textbf{(Supplementary Table 7)}. The antibody-based association on chromosome 16 could be mapped to a single intronic variant, rs77924615, in \textit{PDILT}, the gene upstream of \textit{UMOD}. Another independent set of 10 variants in the locus mapped to \textit{UMOD} \textbf{(Figure 2)}, with the lead SNP rs4293393 experimentally shown to affect \textit{UMOD} transcription \cite{15}. To study whether rs77924615 may be an upstream variant regulating \textit{UMOD} transcription, we generated functional genomic annotation data of chromatin accessibility (ATAC-seq) and gene expression (RNA-seq) from cortex and medulla of native human kidney tissue (Methods) that showed transcription of \textit{UMOD}, more strongly in medulla than in cortex, but not of \textit{PDILT}. Both independent variants, rs77924615 in \textit{PDILT} and rs4293393 in the \textit{UMOD} promoter, mapped into regions of open chromatin in medulla, where \textit{UMOD} transcript levels in human kidney are highest (GTEx Project V8) \cite{31}. These regions aligned with open chromatin in LOH and DCT kidney cells from single-nucleus ATAC-seq data (Methods), that were not observed in several other kidney and immune cell types \textbf{(Figure 2)}. Both variants mapped into the same topological associated domain, with predicted contacts based on chromatin conformation capture (Hi-C, Methods). Thus, the identified SNPs likely are regulatory variants in kidney cell types producing uromodulin.
The lead SNP at the B4GALNT2 locus, rs7224888, was identified with the antibody assay and is a missense variant. Its minor C allele encodes a cysteine-to-arginine substitution (p.Cys466Arg; NP_703147.2) in the encoded enzyme beta-1,4-N-acetylgalactosaminyltransferase 2. The C allele was associated with higher circulating uromodulin in our study, and has been linked to the absence of the Sda antigen (32), a blood group antigen synthesized by B4GALNT2 that is present on uromodulin (33). The B4GALNT2 locus also contained an independent small credible set of three variants. The most likely causal rs72835417 maps into a splice region; its minor allele was associated with higher uromodulin in our study, and with lower B4GALNT2 expression (p=1.8e-7) in micro-dissected kidney tubules (34), further supporting that reduced B4GALNT2 function relates to higher circulating uromodulin.

At SIGLEC9, the major G allele at the most likely causal variant rs2075803 leads to a lysine-to-glutamine substitution in SIGLEC9 (p.Lys100Glu; NP_001185487.1). It was associated with lower aptamer signal in our study (p=3.8e-100), and with lower circulating SIGLEC9 protein (p=6e-2142) (35) and serum C-reactive protein (p=5e-10) (36) in previous studies. The encoded sialic acid-binding Ig-like lectin-9 is an inhibitory receptor mainly present on neutrophils and monocytes. It has been experimentally shown to interact with urinary uromodulin (37), indicating that genetically encoded variation in SIGLEC9 levels relates to differences in the aptamer readout of circulating uromodulin.

Uromodulin-associated loci are associated with distinct sets of biomarkers and diseases

Genetic studies have linked variation in UMOD to monogenic autosomal-dominant tubulointerstitial kidney disease (17, 38), to complex kidney function traits and CKD (4), blood pressure and hypertension (11, 12), uric acid levels and gout (13) as well as to kidney stone
In order to investigate whether any of the 13 significant loci share phenotype association patterns, and to detect additional disease associations that may be mediated by altered uromodulin levels or properties, we performed colocalization with (i) levels of 30 biomarkers and (ii) 1,404 complex traits and diseases based on data from the UK Biobank study, as well as (iii) additional traits previously linked to common UMOD variants – namely eGFR, CKD, systolic (SBP), diastolic blood pressure (DBP) and uromodulin levels in urine (Methods). Interestingly, genetic associations with antibody-based circulating uromodulin at the PRKAG2 and UMOD/PDILT loci shared a very similar pattern of colocalization with numerous kidney-related traits (creatinine, cystatin C, urea, eGFR, CKD, urinary calculus, DBP, SBP, hypertension; Supplementary Table 8, Figure 3). The directions of association of colocalizing traits were consistent with biological knowledge based on studies of uromodulin levels in urine, for example higher serum uromodulin and higher risk of CKD (Figure 3). These observations are consistent with a common biological context of the PRKAG2 and UMOD/PDILT loci in the pathophysiology of CKD, hypertension, and kidney stone disease, and with the earlier identification of the PRKAG2 locus in GWAS of CKD (39). Conditional co-localization of two independent SNP sets at the UMOD locus further supported a shared genetic cause between the levels of circulating and urine uromodulin levels (Figure 3, Supplementary Table 9).

