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Permalink

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Journal

Kidney360, 4(1)

ISSN

2641-7650

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Publication Date

2023

DOI

10.34067/kid.0004172022

Peer reviewed

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Metabolomic Profiling Identifies New Endogenous Markers of Tubular Secretory Clearance

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Key Points

- Proximal tubular secretion is a primary kidney function not reflected by GFRs.
- Secretion is rarely measured due to a paucity of validated markers. This study uses metabolomics to identify candidate endogenous solutes.
- Solutes were compared with the clearance of furosemide and penciclovir, two highly secreted medications, in 50 patients with and without CKD.

Abstract

Background The proximal tubules eliminate protein-bound toxins and drugs through secretion. Measurements or estimates of GFR do not necessarily reflect the physiologically distinct process of secretion. Clinical assessment of this important intrinsic kidney function requires endogenous markers that are highly specific for secretory transport.

Methods We used metabolomics profiling to identify candidate markers of tubular secretory clearance in 50 participants from a kidney pharmacokinetics study. We measured metabolites in three sequential plasma samples and a concurrent 10-hour timed urine sample using hydrophilic interaction liquid chromatography/high-resolution mass spectrometry. We quantified the association between estimated kidney clearance and normalized plasma peak height of each candidate solute to the clearance of administered furosemide, a protein-bound, avidly secreted medication.

Results We identified 528 metabolites present in plasma and urine, excluding pharmaceuticals. We found seven highly (>50%) protein-bound and 49 poorly bound solutes with clearances significantly associated with furosemide clearance and 18 solute clearances favoring an association with furosemide clearance by the 90th percentile compared with GFR. We also found four highly bound and 42 poorly bound plasma levels that were significantly associated with furosemide clearance.

Conclusions We found several candidate metabolites whose kidney clearances or relative plasma levels are highly associated with furosemide clearance, an avidly secreted tracer medication of the organic anion transporters, highlighting their potential as endogenous markers of proximal tubular secretory clearance.

KIDNEY360 4: 23–31, 2023. doi: https://doi.org/10.34067/KID.0004172022

Introduction

The kidneys eliminate retained solutes and medications through glomerular filtration and tubular secretion. Filtration, which removes freely circulating solutes from the circulation, is regulated by hemodynamic and oncotic forces. Conversely, the secretion of endogenous solutes and drugs into the urine, including protein-bound substances, involves active processes that depend not only on blood flow but also the orchestration of cellular transporters and energy.[1,2] These considerations suggest that measurement of tubular secretory clearance could provide important information about kidney health beyond that of glomerular filtration.[3]

Despite the fundamental importance of secretory clearance in maintaining homeostasis, procedures to estimate this intrinsic kidney function remain largely limited to research settings. Existing markers of tubular secretory clearance are subject to overlapping degrees of glomerular filtration, intraindividual variation in circulating concentrations, and require a timed urine collection to calculate kidney clearance, limiting clinical application.[4–6] The development of reliable and facile methods to estimate secretory clearance could

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promote more informative measures of kidney disease activity and improve medication dosing. Secretory clearance measurements may improve understanding of different patterns of kidney disease and may be more highly associated with the clearance of protein-bound toxins, which are implicated in the cardiovascular and other complications of CKD.

We applied metabolomics profiling to advance the discovery of potential endogenous markers of tubular secretory clearance. We estimated the kidney clearances of 528 small molecular solutes using sequentially collected plasma and timed urine samples from a dedicated pharmacokinetics study. We determined associations of estimated solute clearances with the kidney clearance of administered furosemide, an avidly secreted drug that is highly protein bound and minimally filtered.[7,8] We also compare the association with clearance of penciclovir, the highly secreted active metabolite of administered prodrug famciclovir.[9] Concurrently, we evaluated joint associations with iohexol measurements of GFR (iGFR), quantified short-term variation in plasma, and explored whether plasma measurements alone could predict secretory clearance in the absence of urine measurements.

Methods

Study Design and Population

We conducted an ancillary study to the Proximal Tubular Clearance of Renal Medications study, a pharmacokinetic study of 54 adult patients with a wide range of kidney function (eGFRs 21–140 ml/min per 1.73 m^2).[5] Exclusion criteria included dialysis dependence, nephrotic syndrome, cirrhosis, or current use of the study medications (furosemide and famciclovir). On arrival to the study center, participants received a single 5-mg intravenous bolus of furosemide and a single 125-mg oral dose of famciclovir, which is highly bioavailable and rapidly and extensively converted to penciclovir in the liver through first-pass metabolism. Study personnel obtained sequential blood samples through an indwelling intravenous catheter and collected a 10-hour daytime urine sample. Contemporaneously, study personnel measured GFR by plasma iohexol disappearance (iGFR).[10] Participants received three meals and ample fluids throughout the study visit. This study was approved and overseen by the University of Washington Institutional Review Board, and participants provided written informed consent.

Measurement of Small Molecule Solutes

We performed metabolomics profiling in sequential plasma samples collected at 45, 240, and 600 minutes after study drug administration and a concurrent 10-hour urine sample. If the 45-minute plasma sample was depleted, we used the 60-minute sample as a replacement. Sufficient samples were available for 50 of the 54 original Proximal Tubular Clearance of Renal Medications study participants. To determine protein binding, we split the 45-minute plasma sample into two aliquots and passed one through a centrifugal protein separation filter (Millipore Amicon Ultra 0.5-ml 3-kDa filter, MilliPore Sigma, Burlington, MA) at room temperature and $11{,}200 \times g$ for 30 minutes. All samples were shipped to West Coast Metabolomics where they underwent analysis with hydrophilic interaction liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry.[11] Peak heights were standardized using vector normalization on the basis of the sum of peak heights for all identified metabolites in each sample. Peak height is used as an expression of the relative level of a substance after normalization. Data were processed using MS-DIAL 4.0 software and the NIST20 and MassBank of North America libraries to identify metabolites with manual confirmation of adduct ions and spectral scoring accuracy. We excluded unidentified compounds (mass spectrometry imaging >3), known drugs and their metabolites, and compounds that were not identified in both urine and plasma.

