A comprehensive analysis of ontogeny of renal drug transporters: mRNA analyses, quantitative proteomics and localization

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ABSTRACT

Human renal membrane transporters play key roles in the disposition of renally cleared drugs and endogenous substrates but their ontogeny is largely unknown. Using 184 human postmortem frozen renal cortical tissues (preterm newborns – adults) and a subset of 62 tissue samples, we measured the mRNA levels of 11 renal transporters and the transcription factor PXR with RT-qPCR, and protein abundance of 9 transporters using LC-MS/MS SRM, respectively. Expression levels of P-gp, URAT1, OAT1, OAT3, and OCT2 increased with age. Protein levels of MATE2-K and BCRP showed no difference from newborns to adults despite age-related changes in mRNA expression. MATE1, GLUT2, MRP2, MRP4 and PXR expression levels were stable. Using immunohistochemistry, we found that MRP4 localization in pediatric samples was similar to that in adult samples. Collectively, our study revealed that renal drug transporters exhibited different rates and patterns of maturation, suggesting that renal handling of substrates may change with age.

INTRODUCTION

Renal membrane transporters, which are located on the apical and basolateral sides of the tubular epithelium, are key players in tubular secretion and reabsorption of a plethora of endogenous and exogenous compounds in the kidney.[1,2] Because of their role in renal elimination, many transporters in the kidney play critical roles in the disposition, efficacy and toxicity of drugs. Notably, renal drug transporters have received increasing regulatory attention in recent years, highlighting their significance in drug disposition.[3-6]

Interindividual variation in expression levels and functional activities of membrane transporters can affect the homeostasis of endogenous substrates, as well as the pharmacokinetics and pharmacodynamics of drugs.[1] As a result of developmental changes in key transporters and enzymes, levels of endogenous substrates, such as metabolites, nutrients, antioxidants and hormones, change as children grow.[7] Reduced hepatic clearance of the opioid morphine in newborns and young infants was reported.[8] This was suggested due to significantly lower hepatic levels of both the drug metabolizing enzyme uridine 5-diphosphoglucuronic acid glucuronyl transferase (UGT) 2B7 and the organic cation transporter (OCT) 1 in young pediatric populations compared to adults.[9,10] In contrast to the liver, less is known about the maturation and ontogeny pattern of renal membrane transporters. This knowledge gap limits the ability to predict the pharmacokinetics of renally eliminated drugs in children, which may be critical for rational dosing and drug efficacy and safety. Thus, there is an urgent need to understand the ontogeny of human drug transporters in the kidney.

The current study aimed to identify age-related differences in gene expression and protein abundance of renal transporters. We chose to focus on renal transporters with demonstrated clinical relevance in drug disposition, and those that handle various endogenous and exogenous substances important for developing children,[11,12] i.e., breast cancer resistance protein (gene name/protein name *ABCG2*/BCRP), multidrug and toxin extrusion protein (*SLC47A*/MATE) 1 and 2-K, multidrug resistance protein 1 (*ABCB1*/MDR1/P-gp), multidrug resistance-associated protein (*ABCC*/MRP) 2 and 4, and urate transporter 1 (*SLC22A12* /URAT1) on the apical site of the membrane and glucose transporter 2 (*SLC2A2*/GLUT2), organic anion transporter 1 (*SLC22A6*/OAT1) and 3 (*SLC22A8*/OAT3), and *SLC22A2*/OCT2 located on the basolateral site. In an effort to explore a regulatory mechanism for maturation of transporter expression, we also studied renal gene expression of the nuclear pregnane X receptor (PXR*)* in relation to the transporter expression levels.[13]

In addition, altered localization of a transporter may introduce variation in pharmacokinetics of transporter substrates. However, little is known about the localization of transporters during development of the kidney. MRP4 is an apical efflux transporter involved in transport of a range of endogenous molecules, including cyclic nucleotides, urate and conjugated steroid hormones, and drugs that are used in children, including antivirals and diuretics.[14] We performed immunohistochemistry, as a proof-of-concept, to visualize the location of MRP4 in our pediatric kidney tissues.

METHODS

Tissue procurement and sample characteristics

Two sample sets were analyzed and the demographic information of donors is reported in Table 1. Age groups were predefined based on the International Council for Harmonisation guidelines: preterm newborns (0-28 days PNA, <37 weeks GA), newborns (0-28 days PNA), infants (1-24 months old), children (2-12 years old), adolescents (12-16 years old) and adults (>16 years old)[4]. Sample set 1 consisted of postmortem autopsy kidney samples and surgical adult kidney samples from the Erasmus MC Tissue Bank, Rotterdam, the Netherlands. Sample set 2 consisted of 122 human postmortem frozen renal cortical tissues (donors aged 1 day to 30 years old), which were obtained from NIH NeuroBioBank at the University of Maryland, Baltimore, MD, United States. Tissues, which were selected for having no renal abnormalities in pathology and primary diagnosis, were procured at the time of autopsy within 48 hours after death and were stored at -196 °C (Sample set 1) and -80°C (sample set 2) for later use. The quantitative proteomic analysis was done completely in the United States on the subset of samples from Sample set 2 and the immunohistochemistry was performed entirely in the Netherlands on Sample set 1. Gene expression analysis was conducted in both laboratories, and the data from the two sources were first analyzed separately, followed by a combined analysis. Combined analysis was deemed appropriate as no significant differences were observed between the expression levels of six transporter genes (MATE1, MATE2, P-gp, OAT1, OAT3 and OCT2) in adult samples obtained in the United States and in the Netherlands. Further, developmental patterns in expression of the transporters in the two sample sets showed comparable results.

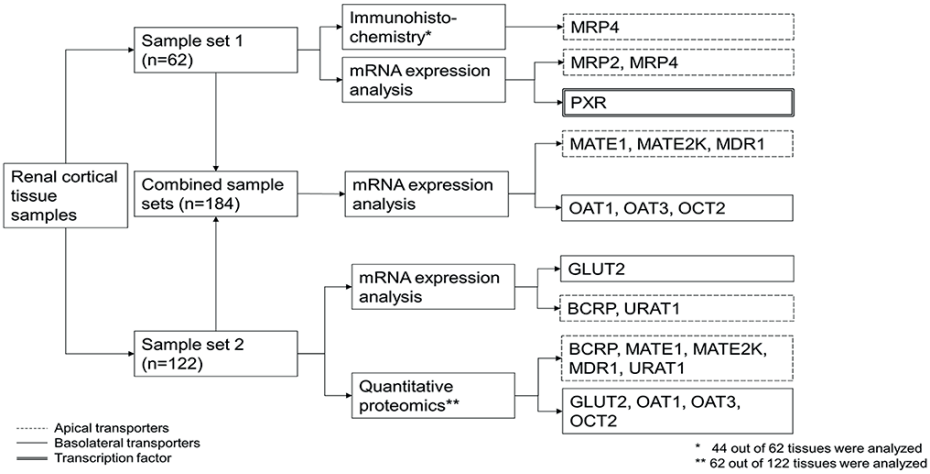
mRNA expression

Figure 1 illustrates the sample analysis scheme. For sample set 1, the protocol on real-time reverse transcription polymerase chain reaction (RT-PCR) is described in Material S1 and Table S6. For sample set 2, the protocol described in Chen et al. was followed with slight modifications (Material S1).[15]

Table 1 Overview of sample size and age range of sample sets 1 and 2

	Number of samples				Age range	
	Sample set 1	Sample set 2				
Age group	Race unknown	Caucasian	African American	Total	Gestational age	Postnatal age
Preterm newborns	9	-	-	9	34.00 (24.00-36.71) wks	1.29 (0.14-4.00) wks
Term newborns	8	10	1	19	NA	1.29 (0.14-3.86) wks
Infants	21	30	30	81	NA	17.86 (4.14-103.00) wks
Children	7	15	16	38	NA	4.74 (2.00-11.56) yr
Adolescents	-	5	5	10	NA	13.38 (12.48-15.26) yr
Adults	17	5	5	27	NA	45.00 (16.75-75.00) yr
Total	62	65	57	184		
		122				

Figure 1 Sample analysis scheme.



The subset of 62 samples from sample set 2 for quantitative proteomics consisted of the 57 African American samples and 5 adult Caucasian samples (See Table 1).