There were several other examples of positive colocalizations supported by biological knowledge: first, genetic associations at the B4GALNT2 locus with antibody-based circulating uromodulin colocalized with the odds of multiple gestation. This is consistent with a role of the B4GALNT2-mediated Sda antigen in embryo implantation in mice (40) (Figure 3). Second, SNPs at SIGLEC9 associated with plasma aptamer uromodulin-readout colocalized with levels of alkaline phosphatase in blood, which is in line with altered bone turnover described in
recent knockout mouse model of the homologous gene (41). These observations suggest that the aptamer-based assay is particularly well suited to generate insights into the generation of glycosylation residues that are present on uromodulin, and how such glycosylation residues are recognized in the circulation. Tests of pairwise interactions of the 13 index SNPs, which could point towards non-additive effects when the same pathway is affected, showed a significant interaction between genotype at the lead SNPs in \textit{ST3GAL6} and \textit{SIGLEC9} on plasma aptamer uromodulin-readout (interaction p-value=3e-07; Methods). These two genes are indeed functionally related, as \textit{ST3GAL6} is involved in the synthesis of sialic acid moiety that is bound by \textit{SIGLEC9}.

Additionally, we tested the aggregate effect of rare (MAF <0.1%), potentially deleterious variants in the genes prioritized at each of the 13 uromodulin-associated genetic loci on 770 complex diseases (Methods). Using data from whole exome sequencing of 173,688 UK Biobank participants, significant associations (p<4.9e-06) were identified between carrier status of rare \textit{UMOD} variants and anemia of chronic disease (OR=2.8, p=6.1e-09), hypertensive CKD (OR=2.6, p=1.8e-06), and CKD (OR=1.89, p=2.0e-09; \textbf{Supplementary Table 10}).

\textit{Prioritization of causal genes in uromodulin-associated loci}

Colocalization analyses of the uromodulin association signals at all 13 significant loci were also performed with the expression of genes in \textit{cis} based on transcriptome-wide RNA-sequencing of 36 non-brain tissues (GTEx Project V8) (31), tubulo-interstitial and glomerular kidney tissue portions (42), as well as with circulating plasma proteins (35) in order to prioritize the most likely causal genes (Methods). Plasma proteomics captures information about the abundance, structure, and context of circulating proteins and, when integrated
with genomics, can reveal new insights into proteins that mediate genetic associations with complex traits and diseases (35, 43, 44). At least one positive colocalization with gene expression or plasma proteins was observed for most loci (Supplementary Table 11, Supplementary Figure 7). For example, the association of genetic variants on chromosome 19 with uromodulin colocalized with their association with SIGLEC9 protein, further supporting that genetic variation in SIGLEC9 relates to changes in the aptamer readout of circulating uromodulin. We confirmed that the aptamer readouts of plasma uromodulin and SIGLEC9 were correlated in the ARIC study (Spearman coefficient 0.53, p<2.2e-16).

Integration of colocalization evidence with additional sources of annotation (Methods) implicated PRKAG2, B4GALT2, and UMOD/PDILT as the genes most likely causing the association with antibody-based uromodulin, and CFH, MGAT5, ST3GAL6, HLA-DRB1, B4GALT1, ABO, DPP7/MAN1B1, ST3GAL4, ASGR1/ASGR2, and SIGLEC9 for the aptamer-based readout (Supplementary Table 12).

Antibody- and aptamer-based measurement of circulating uromodulin differ in their approach (Figure 4A). Whereas the antibody-based methods quantify abundance of the circulating protein as evidenced by the cis-association at UMOD/PDILT, the aptamer-based assay may rather identify differences related to glycan marks known to be present on uromodulin and their receptors, for example because such modifications may lead to differential aptamer binding. In order to detect any shared functions, processes and pathways among the respective genes identified by each assay, enrichment analyses were performed (Methods). Terms and pathways related to protein glycosylation were highly enriched for genes identified via aptamer plasma uromodulin readout (Figure 4B), with the genes that drove the enrichment encoding for enzymes and receptors involved in the biosynthesis and recognition of glycans, respectively (Figure 4C, Supplementary Table 13).
B4GALNT p.Cys466Arg causes reduced enzyme function and processing

To study the possible biological effect of rs7224888, we first generated a homology-based model of B4GALNT2. The arginine insertion at p.Cys466Arg was predicted to reduce protein structural stability by three different programs (Pymol, Site Direct Mutation, Missense3D), likely as a consequence of the higher steric hindrance of arginine (Figure 5A and data not shown). To validate the in silico findings, we transfected Madin-Darby Canine Kidney (MDCK) cells with expression vectors for the two B4GALNT2 allelic variants. Two isoforms were reported for B4GALNT2, long and short (45). For this project, we employed an already described expression vector for B4GALNT2 short isoform, where cysteine 466 corresponds to residue 406. A previous study (32) suggested that the p.Cys466Arg variant affects a region that glycosyltransferases typically use to interact with their substrate, impairing B4GALNT2 activity. Thus, we tested the activity of the enzyme isoforms (wildtype [wt] and Arg406) by taking advantage of the well-established interaction between the Dolichos biflorus agglutinin (DBA) and the Sda antigen (46). By using a rhodamine labelled version of DBA we observed a clear signal, mostly localized on the plasma membrane, in MDCK cells expressing wt B4GALNT2, while virtually no signal could be detected for Arg406 expressing cells (Figure 5B), confirming absent activity. Double staining of wt B4GALNT2 and DBA confirmed the specific presence of Sda antigen only in cells expressing the enzyme (Figure 5C).