Estimation of Kidney Clearance, Protein Binding, and Intraindividual Variation

We estimated the kidney clearance (CL_R) of each identified solute as:

$$CL_x = Ux * V / \Sigma Px$$

where Ux represents peak height of solute x in urine, V represents the urine volume collected in the daytime sample, and $\Sigma\ Px$ represents the time-weighted mean plasma peak height of solute x calculated from the 45- (unfiltered), 240-, and 600-minute samples.

We estimated the protein binding of each solute from the 45-minute plasma sample as:

$$Protein\ binding = (Total - filtered) / Total$$

where *total* and *filtered* represent solute peak heights in the unfiltered and filtered aliquots, respectively.

Creatinine clearance was measured in sample using mass spectrometry peak heights. We calculated the ratio of the estimated kidney clearance of each solute to the kidney clearance of creatinine in the same timed urine sample as:

$$CLr - to - CrCl\ Ratio = (CL_x) / (Clcreatinine)$$

We calculated the intraindividual coefficient of variation (CoV) of each solute in plasma as the standard deviation divided by the mean value from the 45- (unfiltered), 240-, and 600-minute samples.

Statistical Analyses

We quantified associations between the estimated clearance of each solute and the kidney clearance of administered furosemide using linear regression. Potential outlying observations were evaluated and removed from analysis if they were found to have a Cook's distance ≥1 or were >5 standard deviations from the mean. We used a Bonferroni corrected P value of 0.05/528 (equal to 0.000095) to declare statistical significance, and we present results stratified by protein-binding status (>50% versus <50%). Recognizing the expected strong linkage between estimated secretory clearance and GFR, we graphed the $-\log_{10}(P$ value) of regressions for furosemide clearance and iohexol clearance and then selected candidate solutes exceeding the 90th percentile regression favoring furosemide clearance. To explore whether plasma measurements of individual solutes could predict furosemide clearance in the absence of urine measurements, we transformed normalized plasma peak heights as $1/(\text{height})^{0.7}$,

which approximates the 1/(concentration) relationship expected from a retained solute and corresponds with associations of serum creatinine and cystatin C concentrations with GFR in published equations.[12] We repeated the analyses using penciclovir clearance as the outcome variable under identical methods. Analyses were performed using R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study Characteristics and Identified Metabolites

The study population was characterized by a mean age of 56 ± 13 years and 32% women (Table 1). The mean iGFR was 68 ± 25 ml/min per 1.73 m^2, and the median urine albumin-to-creatinine ratio was 7.5 mg/g (interquartile range, 3.7–50.5 mg/g). Participants with lower iGFRs tended to have higher blood pressures, a higher prevalence of diabetes, and use more medications. The mean kidney clearance of furosemide was 86 ± 48 ml/min. Kidney furosemide clearance was correlated with iohexol clearance (ρ=0.80) and with penciclovir clearance (ρ=0.81). After removing participants with 0 plasma or urine values, 595/625 solutes had all 50 observations available, 18 had 49 observations available, and 12 had between 38 and 48 observations available. After applying Cook's distance >1 or standard deviation cutoff of 5, 11 solutes had two observations removed and 133 solutes had one observation removed. Among the 528 solutes under investigation, the median protein binding was 21% (IQR, 12%–37%). The estimated kidney clearance of 49% of tested solutes exceeded creatinine clearance in the same urine sample.

Associations of Estimated Solute Clearances with Furosemide Clearance

The estimated kidney clearances of several endogenous solutes met Bonferroni-corrected statistical significance for the association with furosemide clearance (Figure 1; Table 2). Chief among the top protein-bound solutes (>50% binding) were androstane-3-ol-17-one-3-glucuronide, gentisic acid, 4-pyridoxic acid, o-hydroxyhippuric acid, and kynurenic acid. The estimated kidney clearance of these solutes was 2.3–5.8-fold greater than simultaneously measured creatinine clearance. Among these solutes, intraindividual variation in solute peak heights across the three serial plasma measurements was lowest for androstane-3-ol-17-one-3-glucuronide and kynurenic acid and highest for o-hydroxy hippuric acid. Among the top non–protein-bound solutes, the estimated kidney clearances of L-homocitrulline, 3′-sialyllactose, 2-[(4-aminobenzoyl) amino]acetic acid, (2R)-3-hydroxyisovaleroylcarnitine, and 1-methylxanthine were most strongly associated with furosemide clearance. Each demonstrated an estimated kidney clearance that exceeded that of creatinine. Plasma peak heights of (2R)-3-hydroxyisovaleroylcarnitine exhibited the lowest intraindividual variability over the

Table 1. Patient characteristics, separated by iGFR category (<45, 45–60, 60–90, >90)