Quantitative proteomics using LC-MS/MS with Selective Reaction Monitoring (SRM)

Quantitative proteomics was only performed in sample set 2 (Figure 1). Unless otherwise stated, reagents from MyOmicsDx, Inc (Towson, MD) were used. Details of the LC and MS method and parameters are described in the supplemental documents (Material S2). Briefly, membrane proteins were extracted from the renal cortical tissues using

MyPro-MembraneEx buffer. The total extracted membrane protein concentration was determined using BCA protein assay kit. The membrane protein samples were then processed by MyOmicsDx, Inc (Towson, MD) using Filter-aided Sample Preparation method.[16]

Five peptides were chosen for each transporter as SRM quantifying targets and six best transitions per peptide precursors were selected for SRM quantification (Table S7). Peptide samples that were previously reconstituted in MyPro-Buffer 3 were spiked with MyPro-SRM Internal Control Mixture and were subjected to SRM analysis. The peptide samples were eluted through an online Agilent 1290 HPLC system into the Jet Stream ESI source of an Agilent 6495 Triple Quadrupole Mass Spectrometer (Agilent, Santa Clara, CA).

Quantitative data were imported into Skyline 3.1.[17] The abundance of a target peptide was represented by the area under the curve (AUC) of all its transitions normalized to the total AUC of all transitions from the most nearby (sharing a similar hydrophobicity) heavy isotope-labeled peptide from MyPro-SRM Internal Control Mixture spiked in before the SRM analysis. Absolute quantification of each protein is performed through applying AQUA™ Peptides (Sigma-Aldrich, St. Louis, MO).

Immunohistochemistry

Localization of MRP4 was explored in a representative subpopulation of sample set 1. Immunohistochemistry was performed using an immunoperoxidase staining method for amplified antigen detection. Sections of 4 µm thick cortex were gained from formalin fixed, paraffin-embedded post-mortem kidney tissue blocks, and were mounted on glass slides. They were heated at 60°C for 30 min, deparaffinized in xylene, and rehydrated with a series of graded ethanol. Enhanced antigen retrieval was performed by treating slides in TRIS-EDTA (10mM Tris Base, 1mM EDTA Solution, 0.05% Tween 20, pH 9.0) for 15 min at 98°C. Endogenous peroxidase activity was quenched by incubating slides in 3% H_2O_2 for 30 min at room temperature. The sections were blocked with Avidin/Biotin blocking solution (Vector Laboratories, Burlingame, CA) 15 min each.

Primary antibodies rat anti-MRP4 (ab15598 Abcam) at dilution of 1:20 were incubated over night at 4°C in 1% BSA. A biotinylated secondary rabbit anti-rat serum (Acris Antibodies GmbH, R1371B) at dilution of 1:1000 was then applied for 30 min. Immunoreactive sides were detected using the ABC kit (Vector Laboratories, Burlingame, CA) for 30 min, and 3,3 diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO) solution staining for 15 min. The nuclei were counterstained with Mayers Hematoxylin Solution (Sigma-Aldrich, St. Louis, MO). One negative control staining lacking the primary antibody was performed for every age group.

Data analysis and statistics

Data were expressed as median (range). Kruskal-Wallis tests with Dunn's post-hoc test were used for multiple comparisons of expression levels between age groups, using the p-values adjusted for multiple testing. If no difference in expression was found between age groups, the ontogeny would be referred to as "stable". Sigmoidal Emax models are used often for maturational processes as it allows gradual maturation of clearance in early life and a "mature" clearance to be achieved at a later age.[18] Therefore, Emax models were used to fit the protein abundance data on a continuous scale of age for those transporters that showed between-group differences. The data point from the term newborn in this set of data was excluded prior to fitting to eliminate bias, as it was the only sample quantified for that age group. We first set the median of adult data to be 100%, and then normalized the data points from pediatric samples towards the median of adult data. Potential outliers were assessed and excluded using the Robust Regression followed by Outlier Identification method (ROUT) during the model fit process.[19] The age at which 50% maturation was reached (TM_{50}) was determined from the Emax model. Visual inspection and 95% CI of the Emax parameter estimates were used to assess the goodness of fit of the Sigmoidal model. Spearman's correlation analysis was used to evaluate the relationship between mRNA and protein abundances within the same and among other transporters.

For the analysis of staining intensity after immunohistochemistry, a semi-quantitative scoring system was used, graded by two observers (BG, MB) who independently confirmed cell staining intensity as negative (0), low staining (+1) or high staining (+2). Simultaneously, the localization of MRP4 in the kidney tissue was determined for each sample by the same observers.

Statistical analysis was performed using IBM SPSS Statistics software (version 21.0; Armonk, NY) and a significance level of $p<0.05$ was used throughout the study. Graphical exploration was performed using GraphPad Prism software (version 5.00; La Jolla, CA).

RESULTS

Two sample sets, which provided a total of 184 postmortem renal cortical tissues, were analyzed in this study (Figure 1 and Table 1). Sample set 1 represented 62 samples from individuals of different ages ranging from preterm newborns (gestational age (GA) > 24 weeks, postnatal age (PNA) 1 day) to adult donors (oldest 75 years). The 122 tissues in sample set 2 were from African American and Caucasian term newborns to adults. No statistical difference was observed in gene or protein abundance levels for any of

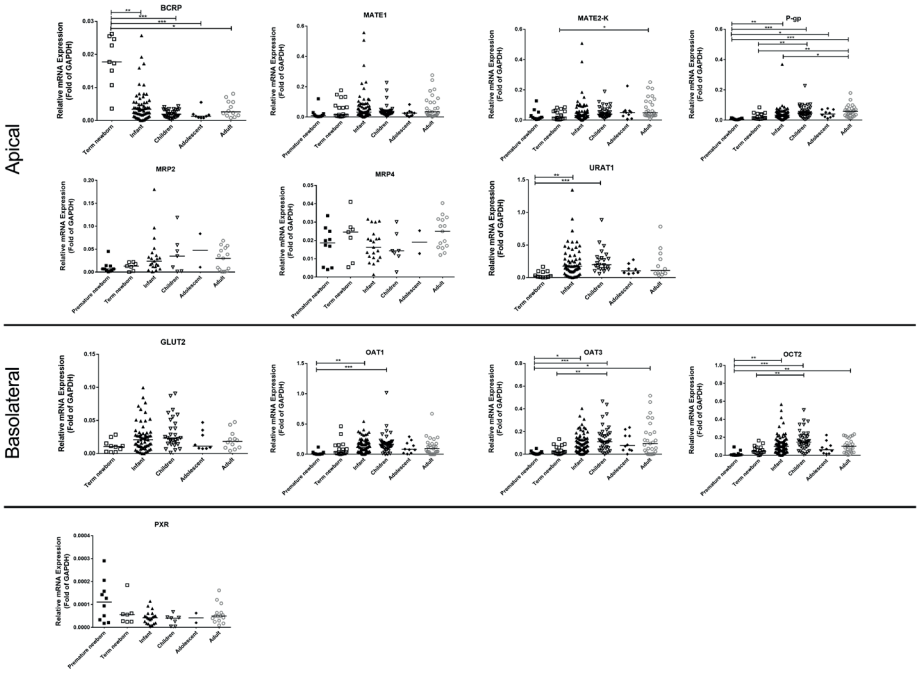
the transporters between males and females, and between African Americans and Caucasians (Table S1, Table S2 and Table S3); hence subsequent analyses were performed by combining both sexes and all ethnic groups.

Relative mRNA quantitation

All 184 tissues were processed for mRNA quantitation (Figure 2 and Table S4). mRNA levels of the selected transporters were detected successfully in all samples, with the exception of MATE1 in two samples. GAPDH mRNA expression did not change with age (r_s = -0.12, p=0.119).

Overall, a large variability in the developmental changes in transporter mRNA level was observed (Figure 2 and Table S4). MATE2-K, P-gp, URAT1, OAT1, OAT3 and OCT2 levels in premature and/or term newborns were significantly lower than in the older age groups. In contrast, term newborns showed significantly higher BCRP mRNA levels than children and adolescents. MATE1, MRP2, MRP4, GLUT2 and PXR levels were not different between all age groups (preterm newborn, term newborn, infants, children, adolescents and adults).