Western blot analysis on cells lysates showed that Arg406 B4GALNT2 had a slightly reduced molecular weight compared with wt protein. Such difference is related to different glycosylation, due to retention of Arg406 B4GALNT2 in the endoplasmic reticulum (ER). Indeed, the lower band observed for the Arg406 isoform was fully sensitive to treatment with Endo H, a deglycosylating enzyme that is specific for high-mannose, ER-type N-glycans, while
only a minor fraction of wt B4GALNT2 was cleaved by Endo H (Figure 6A). To substantiate this finding, we analyzed the intracellular localization of B4GALNT2 by immunofluorescence. While the wt isoform showed the expected predominant localization in the Golgi compartment, the Arg406 isoform fully colocalized with the ER marker KDEL, confirming its ER retention (Figure 6B, C). These results demonstrate that the B4GALNT2 variant p.Cys466Arg is functional and leads to loss of B4GALNT2 function and ER retention, likely due to protein misfolding.

Previous in vitro studies demonstrated that a N-acetyl-β-D-galactosaminyltransferase activity present in microsomal preparations of guinea-pig kidney transfers \(N\)-[\(^{14}\text{C}\)]-acetylgalactosamine to N-linked glycans of UMOD for the synthesis of Sda antigen (47). To assess whether B4GALNT2 directly acts on uromodulin under physiological conditions, we first verified their co-expression in kidney cells using real-time RT-qPCR on RNA extracted from micro-dissected mouse nephron segments and immunofluorescence on mouse and human kidney tissue (Methods). Real-time RT-qPCR demonstrated the presence of B4GALNT2 transcript in TAL and DCT segments where uromodulin is expressed (Figure 7A). These data were confirmed by immunofluorescence analysis that showed a strong B4GALNT2 signal in collecting ducts (AQP2+), and a low but consistent signal in UMOD-positive cells (Figure 7B). B4GALNT2 expression in UMOD-positive cells was also confirmed in human kidney tissue (Figure 7C). To demonstrate the activity of B4GALNT2 on uromodulin glycosylation, we generated MDCK clones stably expressing uromodulin +/- B4GALNT2. Western blot analysis showed that uromodulin has a slightly increased molecular weight in lysates of B4GALNT2-positive cells that is due to different protein glycosylation, as demonstrated by removal of N-glycans through PNGase F treatment (Figure 7D).
Finally, we excluded that the association of B4GALNT2 loss-of-function with higher circulating uromodulin can be ascribed to altered immunoreactivity due to absence of the Sda antigen based on two observations: first, the quantitative, additive effect of UMOD variants on serum uromodulin was clearly detected regardless of the B4GALNT2 genotype (Figure 8A). Second, the immunoreactivity of both ELISA antibodies did not differ from a reference antibody in detecting increasing amounts of uromodulin produced by cells expressing or not expressing B4GALNT2, hence carrying or not the Sda antigen glycan moiety (Figure 8B).

Discussion
This GWAS meta-analysis of circulating uromodulin using complementary antibody-based (N=13,985) and aptamer-based (N=18,070) assays has four principal findings: first, it identifies an upstream variant at the UMOD/PDILT locus for which integration with functional genomics data from primary human kidney tissue supports differential chromatin accessibility and transcription in cells synthesizing uromodulin as underlying its strong association with circulating and urine uromodulin, as well as CKD and hypertension. Second, shared association patterns of uromodulin-associated genes with complex traits and diseases are plentiful and place the PRKAG2 and UMOD loci into the same context with respect to their associations with CKD, hypertension, and kidney stone disease. Third, the missense variant p.Cys466Arg in the uromodulin-glycosylating enzyme B4GALNT2 is a loss-of-function allele leading to higher levels of circulating uromodulin. Fourth, our study reveals enzymes that write glycan marks found on uromodulin and their receptors that may be related to uromodulin’s complex glycosylation pattern, function and clearance.

Previous GWAS of circulating uromodulin quantified with antibody assays only identified the UMOD locus (16, 23). At the UMOD locus, findings were consistent with those
from urine (16) and a previous small GWAS of serum uromodulin (23), e.g., the major allele at the index SNP was associated with higher uromodulin levels. The generation of functional genomic data from kidney tissue now allowed for new insights at this locus, by providing a plausible mechanism by which an intronic variant rs77924615 in the upstream gene PDILT is associated with uromodulin levels (16), despite the absence of PDILT transcription in kidney. Evidence for accessible chromatin at the SNP’s position solely in target kidney cell types for uromodulin synthesis, TAL and DCT cells, and mapping of rs77924615 and the functional UMOD promoter index SNP rs4293393 within the same topological associated domain, are indicative of a regulatory effect of this upstream variant on uromodulin transcription. This mechanism is also likely to underlie the reported associations of rs77924615 with urine uromodulin levels, kidney function and CKD (7).