	iGFR			
	<45	45–60	60–90	>90
N	10	12	17	11
Furosemide kidney clearance (ml/min)[a]	45.6 (23.9)	72.9 (30.8)	125.6 (47.4)	205.1 (61.3)
Age (yr)[a]	61.8 (11.8)	63.2 (11.6)	54.2 (9.2)	44.9 (13.2)
Female (%)	4 (40)	5 (41.7)	3 (17.6)	4 (36.4)
Race (%)				
Black or African American	1 (10)	3 (25)	9 (52.9)	5 (45.5)
White or Caucasian	7 (70)	9 (75)	8 (47.1)	6 (54.5)
Other/prefer not to answer	2 (20)	0 (0)	0 (0)	0 (0)
BMI (kg/m^2)[a]	27.2 (9.4)	30.3 (7.6)	31.6 (4.6)	28.9 (4.6)
Education history (%)				
Less than high school	0 (0)	1 (8.3)	0 (0)	1 (9.1)
High school graduate	2 (20)	1 (8.3)	6 (35.3)	2 (18.2)
Some college	5 (50)	3 (25)	4 (23.5)	4 (36.4)
College graduate or higher	3 (30)	7 (58.3)	7 (41.2)	4 (36.4)
Smoking (%)	3 (30)	2 (16.7)	5 (29.4)	4 (36.4)
Diabetes (%)	2 (20)	3 (25)	0 (0)	1 (9.1)
CHF (%)	0 (0)	1 (8.3)	1 (5.9)	0 (0)
SBP (mm Hg)[a]	142.3 (28)	131.9 (13.8)	139.1 (19.6)	124.1 (14.8)
Urine albumin (mg/mg, spot)[a]	34.4 (30.8)	2.2 (4.9)	10.7 (25.9)	9.8 (21.2)
Serum albumin (g/dl)[a]	4 (0.3)	4.2 (0.2)	3.9 (0.4)	4.2 (0.3)
Medication use (%)				
Insulin	0 (0)	2 (16.7)	0 (0)	1 (9.1)
Statin	5 (50)	6 (50)	5 (29.4)	1 (9.1)
ACE inhibitor	2 (20)	4 (33.3)	4 (23.5)	1 (9.1)
Thiazide diuretic	1 (10)	0 (0)	2 (11.8)	0 (0)
Beta blocker	0 (0)	2 (16.7)	2 (11.8)	0 (0)

Continuous variables are expressed as mean (standard deviation) and categorical variables as *N* (%). iGFR, iohexol GFR; BMI, body mass index; CHF, congestive heart failure; SBP, systolic BP.
[a]Expressed as mean (standard deviation).

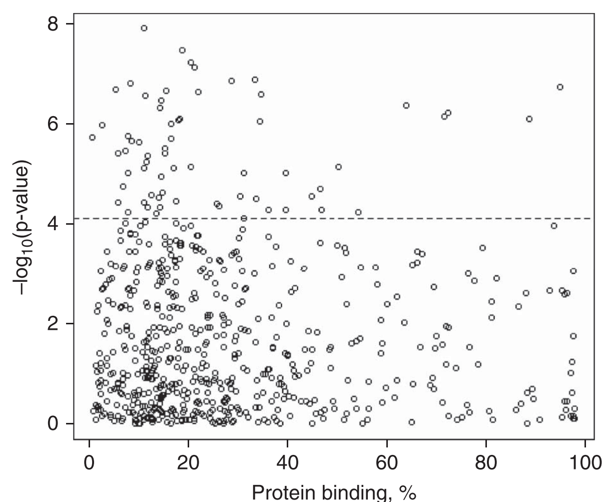

Figure 1. All 528 candidate solutes presented on the basis of their kidney clearances' association with furosemide clearance by −log10(*P*), separated by protein binding. Horizontal dashed line represents the line of significance by Bonferroni correction ($P<0.05/528$). Highly associated solutes presented in Table 2.

10-hour measurement period. Most solutes that reached statistical significance for associations with furosemide clearance were also significantly associated with penciclovir clearance (Supplemental Table 2). Nearly all highly associated solutes had intraindividual variation of <40%. The association of all solutes with furosemide clearance is provided in Supplemental Material (Supplemental Table 1).

Joint Associations of Estimated Solute Clearances with Furosemide and Iohexol Clearance

The estimated kidney clearances of candidate solutes demonstrated broadly similar associations with furosemide clearance and iGFR (Figure 2; Table 3). Nonetheless, the kidney clearances of several solutes favored associations with furosemide clearance on the basis of the 90th percentile line of the paired regressions. Specific solutes demonstrating preferential association with furosemide clearance included 4-hydroxyphenylacetic acid, kynurenic acid, and 2-hydroxyphenylacetic acid (Figure 3).

Associations of Solute Relative Plasma Levels with Furosemide Clearance

In analyses testing whether plasma measurements alone could predict kidney furosemide clearance, in the absence of

Table 2. Candidate solutes are presented according to the association of their kidney clearance with furosemide clearance (−log10[*P*]), separated by protein binding (>50% or ≤50%)

Protein Binding	−log(*P*): Furosemide	RMSE	−log(*P*) iGFR	Compound	Protein Binding (%)	CoV	CLr-to-CrCl Ratio
>50%	6.7	36.0	8.2	Androstan-3-ol-17-one 3-glucuronide	95	0.2	3.8
	6.3	36.6	4.2	Gentisic acid	64	0.4	2.3
	6.2	36.9	6.5	4-Pyridoxic acid	73	0.5	5.8
	6.1	37.2	2.4	o-Hydroxyhippuric acid	72	1.8	5.7
	6.1	37.0	5.8	Kynurenic acid	89	0.2	3.9
	5.1	38.8	5.7	4-Hydroxyphenylacetic acid	50	0.2	1.3
	4.2	40.4	4.3	2-Hydroxyphenylacetic acid	54	0.3	1.2
≤50%	7.9	33.8	4.6	L-Homocitrulline	11	0.3	3.0
	7.5	34.7	5.6	2-[(4-Aminobenzoyl)amino]acetic acid	19	0.3	10.9
	7.2	35.2	5.7	(2R)-3-Hydroxyisovaleroylcarnitine	21	0.1	3.0
	7.1	35.4	5.0	3′-Sialyllactose	21	0.3	5.7
	6.9	35.7	7.6	1-Methylxanthine	33	0.4	5.8
	6.8	35.8	7.4	N2,N2-Dimethylguanosine	29	0.2	5.3
	6.7	35.8	7.5	Guanidinosuccinic acid	5	0.2	6.6
	6.6	36.1	5.7	Galactonic acid	16	0.5	0.8
	6.6	36.2	8	Phenylacetyl-L-glutamine	22	0.3	7
	6.6	36.2	8.7	N2-[2-(1H-Indol-3-yl)acetyl]-L-glutamine	34	0.3	10.8
	6.6	36.2	7	3-Dehydrocarnitine	11	0.1	11.3
	6.4	36.4	5.2	1,4-Cyclohexanedicarboxylic acid	15	0.3	2.4
	6.3	35.8	7.9	Proline-hydroxyproline	14	0.3	3.2
	6.1	37.1	4.5	Inosine	18	0.4	1
	6.1	37.1	6.8	Xanthosine	18	0.2	4.7
	6	36.7	1.5	7-Methyluric acid	34	0.9	5.8
	6	37.2	7.3	6′-Sialyl-N-acetyllactosamine	17	0.2	4.6
	6	37.3	6.6	N-Methylglutamic acid[a]	3	0.3	2.5
	5.7	37.7	7.6	N,N-Dimethylarginine[a]	8	0.2	3.1
	5.7	37.7	5.4	7-Methylguanine	1	0.3	45.9