Figure 2 Relative mRNA expression of 11 renal membrane transporters and PXR in different age groups.



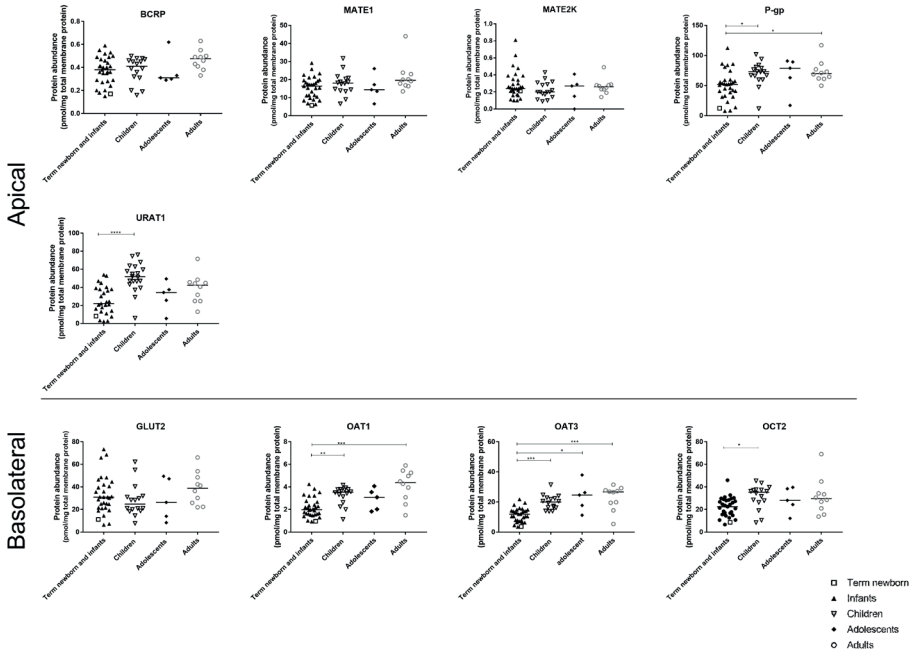
*Transporters are grouped according to their primary localization in the kidney (basolateral or apical). The bar represents the median for each age group. *p<0.05, **p<0.01, ***p<0.001*

Proteomics

62 samples were assessed for transporter protein levels (Figure 3 and Table S5). The median total membrane protein yield for all samples was 49.5 mg/g (range 41.7-62.1 mg/g) renal cortical tissue. All nine transporters were detected and quantified in our samples. P-gp was found to be the most abundant transporter, whereas MATE2-K was the least abundant (Table S5).

P-gp, URAT1, OAT1, OAT3 and OCT2 protein abundance levels were significantly lower in term newborn and infants than in the older age groups (Figure 3). Sigmoidal Emax models were used to fit the protein abundance levels of these five transporters, and all but URAT1 expression data conformed to the model (Figure 4a). OCT2 and P-gp expression increased at a faster rate than OAT1 and OAT3 as evidenced by the younger age at which half of the adult expression was reached (TM_{50}). Moreover, the transporters OCT2 and P-gp, shared a similar maturation pattern, as well as the transporters OAT1 and OAT3 (Figure 4b). No difference in protein abundance levels was found between age groups for BCRP, MATE1, MATE2-K and GLUT2.

Figure 3 Protein abundance levels of nine renal membrane transporters in different age groups.



*The bar represents the median for each age group. Term newborn and infants were combined here for analysis since there was only one term newborn included for this part of the study. *p<0.05, **p<0.01, ***p<0.001*

Figure 4 a) Ontogeny of protein abundance of P-gp, OAT1, OAT3 and OCT2 as described by Sigmoidal Emax model (solid black lines). Dashed lines represent the 95% confidence bands; b) Superimposing the Sigmoidal curves showed that the pair transporters, P-gp /OCT2 and OAT1/OAT3, shared similar maturation patterns.

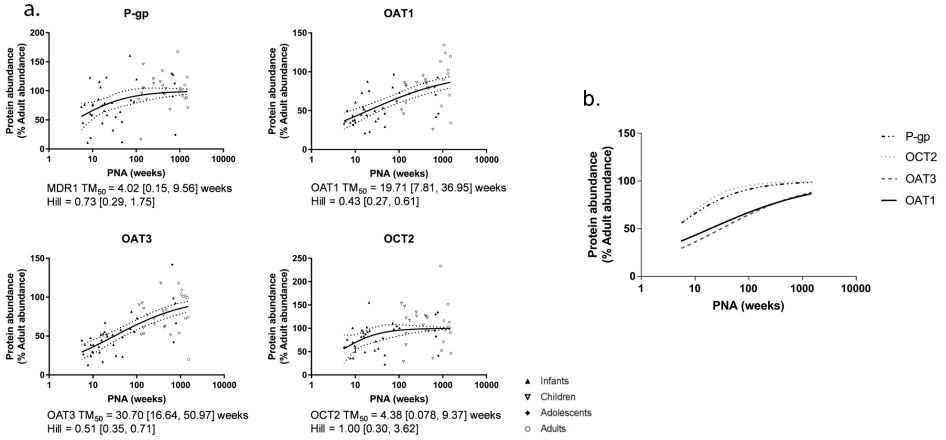


Table 2 Inter-transporter Spearman correlations

		Apical						Basolateral				
		BCRP	MATE1	MATE2-K	MDR1	MRP2	MRP4	URAT1	GLUT2	OAT1	OAT3	OCT2
Apical	BCRP	-	**0.69**		**0.44**	NA	NA			**0.59**		**0.43**
	MATE1		-	*0.63*	*0.36* **0.57**	*0.74*		*0.50* **0.57**	*0.44*	*0.53* **0.61**	*0.54* **0.54**	*0.62* **0.57**
	MATE2-K			-	*0.39*	*0.57*		*0.51*	*0.47*	*0.59*	*0.56*	*0.49*
	MDR1				-	0.47		*0.32* **0.56**	*0.37*	*0.52* **0.72**	*0.49* **0.60**	*0.57* **0.67**
	MRP2					-		NA	NA	*0.79*	*0.83*	*0.67*
	MRP4						-	NA	NA			
	URAT1							-	*0.42*	*0.54* **0.49**	*0.50* **0.49**	*0.46* **0.52**
Basolateral	GLUT2								-	*0.64*	*0.64*	*0.49*
	OAT1									-	*0.85* **0.83**	*0.74* **0.70**
	OAT3										-	*0.73* ***0.72***
	OCT2											-

Italic: mRNA expression; Bold: protein expression; NA=not available. All reached $p < 0.0001$. Data not presented if $p > 0.001$

Correlation between mRNA expression and protein abundance levels

Potential correlation between mRNA expression and protein abundance levels of the transporters was investigated (Figure S1). Significant correlation was found for MATE1, P-gp, URAT1, OAT3 and OCT2.

Inter-transporter correlation

To assess the potential shared expression regulation, we studied the correlation of mRNA expression and protein abundance levels between transporters (Table 2). Levels of OAT1 and OAT3 were the most significantly correlated.

Correlation between PXR and transporter mRNA expression

Weak negative correlations with PXR were found for MATE1 (r_s= -0.27, p = 0.035), MRP2 (r_s = -0.29, p = 0.021) and OCT2 (r_s = -0.26, p = 0.043), whereas no correlation was found for MATE2, P-gp, MRP4, OAT1 and OAT3.

Localization of MRP4 in pediatric kidney tissue

As a proof-of-concept, postmortem kidney tissues of 43 pediatric patients (GA > 24 weeks, PNA 2 days – 14 years old) and 1 adult were analyzed. Positive MRP4 immunostaining was detected as early as 27 weeks of gestation (PNA 9 days) despite negative staining found in 3 tissues from 1 child and 2 adolescents. For all the positive stained samples, MRP4 was found to be located at the apical side of the proximal tubule (Figure 5a and

Figure 5 Apical proximal tubule localization of MRP4 (arrow) by immunohistochemically staining in post mortem tissue of samples with a) GA of 27.7 weeks; PNA age 3.3 weeks, and b) GA of 40.0 weeks; PNA 3.1 year c) represents the negative control, and d) the semi quantification of MRP4 staining in various age groups: negative (0), low staining (+1) or high staining (+2).