There are several potential explanations why a previous meta-analysis of urine uromodulin levels did not detect any of the other loci identified here despite a similar sample size (16). First, uromodulin occurs as a polymer in urine but is present as a monomer in blood. In addition, the 100-fold higher levels in urine as well as the high biological variability of urine concentration and composition may preclude the detection of slight variations that can be observed in plasma. Our study supports a shared genetic basis for urine and circulating uromodulin levels, but the index variant rs77924615 explained more than 10 times as much of the uromodulin variance in the circulation compared to the urine. Thus, circulating uromodulin may be a more attractive biomarker to estimate uromodulin production in the kidney. Second, it is conceivable that receptors recognizing glycan marks present on uromodulin, potentially affecting its stability or clearance, differ between urine and the circulation. Third, previous urine studies did not use aptamer-based assays. Our results suggest that the aptamer detects genetic loci related to the writing of glycans that are present
on many secreted glycoproteins, including uromodulin, and their recognition in the circulation, rather than representing the abundance of the intact protein in blood. Future validation of the uromodulin aptamer, and investigations how the amount of uromodulin protein relates to its glycosylation patterns, are of interest. Regardless, the aptamer readout carries complementary information by delivering insights into the glycan component of this important glycoprotein. Despite the distinct set of loci detected with the two assays, there are also connections: the ABO encoded glycosyltransferase acts on precursor chains that are also substrates of the enzymes encoded by ST3GAL4 and ST3GAL6 to synthesize type 2 monosialyl-galactosylgloboside that is used for the synthesis of sialyl Lewis X antigen, and by B4GALNT2 for the synthesis of the Sda antigen (48, 49).

Concerning B4GALNT2, our functional studies demonstrate that the p.Cys466Arg variant leads to retention of the Arg466 isoform in the ER and its absence in the Golgi compartment. This is likely due to protein misfolding, as suggested by prediction analysis of the effect of the missense change on B4GALNT2 structural stability. The consequence, loss of protein function, is demonstrated by virtual absence of DBA-positivity. These data are consistent with and provide a mechanistic explanation for previous results showing that the Arg466 variant is statistically correlated with absence of the Sda antigen (32).

The role of B4GALNT2 in uromodulin glycosylation is supported by the proteins’ demonstrated co-expression in TAL and DCT segments in mouse and human kidney. Moreover, we show that expression of B4GALNT2 in cells expressing uromodulin leads to addition of a glycan moiety, presumably the Sda antigen. The functional role of the Sda antigen on uromodulin is not known. Through the addition of β1,4-linked GalNAc it may hinder binding of bacterial adhesins and, hence, have a role in pathogen resistance. There are several potential explanations for how B4GALNT2 loss-of-function, i.e. absence of the Sda
antigen, may lead to higher serum levels of uromodulin as quantified by antibody. Neither our experimental nor our population study data provides evidence for an altered immunoreactivity due to absence of the Sda antigen, making this an unlikely option. It is conceivable that the presence of the Sda antigen is associated with lower stability of circulating uromodulin, as observed for von Willebrand factor (vWF) through a mechanism that depends on ASGPR activity (50). Alternatively, the absence of the Sda antigen may influence uromodulin polarized trafficking, partly redirecting the protein towards the basolateral membrane and from there to the circulation.

Common variants at the \textit{PRKAG2} locus showed a striking similarity to those at \textit{UMOD} with respect to shared disease association patterns. Considering that we tested for colocalization with hundreds of human traits and diseases, these mirroring patterns are extremely unlikely to result from chance. Our results imply that genetic variants at \textit{PRKAG2} are associated with higher risk for CKD and hypertension, as well as lower risk for kidney stone disease, through the same biological context as \textit{UMOD}. \textit{PRKAG2} encodes for the regulatory gamma subunit of the AMP-activated protein kinase (AMPK), an enzyme with a key role in regulating multiple processes related to cellular energy metabolism. AMPK has been described to phosphorylate the kidney-specific Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{-} cotransporter (51), the molecular link between CKD-associated \textit{UMOD} variants and hypertension (15). However, although this connection is biologically plausible, we cannot exclude that \textit{GALNT11, GALNT5} or other genes or elements in the \textit{PRKAG2} locus represent the causal link.

The loci identified through aptamer-readout point towards the importance of uromodulin glycosylation in general and sialylation in particular. SIGLEC9 receptor binding to uromodulin depends on the presence of terminal sialic acid on uromodulin N-glycans (37). ASGPR, mainly expressed in liver hepatocytes and to lower extent in several cell types
including monocytes (52), mediates binding, endocytosis and degradation of glycoproteins with decreased sialylation and exposed terminal galactose or N-acetyl galactosamine (GalNAc) residues (52-55). An observed four-fold increase of circulating uromodulin in mice upon ablation of Asgr2 and the mannose receptor (56) as well as the association between a rare ASGR2 variant and the aptamer uromodulin-readout in our study raise the possibility that circulating uromodulin may be another glycoprotein recognized by the ASGPR. The function of SIGLEC9 and ASGPR intersects with that of the ST3Gal family of sialyltransferases, which add sialic acid in alpha2,3 linkage to terminal galactose, thereby generating potential SIGLEC9 ligands, while masking ASPGR ligands (53, 57-59). Overall, these data point at a clear functional relationship between SIGLEC9 and ASGPR, potentially involved in uromodulin-mediated immunomodulatory signaling and clearance, and ST3GAL4 and 6, modulating such activities through sialylation of glycan moieties.