Only the top 20 solutes reaching statistical significance by Bonferroni (0.05/528) are presented. RMSE, root mean squared error from the −log(*P*): furosemide models; iGFR, iohexol GFR; CoV, coefficient of variation; CLr-to-CrCl ratio, solute clearance-to-creatinine clearance ratio.
[a]Did not also reach significance for penciclovir clearance.

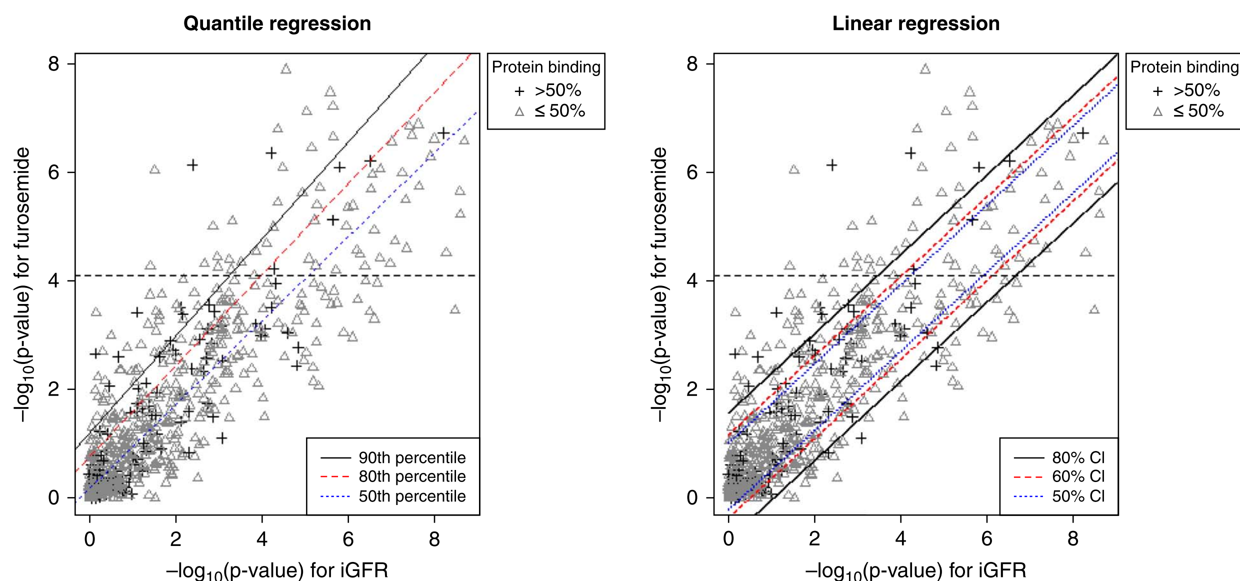

Figure 2. All 528 candidate solute kidney clearances plotted by their association with furosemide clearance (*y* axis) and iGFR (*x* axis). Quantile regression lines are shown for 90th, 80th, and 50th percentile. Solutes favoring furosemide clearance at the 90th percentile line and exceeding Bonferroni correction cutoff (horizontal dashed lined, P values below 0.05/528) are considered significant and presented in Table 3. Horizontal dashed line at $-\log(P)$ cutoff of 4.02, diagonal solid line at 90th percentile of furosemide versus iGFR clearance association. iGFR, iohexol GFR.

urine measurements, transformed normalized plasma peak heights of several solutes were significantly associated with kidney furosemide clearance, including 4-hydroxyphenylacetic acid, kynurenic acid, S-adenosyl-homocysteine, and isoxanthopterin (Table 4, Supplemental Figure 2). Transformed plasma peak heights of these solutes demonstrated similar associations with penciclovir clearance (Supplemental Table 2). Again, nearly all highly associated solutes had very low intraindividual variation.

Discussion

Using metabolomic profiling, we identified several promising endogenous solutes that could serve as potential future markers of tubular secretory clearance. The top identified solutes demonstrated estimated clearances that were strongly associated with empirically determined kidney furosemide clearance, an avidly secreted and minimally filtered medications. Several candidate solutes exhibited a high degree of protein binding, suggesting minimal overlap

Table 3. Solute clearances favoring furosemide clearance compared with iGFR (above 90th percentile regression line)