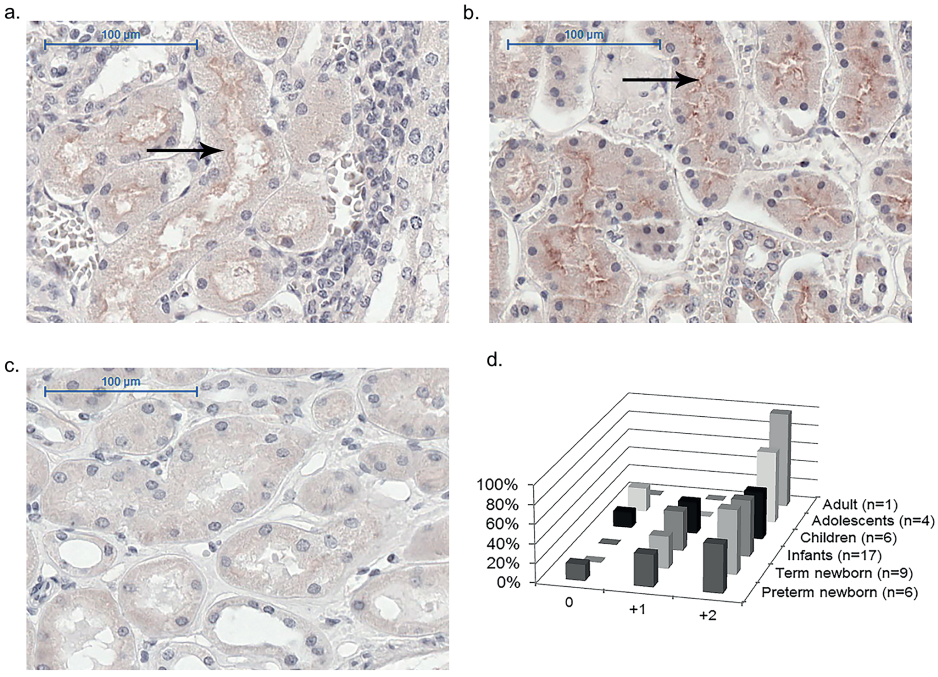


Figure 5b). See Figure 5c for the negative control. Although the examples showed lower staining at 3.3 weeks (Figure 5a) than 3.1 years old (Figure 5b), no statistically significant age-related changes were detected in the semi-quantification of the staining in the whole sample set (Figure 5d).

DISCUSSION

This study, to our best knowledge, is the first to comprehensively describe the ontogeny of human renal membrane transporters via mRNA expression analysis and quantitative proteomics in tissues representing a large span of ages. Albeit data on developmental changes in transporter mRNA expression in animals were reported previously,[20-24] cross species differences limit extrapolation, especially concerning the rates of maturation.[25]

Our study revealed two major findings with respect to the developmental maturation of renal transporters: (i) the expression of most of the transporters characterized in this study increased with age during the earliest developmental periods (< 2 years old); and (ii) maturation pattern was transporter-dependent. Additionally, we observed that: (a) there were maturational differences between mRNA expression and protein abundance; (b) there were correlations between the expression levels of various transporters; (c) PXR seems to play a minimal role in regulating mRNA expression of transporters in the kidney; and (d) stable MRP4 mRNA expression was accompanied by proper apical localization during development.

Transporter-dependent maturation patterns during the earliest developmental periods

The findings that most of the studied transporters showed a transporter-dependent age-related increase in their expression levels, especially during the earliest years of life were expected. Renal membrane transporters play critical roles in elimination and detoxification pathways in the body. They work in concert with enzymes in the kidney, as well as enzymes and transporters in other organs such as the intestine and liver to mediate the removal of ingested potential harmful compounds such as toxins derived from food, environmental toxins, drugs and their metabolites.[7] During infancy, dietary exposure to potential toxins is limited and begins to increase as infants are switched from an exclusively milk diet, to foods that may contain more toxins.[26,27] Thus, detoxification pathways are increasingly needed as the diet of infants expands and diversifies into childhood.

Further, besides changes in dietary intake and nutritional requirement, ontogeny of renal transporters can alter the disposition of endogenous compounds, suggesting important developmental roles for these renal transporters. Both BCRP and URAT1 are thought to play a clear role in uric acid (UA) homeostasis.[28,29] It was previously reported that the fractional excretion of UA (FEUA: the % of filtered UA not reabsorbed by the tubules), was 30-40% in term newborns <5 days old, which then decreased to 8-10% in children of 3 years old.[30-32] Our transporter maturation data, in addition to age-related physiological changes, *e.g.,* urinary acidification and concentration ability, may explain this observation: the decreasing BCRP mRNA expression from birth is accompanied by an increased expression of URAT1, a reabsorptive transporter, from birth till childhood, resulting in a net decrease in UA excretion.[30,32] Interestingly, sex-related differences in FEUA especially during adolescence were reported, which could be due to differences in proximal tubular secretion of UA.[32] Yet, no significant sex-related differences were found in our study consisting mainly of pediatric samples. As the influence of sex appears to be transporter-specific in adults,[33] follow-up studies with more samples and also with other transporters that handle UA, such as GLUT9, would be needed to fully understand the changes in FEUA.

In vivo pharmacokinetic data of drugs that are transporter substrates may be used to support our expression data. However, renal elimination of these drugs is accomplished not only by active tubular secretion facilitated by various transporters, but also by glomerular filtration, which is also subjected to age-dependent changes. As children grow and develop, the glomerular filtration rate (GFR) matures and is predicted to reach 50% of adult values by 2 months PNA and 90% of adult values by 1 year of age.[34] Though the full complement of nephrons in each kidney is complete around GA 36 weeks,[35] GFR continues to develop as a result of increases in kidney blood flow, improvements in filtration coefficients, and maturation of the tubules. Our findings that there are age-related changes in transporter expression early in life (<2 years) support the notion that active tubular secretion matures in parallel with GFR maturation. Thus, observed age-related changes in pharmacokinetics of transporter substrates are likely due to a combination of both maturation in transporter expression and GFR. For instance, after hepatic metabolism, the antiviral drug valacyclovir undergoes renal elimination via glomerular filtration and active tubular secretion likely by OAT1/3.[36] Apparent clearance of valacyclovir in infants < 3 months old is 50% lower than that in young children.[36] The GFR in infants <3 months old is expected to be >50%[34], and therefore this discrepancy may be explained by our findings that the TM_{50} of OAT1 and OAT3 were approximately 4 and 8 months (Figure 4a). Famotidine, an OAT3 substrate used in the treatment of gastritis, provides another example of maturational changes in both GFR and secretory transporters.[37] Given the complex developmental changes in renal elimination processes,

our data could be integrated into physiologically-based pharmacokinetic models to improve prediction of pediatric drug clearance. When doing so, scaling factors should be used to correct for membrane protein yield and total organ weight.

Other observations from data analysis

The abundance pattern of the transporters in our adult samples were assessed. P-gp has the highest abundance, followed by URAT1, GLUT2, OCT2, OAT3, MATE1, OAT1, BCRP and MATE2K. This abundance pattern is different from that reported in Prasad et al [38], where OCT2 was the most abundant transporter, followed by OAT1, MATE1, OAT3 and P-gp. This discrepancy could be due to actual inter-sample variation in expression levels of the transporters, or to differences in the inclusion criteria and quality of the tissue samples used in the studies. In addition, our study also showed much higher absolute abundance for most proteins in our adult samples compared to other studies [39]. As Li et al suggested[40], such inter-laboratory difference may, in part, be explained by different instrumental performance and varying tissue handling techniques.

Differences in the patterns and rates of change among mRNA expression and protein abundance levels of various transporters were noticed. For example, age-related changes were found in BCRP, GLUT2 and MATE2-K mRNA levels but not in protein abundance levels. This may suggest maturational differences in the regulation of gene transcription and post-translational processing. For gene transcription, alternative splicing is suggested to occur due to developmental signals.[41] Some of the alternatively spliced mRNA transcripts may not be translated into the protein of interest but will be quantified by qRT-PCR as the total mRNA expression could be derived from a mixture of different transcripts of the targeted gene. [42] Quantitative proteomics overcomes this challenge by measuring the actual expression of the protein of interest. This process could explain the lack of correlation between mRNA and protein expression.