Our study also provides insights of potential clinical relevance. It indicates that a common genetic basis of urine uromodulin levels with higher risk of CKD and hypertension extends to circulating uromodulin levels, and identifies kidney cell-type specific regulation of uromodulin expression as a mechanism. Interventions aimed at reducing uromodulin synthesis can therefore be expected to have concomitant effects on both urine and serum levels of the protein, which may be of importance given its emerging systemic relevance. The association of genetic variants at PRKAG2 with higher risk for CKD and hypertension as well as lower risk for kidney stone disease suggests a biological link between PRKAG2 and UMOD and suggest that PRKAG2 represents another target to modulate uromodulin-mediated risk of CKD. Finally, therapeutic targeting of (a)sialoprotein receptors may impact on circulating uromodulin, possibly modulating cross-talk between the kidney and the innate and adaptive immune system.
In conclusion, our study provides human genetic evidence of new pathway members of uromodulin and delivers novel insights into its determinants and systemic role in the circulation.
Methods

Study design and participants

Seven prospective studies participated in the genome-wide analyses of serum/plasma uromodulin levels (Supplementary Table 1): the Cardiovascular Health Study (CHS) (60), German Chronic Kidney Disease (GCKD) study (61), the Cooperative Health Research in the Region Augsburg (KORA) study (62), the LLudwigshafen Risk and Cardiovascular Health (LURIC) study (63), the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial (64), the Atherosclerosis Risk in Communities (ARIC) (65) study and the Fenland study (66). Each study contributed data from EA participants. Data from AA and HIS participants were contributed by the CHS study (AA) and the ORIGIN trial (AA, HIS). The ARIC, CHS, Fenland and KORA studies have a population-based design, the GCKD study recruited patients with chronic kidney disease, the LURIC study recruited patients with cardiovascular disease, and the ORIGIN trial patients with impaired glucose tolerance or early type 2 diabetes. Demographic information including age and sex was collected using standardized procedures. The estimated glomerular filtration rate (eGFR) was calculated from IDMS-traceable serum creatinine measurements using the four-variable CKDEpi equation (7).

Genotyping and imputation

Details about genotyping and imputation in each of the six studies are provided in Supplementary Table 2. In brief, all samples were genotyped for genome-wide single nucleotide polymorphisms (SNPs) using Illumina or Affymetrix arrays and called using commercial software. Variant-level quality control and cleaning included removal for low call rate and deviation from Hardy-Weinberg equilibrium. Genotype imputation was then performed using phasing and imputation software, based on the Trans-Omics for Precision
Medicine (TOPMED) haplotypes version r2 (ARIC) or the Haplotype Reference Consortium (HRC) haplotypes version r1.1 (all other studies) reference panels.

Uromodulin quantification

The CHS, GCKD, KORA, and LURIC studies quantified uromodulin from serum using a commercial ELISA (23, 30, 67, 68) (Euroimmun, Medizinische Labordiagnostika AG). The assay is based on a colorimetric sandwich immunoassay, in which the capture antibody was a mouse monoclonal antibody against human uromodulin, and the detection antibody was a biotinylated mouse monoclonal antibody against human uromodulin. The intra-assay precision of the assay at 30–214 ng/mL was 1.8–3.2%, and the inter-assay precision at 35–228 ng/mL 6.6% to 7.8% (21). The ORIGIN study measured serum uromodulin using an immunoassay that is part of the Human DiscoveryMAP panel (Myriad RBM Inc.) with a biotinylated polyclonal detection antibody against human uromodulin (69). In the ARIC and Fenland study, uromodulin was quantified as part of plasma proteome profiling using the SOMAscan assay (Seq-ID 9451-20), a multiplexed modified DNA-based aptamer technology by SomaLogic as described previously (43). The aptamer was raised against amino acids 24-611 of human uromodulin (NP_001008390.1). Its signal-to-noise ratio was 114, and its intra- and inter-assay coefficients of variation were 3.2% and 3.6%, respectively.

GWAS of serum uromodulin levels and meta-analyses

Each study performed four sets of GWAS according to a pre-specified analysis plan: a primary analysis, in which inverse normal rank transformed age-, sex-, and eGFR-adjusted residuals of uromodulin levels were used as the dependent variable and regressed on genotypes as the predictor, controlling for principal components. In addition, three secondary analyses were
carried out: the primary analysis was repeated separately for men and women (without adjustment for sex and only when at least 50 participants were available), and a sex-combined analysis was performed in which the residuals were not adjusted for eGFR. Studies with participants of different ancestries performed separate GWAS for each ancestry group. Sex-stratified GWAS of chromosome X markers, assuming X inactivation and an additive model, were only available from the CHS and GCKD studies. Genome-wide summary statistics were collected in a prespecified format and uploaded to a central server for meta-analysis.