$-\log(P)$: Furosemide	$-\log(P)$: iGFR	Compound	Protein Binding (%)	CoV	CLr-to-CrCl Ratio
7.9	4.6	L-Homocitrulline	11	0.3	3.0
7.5	5.6	2-[(4-Aminobenzoyl)amino]acetic acid	19	0.3	10.9
7.2	5.7	(2R)-3-Hydroxyisovaleroylcarnitine	21	0.1	3.0
7.1	5.0	3′-Sialyllactose	21	0.3	5.7
6.6	5.7	Galactonic acid	16	0.5	0.8
6.4	5.2	1,4-Cyclohexanedicarboxylic acid	15	0.3	2.4
6.3	4.2	Gentisic acid	64	0.4	2.3
6.1	2.4	o-Hydroxyhippuric acid	72	1.8	5.7
6.1	4.5	Inosine	18	0.4	1.0
6.0	1.5	7-Methyluric acid	34	0.9	5.8
5.5	4.4	Ribose-5-phosphate	7	0.2	1.9
5.1	3.3	3-Methylhistidine	17	0.7	1.0
5.0	2.9	Phenylalanine	31	0.5	0.0
4.4	3.0	4-Acetamidobutyric acid	18	0.4	6.1
4.4	3.3	N-α-methylhistamine	26	0.2	13.2
4.3	2.7	Betonicine	14	0.5	1.0
4.3	1.4	Nialamide	47	0.3	0.2
4.3	3.0	N-Methylleucine	40	2.7	0.2

Candidate solutes are ordered by the kidney clearance association with furosemide clearance. iGFR, iohexol GFR; CoV, coefficient of variation; CLr-to-CrCl ratio, solute clearance-to-creatinine clearance ratio.

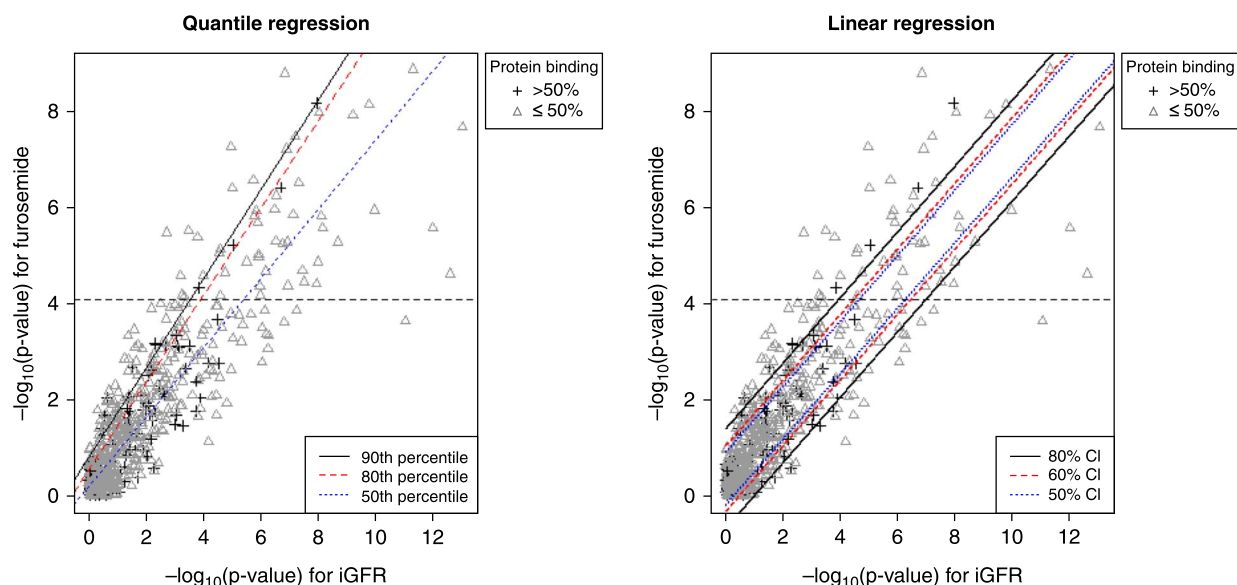

Figure 3. All 528 candidate solute plasma levels plotted by their association with furosemide clearance (*y* axis) and iGFR (*x* axis). Quantile regression lines are shown for 90th, 80th, and 50th percentile. Solutes favoring furosemide clearance at the 90th percentile line and exceeding Bonferroni correction cutoff (horizontal dashed lined, *P* values below 0.05/528) are considered significant and presented in Table 5. Horizontal dashed line at −log(*P*) cutoff of 4.02, diagonal solid line at 90th percentile of furosemide versus iGFR clearance association. iGFR, iohexol GFR.

with glomerular filtration, relatively low short-term variability in plasma, and associations of plasma measurements alone with kidney drug clearances, potentially obviating the need for concomitant urine measurements. These findings were generally consistent with penciclovir clearance, another highly secreted but less protein-bound tracer. Further

Table 4. Candidate solutes are presented according to the association of their plasma concentration (1/concentration$^{0.7}$) with furosemide clearance (−log10[*P*]), separated by protein binding (>50% or ≤50%)

Protein Binding	−log(*P*): Furosemide	−log(*P*): iGFR	Compound	Protein Binding (%)	CoV	CLr-to-CrCl Ratio
>50%	8.2	5.7	4-Hydroxyphenylacetic acid	50	0.2	1.3
	6.4	5.8	Kynurenic acid	89	0.2	3.9
	5.2	4.3	2-Hydroxyphenylacetic acid	54	0.3	1.2
	4.3	3.1	Catechol[a]	65	0.7	0.6
≤50%	8.9	3.2	Glucuronic acid	7	0.2	1.9
	8.8	5.6	2-[(4-Aminobenzoyl)amino]acetic acid	19	0.3	10.9
	8.1	6.5	3-Methylcrotonylglycine	17	0.3	10.7
	8.0	0.5	2-Dimethylamino-6-hydroxypurine	15	0.2	12.6
	7.9	2.2	S-Adenosyl-homocysteine	31	0.2	2.5
	7.7	7.3	6′-Sialyl-N-acetyllactosamine	17	0.2	4.6
	7.3	5.2	1,4-Cyclohexanedicarboxylic acid	15	0.3	2.4
	7.2	5.7	Galactonic acid	16	0.5	0.8
	6.5	6.4	Isoxanthopterin	36	0.2	2.3
	6.4	5	3′-Sialyllactose	21	0.3	5.7
	6.2	7.5	Guanidinosuccinic acid	5	0.2	6.6
	6	6.6	3-Methoxy-4-hydroxyphenylglycol sulfate	18	0.2	8.2
	5.9	8.6	Acetyl-L-threonine	9	0.2	1.3
	5.9	1.6	Mandelic acid	44	0.3	1
	5.8	4	5-Fluoro-1-methyl-1H-1,3-benzodiazole	22	0.2	9.6
	5.8	6.2	Urea	6	0.2	1
	5.7	3	4-Acetamidobutyric acid	18	0.4	6.1
	5.6	7.4	N2,N2-Dimethylguanosine	29	0.2	5.3
	5.5	2.6	Phenylacetylglycine	18	0.3	4.3
	5.5	0.2	Choline[a]	6	0.2	0.2