Our data showed that transporter expression is correlated among various transporters. The strong correlation between expression of OAT1 and OAT3 is not surprising as they are located in adjacent regions on chromosome 11.[43] Moreover, they are both regulated by the transcription factors hepatocyte nuclear factor (HNF) 1α and 1β, which increase their transcription.[44] Our study of transcription factors was confined to PXR, and in agreement with previously reported findings; PXR mRNA levels in kidney were low in all age groups compared to the mRNA levels of the studied transporters.[33,45,46] Thus, PXR seems to have a minor role in regulating transporter gene expression in the kidney than in other organs. This is supported by findings in mice, where the potent rodent PXR activator pregnenolone-16α-carbonitrile induced transporter expression in liver and intestine, but not in kidney.[45] More research is needed to identify the developmental

triggers by which transcription of transporters increase and decrease. Moreover, the relationship between transcription factors maintaining basal expression level, like the HNF family, and renal transporter expression, should be studied.

Detoxification involves the interplay between enzymes and transporters that are ubiquitously expressed in tissues throughout the human body. P-gp expression levels in the liver only reached 50% of adult expression levels at 2.9 years of age[9] whereas our results suggested that full adult levels were achieved in the kidney by that age. For BCRP, our results for mRNA expression in the kidney were consistent with that in the liver, which showed a decline from newborns to adults.[47] As more transporter ontogenetic data in different organs become available, more reliable prediction in transporter-mediated substrate disposition on the whole-body level during development will be achieved.

Potential limitations

Certain limitations are present in this study. In addition to age, there are other potential factors, such as the use of co-medications and inflammation that can influence transporter expression and thereby contribute to the expression variability.[48] The impact of acute and chronic inflammation on transporter expression and activity is related to the activity of multiple proinflammatory cytokines. [49] The exact mechanism remains unknown, and may be related to various nuclear receptors and transcription factors. Similarly, certain medications and environmental toxins could lead to activation of nuclear receptor pathway, and could therefore influence the transporter expression.[49,50] The underlying reason for death of our tissue donors is heterogeneous, and so as the exposure of drugs and environmental toxins. Yet, despite all these inevitable differences, significant changes in the expression levels by age were still observed. However, due to the lack of detailed clinical data available for our samples, these factors could not be explored. Though protein and mRNA levels in the post-mortem samples used in our study were excellent, the amount of degradation in these levels from death to freezing is not known. Degradation may vary among samples, and may result in reduced absolute levels and increased variability in expression level measurements. Moreover, with the exception of PXR, we did not study the ontogeny of other transcription factors and proteins involved in gene and protein regulation; therefore, the mechanisms underlying the ontogeny of transporters observed in this study are not known. Finally, mRNA expression, protein abundance and transporter activity *ex-vivo* and *in-vivo* studies are needed to confirm the implications of our results to drug disposition in the kidney.

CONCLUSIONS

These results showed that the ontogeny of certain renal membrane transporters displayed an age-dependent pattern, suggesting that the clearance of exogenous and endogenous substrates for these kidney transporters are subject to transporter-specific age-related changes. Though future work is clearly needed in refining predictive models for pediatric drug disposition, leveraging our expression data in modeling and simulation strategies may improve predictability of pediatric drug disposition and exposure models. Importantly, our findings set the stage for future research in understanding the mechanisms of developmental changes in renal drug transporters.

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SUPPLEMENTAL INFORMATION

Material S1 RT-PCR protocol sample set 1 and sample set 2

mRNA isolation and cDNA synthesis

Total RNA was isolated from the tissue using a Nucleospin® RNA II kit (Machery-Nagel, Düren, Germany) for sample set 1 and RNeasy Mini Kit (Qiagen, Valencia, CA) for sample set 2, according to the manufacturer's instructions. Approximately 5-30 mg of frozen tissue was manually homogenized on ice in an Eppendorf tube using pellet pestles to yield 22 – 570 ng/Ul (range) RNA. Quality Control standards were applied to all RNA samples in this study. Purity was assessed both with A260nm/A280nm 1.9-2.1. Absorbance measurements at 260 nm in water were used to adjust the stock concentrations of all RNA samples to 1 Ug/Ul.

For reverse transcription, samples were treated with DNAse to digest contaminating DNA. For sample set 1 cDNA was obtained following local protocol, and for sample set 2 using SuperScript VILO cDNA Synthesis Kit (Life Technologies) per manufacturer's protocol.

Quantitative RT-PCR

For sample set 1, expression was measured by a SYBR green (SensiMix SYBR Hi-ROX kit; Bioline) quantitative RT-PCR using a 7900 Sequence detector (Applied Biosystems) on a 96 well optical reaction plate (Applied Biosystems). In house designed primer sequences, with confirmed specificity in appropriate melting curves for each PCR, can be found in Table S1 and were derived from Eurogentec (Eurogentec Netherlands, Maastricht, Netherlands). PCR efficiency of each primer pair was determined using serial dilutions of cDNA from the Caco-2 (colon carcinoma) cell line and from peripheral blood mononuclear cells. Non template controls confirmed the absence of exogenous contaminated DNA.

For sample set 2 RT-PCR was carried out in 384-well reaction plates using 2X Taqman Fast Universal Master Mix (Applied Biosystems, Foster City, CA), 20X Taqman specific gene expression probes and 10ng of the cDNA template. The reactions were carried out on an Applied Biosystems 7500 Fast Real-Time Polymerase Chain Reaction System (Applied Biosystems).

Transporter mRNA expression levels for all samples were normalized to GAPDH mRNA expression levels (ratio transporter/GAPDH) and relative expression was compared across the age range. Quality was assessed by measuring the RNA integrity number (RIN)

by microfluidic capillary electrophoresis on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA), whereby RIN's below 5 were to be excluded from the analysis.

Material S2 Detail description of LC-MS sample preparation and method parameters for quantitative proteomics

Quantitative proteomics was only performed in sample set 2 (Figure 1). Unless otherwise stated, reagents from MyOmicsDx, Inc (Towson, MD) were used. Membrane proteins were extracted from the renal cortical tissues using MyPro-MembraneEx buffer. The total extracted membrane protein concentration was determined using BCA protein assay kit. The membrane protein samples were then processed by MyOmicsDx, Inc (Towson, MD) using Filter-aided Sample Preparation method.[16] Briefly, protein samples in 9M urea were reduced with 5mM TCEP at 37°C for 45 min and reduced cysteines were blocked using 50mM iodoacetamide at 25°C for 15min. Protein samples were then cleaned using 10kDa Amicon Filter (UFC 501096, Millipore) three times using 9M urea and two times using MyPro-Buffer 1 (MyOmicsDx, Inc., Towson, MD). Samples were then proteolyzed with trypsin (V5111, Promega) for 12 hours at 37°C. The peptide solution was acidified by adding 1% trifluoroacetic acid (TFA) and was incubated at room temperature for 15 min. A Sep-Pak light C18 cartridge (Waters Corporation) was activated by loading 5mL 100% (vol/vol) acetonitrile and was washed by 3.5mL 0.1% TFA solution two times. Acidified digested protein solution was centrifuged at 1,800 x g for 5 min and the supernatant was loaded into the cartridge. To desalt the peptide bound to the cartridge, 1mL, 3mL, and 4mL of 0.1% TFA were added sequentially. 2mL of 40% (vol/vol) acetonitrile with 0.1% TFA was used to elute the peptides from the cartridge. The eluted peptides were lyophilized overnight and reconstituted in 37 µL MyPro-Buffer 3 (MyOmicsDx, Inc., Towson, MD).

Five peptides were chosen for each transporter as SRM quantifying targets from MyOmicsDx's SRM target peptide database, MyPro-SRM Map, based on their performance in documented experiments. Transition parameters and retention times of the 45 peptides were established individually using an Agilent 6495 Triple Quadrapole Mass Spectrometer for 1+, 2+, 3+ and 4+ charged precursor ions. Six best transitions per peptide precursor were selected for SRM quantification (Table S2).