Prior to meta-analysis, GWAS summary statistics from individual studies were subjected to thorough quality control using GWAtoolbox (70). The variant identifiers in each GWAS file were harmonized to the format chromosome:position:ref:alt, where ref and alt are the REF and ALT alleles in the HRC r1.1 reference site file. Genome-wide summary statistics were combined for studies with antibody-based uromodulin quantification using inverse-variance weighted meta-analysis of effect estimates. Previous meta-analyses of uromodulin levels in urine quantified from ELISA and the RBM immunoassay showed little heterogeneity and comparable results when using inverse variance weighted and sample size weighted meta-analysis (16). The primary meta-analysis was a trans-ethnic analysis, combining data from EA, AA, and HIS participants, and performed using metal (71). Sex-specific summary statistics of chromosome X were combined via meta-analysis. Genomic control was applied to individual GWAS files when the inflation parameter was >1. Genome-wide significance was defined as p<5e-08. EA-specific meta-analyses were also performed, as EA participants were the largest subsample. SNP-based heritability was estimated using LDSC v1.0.1 with the option --h2. Pre-computed LD scores from 1000 Genomes European data were used as reference. Input files for LDSC were GWAS summary statistics from the primary association analyses filtered for MAF >0.01 (antibody-based assay) or MAF >0.005 (aptamer-based assay).
**Downstream characterization of GWAS meta-analysis results**

Several complementary approaches to characterize genetic loci identified through genome-wide screens of circulating uromodulin were employed, with detailed Methods described in the Supplementary Material. These include (i) associations with urine uromodulin levels, (ii) annotation, enrichment analyses and functional genomics, (iii) independent SNP selection, statistical fine mapping and credible set annotation, (iv) colocalization with gene expression, plasma protein levels, biomarkers and diseases, (v) PheWAS of serum uromodulin levels, as well as (vi) gene-by-gene interaction analyses.

**3D modelling of B4GALNT2 and prediction of the effect of p.Cys466Arg**

The sequence of B4GALNT2 isoform 2 (Uniprot Q8NHY0-2) was analyzed in PFAM ([http://pfam.xfam.org](http://pfam.xfam.org)) to map functional domains. The region 254-464 containing the glycosyltransferase domain was analyzed in iTasser (72) to generate a homology-based 3D model. The effect of the p.Cys466Arg substitution was analyzed in Pymol (version 2.3.4) (Schrödinger) with the Mutagenesis Wizard function, in Site Direct Mutator (73) and in Missense3D (74).

**Experimental studies of B4GALNT2**

Detailed information regarding functional studies of B4GALNT2 (constructs, cell lines and culture conditions, protein extracts and Western blot analysis, RNA isolation and quantitative RT-PCR, immunofluorescence analysis, antibodies) are reported in Methods section in the Supplementary Material. Protein and RNA extracts, Western blot, quantitative RT-PCR and immunofluorescence analyses were carried out as previously described (9, 75).
Study approval

All participants of CHS, GCKD, KORA, LURIC, ORIGIN, ARIC and Fenland studies provided written informed consent, and the studies were approved by their local ethics committees as outlined in their respective design publications (60-66). The use of human kidney biopsies in experimental studies of B4GALNT2 has been approved by the UCLouvain Ethical Review Board. Human kidney tissues used in functional genomics were collected in deidentified fashion through Northwest Biotrust at the University of Washington Medical Center (Seattle, WA) with local IRB approval (Study 1297).

All mouse experiments were performed in accordance with the ethical guidelines at University of Zurich (Zurich, Switzerland) and the legislation of animal care and experimentation of Canton Zurich (Kanton Zürich Gesundheitsdirektion Veterinäramt; protocol ZH049/17).
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Author contributions

Conception and design of the study: Y.L., Y.C., F.C., L.R., A.K.; Statistical analysis: Y.L., Y.C., M.R.C., M.P., N.Q.H.N., N.S., M.L.B., M.E.K., B.G., Pe.S., I.S., Pa.S., M.W., S.A., L.R., A.K.; Management of an individual study: M.E.K., M.P., J.A.B., M.E.G., C.H., U.T.S., B.K.K., F.K., A.P., J.S., D.S., C.T., KU.E., C.G., E.B., B.M.P., J.C., C.L., B.Y.; Measurement of uromodulin: J.S., D.S., C.T., W.M., G.P., J.E.S.; Bioinformatic data integration: Y.L., Y.C., M.E.K., N.S., C.B.J., M.W.; Interpretation of results: Y.L., Y.C., F.C., G.S., P.J.O., S.A., O.D., L.R., A.K.; Experimental studies: F.C., G.S., S.A., O.D., L.R.; Initial manuscript draft: Y.L., Y.C., F.C., G.S., S.A., L.R., A.K.; Critical revision of the manuscript: all authors. The order of the co-first authors was determined based on the overall analytical and conceptual contribution to the project.

Competing interest statement
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Figures