Only the top solutes reaching statistical significance by Bonferroni (0.05/528) are presented. iGFR, iohexol GFR; CoV, coefficient of variation; CLr-to-CrCl ratio, solute clearance-to-creatinine clearance ratio.
[a]Did not also reach significance for penciclovir clearance.

Table 5. Solute plasma levels favoring furosemide clearance compared with iGFR (above 90th percentile regression line)

−log(*P*): Furosemide	−log(*P*): iGFR	Compound	Protein Binding (%)	CoV	CLr-to-CrCl Ratio
8.8	6.9	2-[(4-Aminobenzoyl)amino]acetic acid	19	0.3	10.9
7.3	5	1,4-Cyclohexanedicarboxylic acid	15	0.3	2.4
6.6	5.8	Biotin	2	0.3	2.7
6.4	5	3′-Sialyllactose	21	0.3	5.7
5.5	3.5	Phenylacetylglycine	18	0.3	4.3
5.5	2.7	Choline	6	0.2	0.2
5.4	3.8	3-Dehydrocarnitine	11	0.1	11.3
5.1	4.6	2′-O-Methylinosine	4	0.5	0.6
4.6	4	3-Hydroxyanthranilic acid	15	0.3	3.2
4.6	3.2	2-[(4-Aminobenzoyl)amino]acetic acid	8	0.5	21.3
4.2	3.3	N-Isovalerylglycine	24	0.5	10.2
4.1	3.3	Picolinuric acid	37	0.8	47.6
4	3.4	α-Galactosamine-1-phosphate	17	0.2	3.1
4	2.5	Hexanoyl-L-carnitine	30	0.4	1.9
4	3	Pantothenic acid	24	0.6	0.5
3.9	2.9	Glu-Val	15	0.3	1
3.9	3.1	Isomaltose A	18	0.9	3.3
3.9	2.2	N-Acetylmannosamine	12	0.3	0.9
3.7	2.7	4-Hydroxyhippuric acid	25	0.3	3.6
3.5	1.6	N-Acetylneuraminic acid	4	0.4	2.4

Candidate solutes are ordered by the kidney clearance association with furosemide clearance. iGFR, iohexol GFR; CoV, coefficient of variation; CLr-to-CrCl ratio, solute clearance-to-creatinine clearance ratio.

work to investigate the identified solutes in larger populations using targeted assays holds promise for improving the quality and applicability of procedures to measure tubular secretory clearance.

Our previous work developed a targeted assay for secretory solutes identified from the published literature. These solutes were selected on the basis of reported high affinities for tubular organic anion transporters in experimental models and either a high degree of protein binding or a kidney clearance that exceeded GFR, suggesting secretion as the primary modality of elimination. We found the kidney clearances of these solutes to be associated with the progression of CKD, the extent of tubulointerstitial fibrosis, and uremic symptoms in population and clinic-based studies after adjustment for GFR and albuminuria.[4,6,13,14] Nonetheless, these targeted solutes are limited by imperfect specificity for tubular secretion, variability in circulating plasma concentrations, and the need for a timed urine collection to calculate clearance. New endogenous markers that address these limitations are needed to expand the clinical and research applications of tubular secretory clearance.

Most endogenous secretory solutes are members of complex metabolic pathways that are regulated by processes other than kidney function, limiting specificity. The kidney clearance of a marker, instead of its plasma concentration alone, theoretically circumvents potential differences in synthesis and catabolism; however, valid measurements of clearance require steady state concentrations in plasma and a concurrent timed urine collection, which is subject to collection errors. Some of the identified solutes in this study demonstrated associations with kidney furosemide clearance on the basis of plasma measurements alone. Validation of these results in other studies would represent an important step toward facilitating secretory clearance measurements in clinical settings.

There is burgeoning interest in nonglomerular kidney functions and their potential clinical applications.[3,15] Differences in the broad mechanisms governing glomerular filtration and tubular secretion suggest a potential role for secretory clearance measurements in the detection of early disease, when GFR may remain normal because of hyperfiltration. Moreover, secretory clearance measurements may improve understanding of different patterns of kidney disease, particularly for processes that preferentially affect the tubules. Secretory clearance is a major mechanism for eliminating protein-bound uremic toxins, the retention of which is implicated in cardiovascular and other complications of CKD. The negative effects of protein-bound toxins in context of tubular dysfunction were recognized in a recent consensus conference to modernize the definitions of uremic toxins.[16] These considerations bear directly on current dialysis therapies, which can recapitulate only filtration. Finally, the primary role of secretory clearance in the kidney elimination of administered medications suggests potential importance in improving kidney drug dosing.