Peptide samples previously reconstituted in MyPro-Buffer 3 were spiked with MyPro-SRM Internal Control Mixture which composed of a pool of 1 femto mole heavy isotope labeled peptides covering a large hydrophobicity window and a large M/z range of 200~1300, and were subject to SRM analysis. The peptide samples were eluted through

an online Agilent 1290 HPLC system into the Jet Stream ESI source of an Agilent 6495 Triple Quadrupole Mass Spectrometer (Agilent, Santa Clara, CA).

The Agilent 6495 Triple Quadrupole Mass Spectrometer was tuned using the manufacturer's tuning mixture by MyPro-SRM Tuning Booster after every preventive maintenance. Before and after each batch of SRM analysis, to ensure the stable and consistent performance of the mass spectrometer throughout the entire study, MyPro-SRM Performance Standard, a mixture of standard peptides across a wide range of mass (M/Z 100-1400) and a broad range of hydrophobicity were analyzed.

Figure S1 Correlations between mRNA expressions and protein expressions (Spearman's correlation coefficient, r_s)

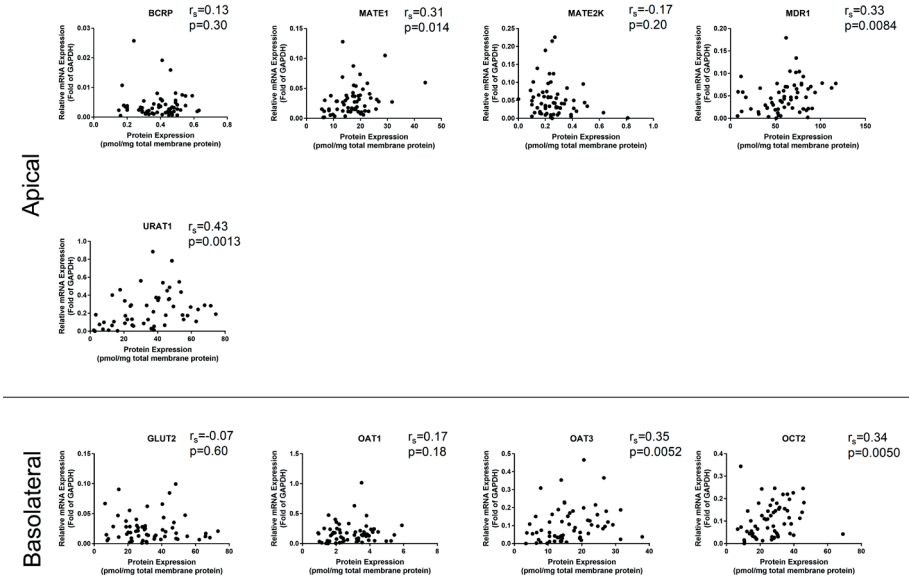

Table S1 Mann-Whitney U test to examine the differences in mRNA and protein expression between female and male

	mRNA Expression Analysis			Quantitative Proteomics		
	Median (range)		Mann-Whitney U test p-value	Median (range)		Mann-Whitney U test p-value
	Female	Male		Female	Male	
BCRP	0.00304 (0.00012-0.12)	0.0031 (0.00014-0.026)	0.71	0.41 (0.18-0.62)	0.37 (0.15-0.63)	0.52
MATE1	0.028 (0.0012-0.56)	0.032 (0-0.51)	0.44	17.24 (6.211-29.13)	16.12 (5.72-31.72)	0.4
MATE2-K	0.032 (0.0032-0.39)	0.034 (0.0022-0.51)	0.89	0.25 (0.14-0.81)	0.24 (0.00-0.31)	0.27
MRP2	0.013 (6.32E-5 - 0.083)	0.018 (0.00089 - 0.18)	0.32	NA	NA	NA
MRP4	0.019 (0.0042-0.031)	0.017 (0.0014-0.041)	0.91	NA	NA	NA
MDR1	0.031 (0.00032-0.37)	0.034 (0.0016-0.23)	0.52	66.12 (11.68-102)	54.6 (7.84-112.4)	0.2
URAT1	0.14 (0.0082-0.7204)	0.1582 (5.65E-5 - 1.35)	0.89	39.1 (2.17-76.05)	35.26 (2.79-74.50)	0.54
GLUT2	0.017 (0.0012-0.50)	0.024 (0.0012-0.099)	0.35	32.01 (7.21-73.94)	26.12 (6.41-68.55)	0.39
OAT1	0.076 (2.3E-5 - 0.36)	0.11 (0.00037-0.67)	0.18	3.14 (0.93 - 4.49)	2.35 (0.96 - 5.45)	0.87
OAT3	0.052 (0-0.40)	0.071 (0.00039-0.44)	0.21	13.91 (4.54-27.23)	15.17 (3.43-37.92)	0.6
OCT2	0.087 (3.37E-5 - 0.57)	0.094 (0.00061-0.50)	0.54	27.82 (8.48-45.94)	26.62 (6.66-45.44)	0.78
PXR	4.84E-5 (1.51E-5 - 0.00018)	4.34E-5 (4.58E-6 - 0.00029)	0.64	NA	NA	NA

Table S2 Kruskal Wallis followed by multiple comparisons to examine the differences in mRNA expression between African American and Caucasians in different age groups. All p-values > 0.9999, suggesting no statistical differences in the mRNA expression between these two ethnic groups in all age groups.

	Preterm to term newborn*	Infant*	Children*	Adolescent*	Adults*
BCRP	-6.7	5.68	11.32	26.00	-29.40
MATE1	16.07	35.92	11.98	1.00	23.20
MATE2-K	-76.09	20.14	3.00	-36.70	20.40
MDR1	5.08	17.25	-30.64	-38.4	62.60
URAT1	20.4	-0.87	27.52	9.30	22.80
GLUT2	51.17	11.15	-20.51	14.00	2.60
OAT1	61.50	-1.39	23.31	22.58	23.00
OAT3	-34.70	19.54	-6.32	16.60	18.00
OCT2	-27.98	14.69	1.44	-16.28	24.60

** mean rank differences*

Table S3 Mann-Whitney U test to examine the differences in protein expression levels between African American adults and Caucasian adults

	Median (range)		Mann-Whitney U test p-value
	African American	Caucasian	
BCRP	0.49 (0.33-0.63)	0.46 (0.38-0.5)	0.60
MATE1	16.8 (13.47-20.08)	23.01 (17.83-44.01)	0.095
MATE2-K	0.28 (0.14-0.49)	0.23 (0.19-0.27)	0.14
MDR1	64.57 (49.76-86.57)	70.98 (61.82-117.00)	0.55
URAT1	31.72 (24.79-45.64)	44.2 (12.95-71.51)	0.55
GLUT2	36.47 (22.02-42.34)	48.5 (22.37=66.12)	0.31
OAT1	4.49 (1.5-5.45)	3.95 (2.46-5.9)	0.84
OAT3	19.84 (5.39-27.23)	27.75 (14.43-31.51)	0.22
OCT2	28.6 (13.76)	33.52 (15.47-69.08)	0.69