Figure 1: Manhattan plot of GWAS of antibody-based and aptamer-based circulating uromodulin. The plots represent, for each SNP, the p-value from meta-analyses of GWAS of antibody-based (N=13,985, dark blue; panel A) and aptamer-based circulating uromodulin (N=18,070, light blue, panel B). The x-axis shows chromosomal location and the y-axis the \(-\log_{10}(p\text{-value})\) of SNP associations with circulating uromodulin. The plots were generated using the R package EasyStrata v8.6. Meta-analyses of X-chromosomal markers did not yield any significant findings.
Figure 2: Functional genomic annotation of significantly associated independent variants at the UMOD/PDILT locus using gene expression and chromatin accessibility data from primary human kidney. The upper part shows the regional association plot of the UMOD/PDILT locus, using the two independent variants as reference SNPs. For non-reference SNPs, the extent of LD with the reference SNP with higher correlation is shown by color gradients. Genetic positions (x-axis) represent GRCh38 coordinates. Open chromatin peaks in different kidney cell type tracks based on single nuclear (sn)ATAC-seq are shown underneath the regional association plot. Gene expression and open chromatin tracks of cortex (light blue tracks) and medulla (dark green tracks) based on bulk RNA-seq and ATAC-seq are shown in the lower part as density peaks. SNPs in the two independent credible sets are marked by ticks (purple) and the 10 kb windows encompassing them indicated by the black horizontal bars. Hi-C data generated from kidney cortex was analyzed for contacts (orange arcs) between the 10kb windows encompassing the indicated SNPs with contacts closest to the causal SNPs arbitrarily shown in bold. Intervals for DomainCaller computed topology associated domains (TADs) are shown as black bars below contact arcs. A heatmap of all Hi-C contacts encompassing this region is shown at the bottom (purple). Podo: podocyte, PT: proximal tubule, LOH: loop of Henle, DCT: distal convoluted tubule, CDPC: collecting duct principal cells, CDIC: collecting duct intercalated cells.
Figure 3: Summary of findings from colocalization of uromodulin signals with associations from GWAS of biomarkers and diseases. The colocalization analyses findings are shown in two categories, biomarkers and diseases. The x-axis indicates the index SNPs with the likely causal genes. The y-axis shows the traits for biomarkers and diseases, and only top level UKB PheCodes are shown. Within each category, horizontal lines separate different data sources (Methods). Included traits had at least one positive colocalization signal (H4>0.8, Methods). Dots are black when H4>0.8 and grey otherwise, and scaled in size to reflect the different ranges of H4. The trait-to-uromodulin effect size ratios are shown as gradient background for positive colocalization signals, with red indicating positive and blue negative changes per unit higher uromodulin levels. The colocalization with urine uromodulin (uUMOD) was based on conditional association statistics (Methods). H4: hypothesis that one shared SNP underlies the association with two traits; PP: posterior probability.
Figure 4: Biological context of genes associated with circulating uromodulin and conceptual model. A, Schematic of antibody- and aptamer-based measurement of circulating uromodulin. B, Dot plot shows Gene Ontology (GO) terms - grouped into three categories (BP: biological process, MF: molecular function, CC: cellular component) - and KEGG pathways enriched for uromodulin-associated genes from the aptamer assay on the y-axis. X-axis shows the proportion of the genes in the corresponding GO term or KEGG pathway. Only terms and pathways with more than two uromodulin associated-genes are displayed. The color intensity of the dots’ scales with the -log_{10}(Benjamini Hochberg-adjusted p-value). C, Conceptual model placing the most likely causal genes associated with circulating uromodulin into their biological context. Loci detected with the aptamer assay predominantly affect differential synthesis or recognition of glycan marks present on uromodulin.
**Figure 5: B4GALNT2 p.Cys466Arg is a functional allele.**

A. Homology-based model of the tridimensional structure of B4GALNT2 enzyme. The partial sequence of B4GALNT2 isoform 2 (Uniprot Q8NHY0-2) containing the glycosyltransferase domain (residues 254-464) was analyzed with iTasser. The top scoring model is shown. The position of cysteine 406 (corresponding to position 466 in B4GALNT2 isoform 1, Uniprot Q8NHY0-1) in the reference sequence is shown. The effect of the p.Cys406Arg substitution was analyzed in Pymol with the Mutagenesis Wizard function. For each isoform, the most likely stereoisomer is shown. Visible red disks indicate significant contacts and bumps. The arginine substitution at position 406 is predicted to increase steric clashes, destabilizing protein structure.

B. Representative immunofluorescence analysis showing DBA signal (red) on the plasma membrane of unpermeabilized MDCK cells, transiently transfected with wt or p.Cys406Arg human B4GALNT2 (N=3).

C. Representative immunofluorescence analysis showing wt B4GALNT2 (green) and DBA (red) in stably transfected MDCK cells. DBA signal is mostly evident in B4GALNT2-expressing cells (N=3).
Figure 6: B4GALNT2 p.Cys466Arg is retained in the ER. A, Representative western blot analysis showing B4GALNT2 wt or p.Cys406Arg in stably transfected MDCK cells lysates, untreated or after deglycosylation with Endo H or PNGase F (N=3). B,C, Representative immunofluorescence analysis showing intracellular signal of wt (B) or p.Cys406Arg (C) B4GALNT2 (red) and GM130 (Golgi marker, green) or KDEL (ER marker, green) and merged pictures (N=3).
Figure 7: B4GALNT2 and uromodulin expression analysis. A, RT-qPCR analysis of B4galnt2 expression in isolated mouse nephron segments. Glom, glomerulus; PCT, proximal convoluted tubule; PST, proximal straight tubule; TAL, thick ascending limb; DCT, distal convoluted tubule; CD, collecting duct. Bars indicate mean ± SEM. n ≥ 3 fractions. B, Upper panels: Immunofluorescence analysis showing UMOD (green) and B4GALNT2 (red) on paraffin-embedded kidney sections from wild-type mouse (N=2). Right panels show high magnification pictures of UMOD-positive and UMOD-negative tubules. Nuclei are counterstained with DAPI. Lower panels: Immunofluorescence analysis showing AQP2 (green) and B4GALNT2 (red) on mouse kidney, showing a strong signal in the intercalated cells of collecting ducts. Right panels show high magnification pictures of AQP2- and B4GALNT2-positive tubules. Nuclei are counterstained with DAPI. C, Immunofluorescence analysis showing UMOD (green) and B4GALNT2 (red) on paraffin-embedded kidney sections from a normal human kidney. Right panels show high magnification pictures of UMOD-positive and UMOD-negative tubules. Nuclei are counterstained with DAPI. D, Western blot analysis showing uromodulin (UMOD) and B4GALNT2 in lysates of MDCK cell clones expressing UMOD with or without B4GALNT2 (see Methods), untreated or after deglycosylation with PNGase F. Actin is shown as a normalizer.
Figure 8: ELISA-based uromodulin quantification is not affected by presence of Sda antigen. 