A strength of this study is the use of empirically measured furosemide clearance as a gold standard marker of secretory clearance. Furosemide is avidly secreted by organic anion transporters 1 and 3 (OAT1 and OAT3) on the basolateral surface of proximal tubules, highly protein bound, minimizing glomerular filtration, and negligibly cleared by other organ systems. By contrast, para-aminohippuric acid, a derivative of hippuric acid that is used to measure kidney blood flow has relatively low protein binding, introducing some overlap with glomerular filtration.[17] Highly protein-bound and therefore minimally filtered drugs with high specificity for tubular transporters are particularly useful for empiric testing, and furosemide is US Food and Drug Administration recognized as a tracer medication for the kidney organic anion transporters.[8] Furosemide in particular has long been recognized

as a specific substrate for OAT1/3 both *in vitro* and *in vivo*, with apical transport provided by multidrug resistance protein 4 similar to many uremic solutes; however, most solutes will have some affinity for multiple transporters.[18-21] As such, heterogeneity of tubular transport systems likely precludes a single gold standard marker of secretory clearance.

There is considerable overlap between several of the top solutes identified in this study and known accumulation in OAT1 and OAT3 knockout animal models, suggesting the proximal tubules are important sites of elimination.[22] Furthermore, recent metabolomic studies by Nigam *et al.* identified overlapping solutes that accumulate in both subtotal nephrectomy and probenecid blocking of OAT1/3, many of which were highly correlated in our study with clearance of the OAT1/3 substrates furosemide and penciclovir.[23,24] Furosemide itself has been recognized to increase the levels of several highly associated metabolites in human patients.[20] Taken together, the mechanistic concordance suggests that many identified solute levels are at least partially determined by proximal tubular organic anion clearance and therefore may be useful as functional biomarkers.

Weaknesses of this study include the relatively small sample size, lack of replication, and inability to directly quantify concentrations of the identified metabolites. Variability was measured within the 10-hour sampling window; however, longer-term variability of individual solutes may limit applicability to secretory clearance particularly as single plasma measurements. The top solutes identified here may not perform as well in other populations. Procedures to create GFR estimating equation on the basis of serum creatine and cystatin C concentrations required large diverse populations with heterogeneous disease entities. Moreover, the top solutes identified in this study were only moderately associated with furosemide clearance, despite strong statistical evidence of association using a very conservative Bonferroni threshold. This limitation may be partly addressed by the future development of targeted assays with specific internal standards, which improves accuracy and precision compared with untargeted profiling and can quantify absolute solute concentrations. Furthermore, plasma levels may depart from the reciprocal relationship to kidney clearance as expected with eGFR markers such as creatinine or cystatin C due to factors such as reduced production or extrarenal clearance. Given these limitations, this study represents an early step in translating new biomarkers into routine use.

In summary, we identified several endogenous solutes that warrant further investigation as potential markers of tubular secretory solute clearance. Follow-up work to investigate and further develop markers of secretory solute clearance could advance the assessment of kidney function and drug dosing.

Disclosures

O. Fiehn reports the following: Consultancy: Guidepoint Global and Research Funding: Agilent. Y. Chen reports the following: Consultancy: Abbvie, AstraZeneca, Bayer, Bristol Myers Squibb, GlaxoSmithKline, Merck, Seagen. C.K. Yeung reports the following: Consultancy: Nortis Inc and Honoraria: National Institutes of Health. A.N. Hoofnagle reports the following: Consultancy: Kilpatrick Townsend & Stockton LLP; Ownership Interest: Seattle Genetics; Research Funding: Waters; Patents or Royalties: SISCAPA Assay Technologies; Advisory or Leadership Role: Clinical Chemistry (Associate Editor); and Other Interests or Relationships: Expert witness for Kilpatrick, Townsend, and Stockton, LLC. B. Kestenbaum reports the following: Consultancy: Reatta Pharmaceuticals and Honoraria: Reatta Pharmaceuticals. All remaining authors have nothing to disclose.

Funding

Funding for this project was provided by National Institutes of Health (NIH)/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) grant 1K24DK103986-01A1 awarded to B. Kestenbaum. Funding for the primary author was provided by the University of Washington training grant through NIH grant T32DK007467-37 and NIDDK grant P30DK081943 awarded by the University of Michigan O'Brien Kidney Translational Core Center. Metabolomics was performed by West Coast Metabolomics at the University of California—Davis, which is directed by co-author Oliver Fiehn. CKY acknowledges funding from NIH NIGMS grant R01GM121354.

Author Contributions

Y. Chen, M.L. Granda, A.N. Hoofnagle, B. Kestenbaum, and C.K. Yeung conceptualized the study; Y. Chen, O. Fiehn, M.L. Granda, A.N. Hoofnagle, and B. Kestenbaum were responsible for data curation; O. Fiehn, M.L. Granda, B. Kestenbaum, and D.K. Prince were responsible for formal analysis; B. Kestenbaum was responsible for funding acquisition; M.L. Granda, A.N. Hoofnagle, and B. Kestenbaum were responsible for investigation and methodology; M.L. Granda and B. Kestenbaum were responsible for project administration and validation; O. Fiehn was responsible for resources; D.K. Prince was responsible for software; A.N. Hoofnagle, B. Kestenbaum, and C.K. Yeung provided supervision; M.L. Granda was responsible for visualization; O. Fiehn, M.L. Granda, B. Kestenbaum, D.K. Prince, and T. Rajabi wrote the original draft; and Y. Chen, O. Fiehn, A.N. Hoofnagle, B. Kestenbaum, D.K. Prince, T. Rajabi, and C.K. Yeung reviewed and edited the manuscript.

Supplemental Materials

This article contains the following supplemental material online at http://links.lww.com/KN9/A247.

Supplemental Table 1. Expanded version of Table 2 depicting all candidate solutes according to their kidney clearance association with furosemide clearance.

Supplemental Table 2. All candidate solutes presented according to the association of kidney clearance with penciclovir clearance.

Supplemental Figure 1. Histogram of all solutes' mean protein binding.

Supplemental Figure 2. All 528 candidate solutes presented on the basis of their transformed plasma concentration ($1/\text{plasma}^{0.7}$) association with furosemide clearance by $-\log10(P)$, separated by protein binding. Highly associated solutes presented in Table 4.