Table S4 Relative mRNA expression of 11 transporters and PXR in age-groups

	Apical						Basolateral					
	BCRP	MATE1	MATE2-K	MDR1	MRP2	MRP4	URAT1	GLUT2	OAT1	OAT3	OCT2	PXR
All samples												
Median	0.0028	0.029	0.034	0.037	0.016	0.020	0.15	0.020	0.11	0.067	0.096	4.57E-05
Minimum	0.00	0.00	1.78E-05	2.91E-04	6.32E-05	0.0014	0.0001	0.0012	2.30E-05	0.00	3.37E-05	4.58E-06
Maximum	0.026	0.56	0.51	0.37	0.18	0.041	1.35	0.099	1.02	0.68	0.88	2.90E-04
Preterm newborns												
Median		0.010	0.019	0.0062	0.0065	0.020			0.0055	0.0072	0.0062	9.39E-05
Minimum		0.0012	0.0052	3.16E-04	8.59E-04	0.0042			2.30E-05	2.51E-05	3.37E-05	1.79E-05
Maximum		0.12	0.13	0.015	0.045	0.034			0.12	0.052	0.094	2.90E-04
Term newborn												
Median	0.018	0.015	0.017	0.014	0.013	0.023	0.026	0.011	0.038	0.023	0.049	5.56E-05
Minimum	0.0036	0.0023	0.0032	0.0023	6.32E-05	0.0053	0.0024	0.0022	4.37E-04	2.59E-04	1.11E-04	2.47E-05
Maximum	0.026	0.18	0.085	0.20	0.022	0.041	0.17	0.029	0.46	0.13	0.16	1.84E-04
Infants												
Median	0.0034	0.032	0.032	0.033	0.024	0.016	0.18	0.022	0.11	0.071	0.10	4.15E-05
Minimum	0.00	0.0001	1.78E-05	2.91E-04	8.85E-04	0.0014	5.65E-05	0.0012	4.71E-05	0.00	1.85E-04	7.69E-06
Maximum	0.026	0.56	0.51	0.37	0.18	0.032	1.35	0.099	0.47	0.42	0.88	1.14E-04
Children												
Median	0.0019	0.028	0.040	0.047	0.035	0.014	0.20	0.024	0.17	0.11	0.15	3.99E-05
Minimum	5.16E-04	0.0018	0.010	0.0057	0.0013	0.0028	0.058	0.0012	5.63E-04	3.87E-04	0.015	4.58E-06
Maximum	0.0043	0.23	0.19	0.23	0.12	0.030	0.89	0.091	1.02	0.68	0.50	6.75E-05
Adolescents												
Median	0.0013	0.025	0.051	0.041	0.047	0.019	0.10	0.011	0.081	0.077	0.062	4.12E-05
Minimum	7.96E-04	0.0037	0.0070	0.011	0.011	0.013	0.058	0.0074	0.0041	0.0015	0.017	2.01E-05

Table S4 Relative mRNA expression of 11 transporters and PXR in age-groups (continued)

	Apical						Basolateral					
	BCRP	MATE1	MATE2-K	MDR1	MRP2	MRP4	URAT1	GLUT2	OAT1	OAT3	OCT2	PXR
Maximum	0.0055	0.083	0.23	0.078	0.084	0.025	0.28	0.047	0.29	0.24	0.23	6.23E-05
Adults												
Median	0.0026	0.034	0.048	0.057	0.030	0.025	0.11	0.018	0.10	0.10	0.10	4.96E-05
Minimum	8.22E-04	0.0011	0.0062	0.014	6.69E-04	0.012	0.042	0.0041	2.66E-05	8.45E-05	0.0029	8.97E-06
Maximum	0.0081	0.28	0.25	0.18	0.069	0.040	0.78	0.048	0.67	0.52	0.24	1.61E-04

Table S5 Absolute abundance of nine selected renal membrane transporters[+]

	Apical				Basolateral				
	BCRP	MATE1	MATE2-K	MDR1	URAT1	GLUT2	OAT1	OAT3	OCT2
All samples									
median^	0.40	17.31	0.24	60.51	37.84	30.02	2.65	14.86	27.14
Range	0.15-0.63	5.72-44.01	0-0.81	7.84-117.00	2.17-76.05	6.41-73.34	0.93-5.90	3.43-37.92	6.66-69.08
Term newborn and infants									
median^	0.38	16.35	0.24	51.79	24.35	30.97	1.98	11.92	22.48
Range	0.15-0.59	5.72-29.13	0.10-0.81	7.84-112.4	2.17-63.98	6.41-73.34	0.93-4.25	3.43-21.76	6.66-45.94
Children									
median^	0.41	18.00	0.20	73.01	47.09	24.74	3.55	19.96	35.32
Range	0.16-0.50	6.59-31.72	0.090-0.43	11.68-102.00	5.86-76.05	7.76-62.25	1.14-4.17	13.71-31.53	8.48-45.44
Adolescents									
median^	0.31	14.36	0.27	78.91	34.33	26.27	3.08	24.61	28.09
Range	0.29-0.62	6.56-26.05	0.00-0.41	17.03-90.75	5.48-49.34	8.38-49.50	1.83-4.07	11.21-37.92	12.15-39.61
Adults*									
median^	0.48	19.56	0.26	69.92	42.42	38.81	4.39	26.71	29.59
Range	0.33-0.63	13.47-44.01	0.14-0.49	49.76-117.00	12.95-71.51	22.02-66.12	1.50-5.90	5.39-31.51	13.76-69.08

[+]*Term newborn and infants were combined for analysis because there was only one term newborn included in this part of the study. Levels of significance are shown in Figure 3.*

*^pmol/mg total membrane protein *African American and Caucasian adults*

Table S6 Primer sequences sample set 1

Transporter	Sequence
MDR1	Forward: 5'TTGCCACCACGATAGC 3' Reverse: 5'GCCAAGGGGTCGTAGA 3'
MRP2	Forward: 5'TGGGACCAAAAAAGATGTT 3' Reverse: 5'CCAGGGATTTGTAGCAGTT 3'
MRP4	Forward: 5' CGGTTTGGTCTCAACAAT 3' Reverse: 5'CCTCCTCCATTTACAGTGAC 3'
MATE1	Forward: 5'CCTGCAACCTTTCTTTATATG 3' Reverse: 5'CGAGGGCATTGACAAG 3'
MATE2-K	Forward: 5'GCCCAGGCTGTCATCT 3' Reverse: 5'CTTGGCCTGCACAGTATC 3'
PXR	Forward: 5'TCTCCCATTTCAAGAATTTC 3' Reverse: 5'ATGCCTTTGAACATGTAGGT 3'
OAT1	Forward: 5'GCCGGAAGGTACTCATCT 3' Reverse: 5'ATCCACTCCACATTCAGTGT 3'
OAT3	Forward: 5'CGTGCTTGGAGACCTGT 3' Reverse: 5'GGTCCGTGAGGCTGTAG 3'
OCT2	Forward: 5'ATCATTAAGCACATCGCAA 3' Reverse: 5'AGCTCGTGAACCAGTTGTAC 3'

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
OCT2	VSLQLLR.light	414.7687	729.4618	380	6	5	Positive
OCT2	VSLQLLR.light	414.7687	642.4297	380	10	5	Positive
OCT2	VSLQLLR.light	414.7687	529.3457	380	10	5	Positive
OCT2	VSLQLLR.light	414.7687	401.2871	380	10	5	Positive
OCT2	VSLQLLR.light	414.7687	321.7185	380	8	5	Positive
OCT2	LNPSFLDLVR.light	587.335	946.5356	380	12	5	Positive
OCT2	LNPSFLDLVR.light	587.335	849.4829	380	24	5	Positive
OCT2	LNPSFLDLVR.light	587.335	762.4509	380	24	5	Positive
OCT2	LNPSFLDLVR.light	587.335	530.7929	380	12	5	Positive
OCT2	LNPSFLDLVR.light	587.335	473.7715	380	8	5	Positive
OCT2	LNPSFLDLVR.light	587.335	228.1343	380	8	5	Positive
OCT2	LNPSFLDLVR.light	391.8924	559.2875	380	1	5	Positive
OCT2	LNPSFLDLVR.light	391.8924	502.2984	380	1	5	Positive
OCT2	LNPSFLDLVR.light	391.8924	473.7715	380	1	5	Positive
OCT2	LNPSFLDLVR.light	391.8924	387.2714	380	5	5	Positive
OCT2	LNPSFLDLVR.light	391.8924	336.6894	380	1	5	Positive
OCT2	SPGVAELSLR.light	514.7904	844.4887	380	18	5	Positive
OCT2	SPGVAELSLR.light	514.7904	688.3988	380	14	5	Positive
OCT2	SPGVAELSLR.light	514.7904	471.2744	380	18	5	Positive
OCT2	SPGVAELSLR.light	514.7904	341.1819	380	14	5	Positive
OCT2	SPGVAELSLR.light	514.7904	242.1135	380	18	5	Positive
OCT2	SPGVAELSLR.light	343.5293	541.2617	380	4	5	Positive
OCT2	SPGVAELSLR.light	343.5293	488.3191	380	4	5	Positive
OCT2	SPGVAELSLR.light	343.5293	412.2191	380	8	5	Positive
OCT2	SPGVAELSLR.light	343.5293	375.235	380	8	5	Positive
OCT2	SPGVAELSLR.light	343.5293	309.1845	380	8	5	Positive
OCT2	KLNPSFLDLVR.light	434.5907	687.3824	380	3	5	Positive
OCT2	KLNPSFLDLVR.light	434.5907	615.3824	380	3	5	Positive
OCT2	KLNPSFLDLVR.light	434.5907	502.2984	380	3	5	Positive
OCT2	KLNPSFLDLVR.light	434.5907	400.7369	380	3	5	Positive
OCT2	KLNPSFLDLVR.light	434.5907	308.1949	380	3	5	Positive
OCT2	VVAGVADAL.light	407.7371	683.3723	380	2	5	Positive
OCT2	VVAGVADAL.light	407.7371	612.3352	380	2	5	Positive
OCT2	VVAGVADAL.light	407.7371	426.2711	380	2	5	Positive
OCT2	VVAGVADAL.light	407.7371	389.2031	380	2	5	Positive
OCT2	VVAGVADAL.light	407.7371	342.1898	380	2	5	Positive
OCT2	VVAGVADAL.light	407.7371	203.139	380	2	5	Positive
OAT1	IYLTLLR.light	446.2867	778.4822	380	11	5	Positive