A, Uromodulin serum levels in individuals carrying GG or GA/AA genotype at UMOD variant rs77924615, stratified for their genotype at B4GALNT2 variant rs7224888 (CC, Sda+; TT, Sda-). The expected differences in uromodulin levels are detected regardless of the presence/absence of Sda antigen. The start and end of boxes represent the 25th and 75th percentiles of the uromodulin distribution. The line inside the box represents the median, and the dots indicate outliers above the 75% + 1.5 × (interquartile range) of uromodulin values. B, Representative western blot analysis (left) and relative quantification (right) of uromodulin in lysates of MDCK cells transduced with lentiviral vector expressing HA-tagged uromodulin (lv.HA-hUMOD) and stably expressing B4GALNT2 (Sda+) or not (Sda-). The immunoreactivity of 3 different antibodies (anti-HA, and the two antibodies of the Euroimmun ELISA anti-UMOD capture and anti-UMOD detector) was assessed by loading and quantifying increasing amount of cell lysate. Each value represents the ratio between B11 (negative clone) and B9 (positive clone) expressed as fold relative to the ratio measured with 25 μg of cell lysate (n=3 independent experiments). Data are represented as vertical scatter plot expressed as mean ± S.D. (one way ANOVA; p=0.66). The ratios obtained for the different antibodies are comparable, suggesting similar immunoreactivity that is not modified by the presence of the Sda antigen.
Table 1: Summary of genomic loci with genetic variants significantly associated with uromodulin levels. The SNP with the lowest association p-value (index SNP) at each of the genomic loci is shown.

| Uromodulin assay | SNP     | chr | Position (b37) | Locus      | Effect allele / non-effect allele | Effect allele frequency | Effect StdErr | P-value          | Sample size | Proportion of explained variance |
|------------------|---------|-----|----------------|------------|----------------------------------|------------------------|---------------|-----------------|-------------|----------------------------------|
| antibody         | rs55791829 | 7   | 151407429      | PRKAG2     | C/G                              | 0.72                   | -0.08         | 0.014           | 2.89E-09   | 13956                             |
| antibody         | rs77924615 | 16  | 20392332       | UMOD/PDILT | A/G                              | 0.19                   | -0.76         | 0.015           | 6.39e-577  | 13956                             |
| antibody         | rs7224888 | 17  | 47246163       | B4GALNT2   | T/C                              | 0.90                   | -0.25         | 0.021           | 1.77E-32   | 13956                             |
| aptamer          | rs10922098 | 1   | 19664651       | CFH        | T/C                              | 0.61                   | 0.08          | 0.011           | 2.30E-12   | 18070                             |
| aptamer          | rs7601756 | 2   | 134893447      | MGAT5      | A/G                              | 0.28                   | -0.07         | 0.012           | 8.81E-09   | 18070                             |
| aptamer          | rs34211178| 3   | 98383562       | ST3GAL6    | A/G                              | 0.45                   | -0.46         | 0.010           | 6.88e-442  | 18070                             |
| aptamer          | rs3117139 | 6   | 32364667       | HLA-DRB1   | A/G                              | 0.13                   | 0.14          | 0.016           | 2.60E-19   | 18070                             |
| aptamer          | rs10738906| 9   | 31315634       | B4GALT1    | T/C                              | 0.69                   | -0.09         | 0.011           | 8.44E-15   | 18070                             |
| aptamer          | rs532436 | 9   | 136149830      | ABO        | A/G                              | 0.20                   | -0.14         | 0.013           | 4.74E-26   | 18070                             |
| aptamer          | rs9411249 | 9   | 139992907      | MAN1B1/DPP7| T/G                              | 0.36                   | 0.06          | 0.011           | 1.77E-08   | 18070                             |
| aptamer          | rs3967200 | 11  | 12623285       | ST3GAL4    | T/C                              | 0.14                   | -0.15         | 0.015           | 9.24E-24   | 18070                             |
| aptamer          | rs146261845| 17  | 7012254        | ASGR1/ASGR2| T/C                              | 0.01                   | 1.05          | 0.080           | 6.74E-39   | 16741                             |
| aptamer          | rs2075803 | 19  | 51628529       | SIGLEC9    | A/G                              | 0.44                   | 0.37          | 0.011           | 2.40E-280  | 18070                             |
Data availability
Genome-wide summary statistics of the meta-analyses of circulating uromodulin are available at web page https://nxc-1453.imbi.uni-freiburg.de/s/gReQNkMJtkYYLxa.