References

1. Masereeuw R, Mutsaers HAM, Toyohara T, et al. The kidney and uremic toxin removal: glomerulus or tubule? *Semin Nephrol.* 2014;34(2):191–208. doi:10.1016/j.semnephrol.2014.02.010
2. Sirich TL, Aronov PA, Plummer NS, Hostetter TH, Meyer TW. Numerous protein-bound solutes are cleared by the kidney with high efficiency. *Kidney Int.* 2013;84(3):585–590. doi:10.1038/ki.2013.154
3. Wang K, Kestenbaum B. Proximal tubular secretory clearance. *Clin J Am Soc Nephrol.* 2018;13(8):1291–1296. doi:10.2215/cjn.12001017

4. Chen Y, Zelnick LR, Wang K, et al. Kidney clearance of secretory solutes is associated with progression of CKD: the CRIC study. *J Am Soc Nephrol.* 2020;31(4):817–827. doi:10.1681/asn.2019080811
5. Chen Y, Zelnick LR, Hoofnagle AN, et al. Prediction of kidney drug clearance: a comparison of tubular secretory clearance and glomerular filtration rate. *J Am Soc Nephrol.* 2021;32(2): 459–468. doi:10.1681/asn.2020060833
6. Wang K, Nguyen M, Chen Y, et al. Association of tubular solute clearance with symptom burden in incident peritoneal dialysis. *Clin J Am Soc Nephrol.* 2020;15(4):530–538. doi:10.2215/cjn.11120919
7. Huang X, Dorhout Mees E, Vos P, Hamza S, Braam B. Everything we always wanted to know about furosemide but were afraid to ask. *Am J Physiol Ren Physiol.* 2016;310(10):F958–F971. doi:10.1152/ajprenal.00476.2015
8. US Food & Drug Administration. *Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers. FDA [Internet].* 2021. https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers
9. Boike SC, Pue MA, Freed MI, et al. Pharmacokinetics of famciclovir in subjects with varying degrees of renal impairment. *Clin Pharmacol Ther.* 1994;55(4):418–426. doi:10.1038/clpt.1994.51
10. Delanaye P, Ebert N, Melsom T, et al. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: how to measure glomerular filtration rate with iohexol? *Clin Kidney J.* 2016;9(5):682–699. doi:10.1093/ckj/sfw070
11. Blaženović I, Kind T, Sa MR, et al. Structure annotation of all mass spectra in untargeted metabolomics. *Anal Chem.* 2019;91(3): 2155–2162. doi:10.1021/acs.analchem.8b04698
12. Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and cystatin C–based equations to estimate GFR without race. *N Engl J Med.* 2021;385(19):1737–1749. doi:10.1056/nejmoa2102953
13. Chen Y, Zelnick LR, Wang K, et al. Association of tubular solute clearances with the glomerular filtration rate and complications of chronic kidney disease: the Chronic Renal Insufficiency Cohort Study. *Nephrol Dial Transplant.* 2020;36(7):1271–1281. doi:10.1093/ndt/gfaa057
14. Garimella PS, Katz R, Waikar SS, et al. Kidney tubulointerstitial fibrosis and tubular secretion. *Am J Kidney Dis.* 2022;79(5): 709–716. doi:10.1053/j.ajkd.2021.08.015. https://www.ajkd.org/article/S0272-6386(21)00888-X/fulltext.
15. Lowenstein J, Grantham JJ. The rebirth of interest in renal tubular function. *Am J Physiol Renal Physiol.* 2016;310(11): F1351–F1355. doi:10.1152/ajprenal.00055.2016
16. Rosner MH, Reis T, Husain-Syed F, et al. Classification of uremic toxins and their role in kidney failure. *Clin J Am Soc Nephrol.* 2021;16(12):1918–1928. doi:10.2215/cjn.02660221
17. Taggart JV. Protein binding of p-aminohippurate in human and dog plasma. *Am J Physiol.* 1951;167(1):248–254. doi:10.1152/ajplegacy.1951.167.1.248
18. Burckhardt BC, Burckhardt G. Transport of organic anions across the basolateral membrane of proximal tubule cells. *Rev Physiol Biochem Pharmacol.* 2003;146:95–158. doi:10.1007/s10254-002-0003-8
19. Vallon V, Rieg T, Ahn SY, Wu W, Eraly SA, Nigam SK. Overlapping in vitro and in vivo specificities of the organic anion transporters OAT1 and OAT3 for loop and thiazide diuretics. *Am J Physiol Ren Physiol.* 2008;294(4):F867–F873. doi:10.1152/ajprenal.00528.2007
20. André C, Mernissi T, Choukroun G, et al. The prescription of drugs that inhibit organic anion transporters 1 or 3 is associated with the plasma accumulation of uremic toxins in kidney transplant recipients. *Toxins.* 2021;14(1):15. doi:10.3390/toxins14010015
21. Hasegawa M, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Multidrug resistance-associated protein 4 is involved in the urinary excretion of hydrochlorothiazide and furosemide. *J Am Soc Nephrol.* 2007;18(1):37–45. doi:10.1681/asn.2005090966
22. Wu W, Bush KT, Nigam SK. Key role for the organic anion transporters, OAT1 and OAT3, in the in vivo handling of uremic toxins and solutes. *Sci Rep.* 2017;7(1):4939. doi:10.1038/s41598-017-04949-2
23. Bush KT, Singh P, Nigam SK. Gut-derived uremic toxin handling in vivo requires OAT-mediated tubular secretion in chronic kidney disease. *JCI Insight.* 2020;5(7):e133817. doi:10.1172/jci.insight.133817
24. Granados JC, Bhatnagar V, Nigam SK. Blockade of organic anion transport in humans after treatment with the drug probenecid leads to major metabolic alterations in plasma and urine. *Clin Pharmacol Ther.* 2013;94(1):27–29. doi:10.1038/clpt.2013.82

Received: June 15, 2022 **Accepted:** September 15, 2022