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters (continued)

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
OAT1	IYLTLLR.light	446.2867	615.4188	380	11	5	Positive
OAT1	IYLTLLR.light	446.2867	502.3348	380	7	5	Positive
OAT1	IYLTLLR.light	446.2867	390.2387	380	7	5	Positive
OAT1	IYLTLLR.light	446.2867	277.1547	380	7	5	Positive
OAT1	LVGFLVINSLGR.light	644.3928	1075.626	380	18	5	Positive
OAT1	LVGFLVINSLGR.light	644.3928	871.536	380	22	5	Positive
OAT1	LVGFLVINSLGR.light	644.3928	758.4519	380	14	5	Positive
OAT1	LVGFLVINSLGR.light	644.3928	659.3835	380	18	5	Positive
OAT1	LVGFLVINSLGR.light	644.3928	417.2496	380	10	5	Positive
OAT1	LVGFLVINSLGR.light	644.3928	213.1598	380	22	5	Positive
OAT1	NGGLEVWLPR.light	570.8116	799.4461	380	20	5	Positive
OAT1	NGGLEVWLPR.light	570.8116	670.4035	380	8	5	Positive
OAT1	NGGLEVWLPR.light	570.8116	385.2558	380	12	5	Positive
OAT1	NGGLEVWLPR.light	570.8116	342.1772	380	8	5	Positive
OAT1	NGGLEVWLPR.light	570.8116	272.1717	380	12	5	Positive
OAT1	NGGLEVWLPR.light	570.8116	229.0931	380	24	5	Positive
OAT1	GQASAMELLR.light	538.2819	890.4764	380	11	5	Positive
OAT1	GQASAMELLR.light	538.2819	819.4393	380	15	5	Positive
OAT1	GQASAMELLR.light	538.2819	732.4073	380	11	5	Positive
OAT1	GQASAMELLR.light	538.2819	661.3702	380	11	5	Positive
OAT1	GQASAMELLR.light	538.2819	257.1244	380	11	5	Positive
OAT1	TSLAVLGK.light	394.7475	600.4079	380	9	5	Positive
OAT1	TSLAVLGK.light	394.7475	487.3239	380	9	5	Positive
OAT1	TSLAVLGK.light	394.7475	416.2867	380	13	5	Positive
OAT1	TSLAVLGK.light	394.7475	317.2183	380	13	5	Positive
OAT1	TSLAVLGK.light	394.7475	204.1343	380	17	5	Positive
OAT3	TFSEILNR.light	490.264	731.4046	380	13	5	Positive
OAT3	TFSEILNR.light	490.264	644.3726	380	13	5	Positive
OAT3	TFSEILNR.light	490.264	515.33	380	13	5	Positive
OAT3	TFSEILNR.light	490.264	402.2459	380	13	5	Positive
OAT3	TFSEILNR.light	490.264	289.1619	380	13	5	Positive
OAT3	TFSEILNR.light	490.264	249.1234	380	9	5	Positive
OAT3	INLQKEI.light	429.2582	744.425	380	7	5	Positive
OAT3	INLQKEI.light	429.2582	726.4145	380	11	5	Positive
OAT3	INLQKEI.light	429.2582	630.3821	380	11	5	Positive
OAT3	INLQKEI.light	429.2582	517.298	380	7	5	Positive
OAT3	INLQKEI.light	429.2582	228.1343	380	7	5	Positive
OAT3	VAVLDGK.light	351.2132	602.3508	380	8	5	Positive

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters (continued)

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
OAT3	VAVLDGK.light	351.2132	531.3137	380	8	5	Positive
OAT3	VAVLDGK.light	351.2132	432.2453	380	8	5	Positive
OAT3	VAVLDGK.light	351.2132	319.1612	380	16	5	Positive
OAT3	VAVLDGK.light	351.2132	204.1343	380	16	5	Positive
OAT3	YTASDLFR.light	486.7429	809.4152	380	13	5	Positive
OAT3	YTASDLFR.light	486.7429	708.3675	380	9	5	Positive
OAT3	YTASDLFR.light	486.7429	637.3304	380	13	5	Positive
OAT3	YTASDLFR.light	486.7429	435.2714	380	21	5	Positive
OAT3	YTASDLFR.light	486.7429	265.1183	380	5	5	Positive
OAT3	TVLAVFGK.light	417.7578	634.3923	380	6	5	Positive
OAT3	TVLAVFGK.light	417.7578	521.3082	380	10	5	Positive
OAT3	TVLAVFGK.light	417.7578	450.2711	380	14	5	Positive
OAT3	TVLAVFGK.light	417.7578	351.2027	380	18	5	Positive
OAT3	TVLAVFGK.light	417.7578	201.1234	380	6	5	Positive
MATE1	QEEPLPEHPQDGAK.light	525.5864	659.3253	380	10	5	Positive
MATE1	QEEPLPEHPQDGAK.light	525.5864	594.804	380	10	5	Positive
MATE1	QEEPLPEHPQDGAK.light	525.5864	546.2776	380	10	5	Positive
MATE1	QEEPLPEHPQDGAK.light	525.5864	489.7356	380	6	5	Positive
MATE1	QEEPLPEHPQDGAK.light	525.5864	439.886	380	6	5	Positive
MATE1	QEEPLPEHPQDGAK.light	525.5864	396.8718	380	10	5	Positive
MATE1	TGEPQSDQQMR.light	638.783	989.4469	380	18	5	Positive
MATE1	TGEPQSDQQMR.light	638.783	559.7484	380	10	5	Positive
MATE1	TGEPQSDQQMR.light	638.783	495.2271	380	18	5	Positive
MATE1	TGEPQSDQQMR.light	638.783	486.2069	380	22	5	Positive
MATE1	TGEPQSDQQMR.light	638.783	288.119	380	18	5	Positive
MATE1	VGNALGAGDMEQAR.light	694.833	1047.489	380	20	5	Positive
MATE1	VGNALGAGDMEQAR.light	694.833	934.4047	380	20	5	Positive
MATE1	VGNALGAGDMEQAR.light	694.833	806.3461	380	20	5	Positive
MATE1	VGNALGAGDMEQAR.light	694.833	455.2613	380	16	5	Positive
MATE1	VGNALGAGDMEQAR.light	694.833	342.1772	380	24	5	Positive
MATE1	VGNALGAGDMEQAR.light	463.5577	467.706	380	4	5	Positive
MATE1	VGNALGAGDMEQAR.light	463.5577	455.2613	380	4	5	Positive
MATE1	VGNALGAGDMEQAR.light	463.5577	439.1953	380	4	5	Positive
MATE1	VGNALGAGDMEQAR.light	463.5577	411.5278	380	4	5	Positive
MATE1	VGNALGAGDMEQAR.light	463.5577	374.2146	380	4	5	Positive
MATE1	VGNALGAGDMEQAR.light	463.5577	246.1561	380	4	5	Positive
MATE1	GGPEATLEVR.light	514.7722	688.3988	380	14	5	Positive
MATE1	GGPEATLEVR.light	514.7722	617.3617	380	18	5	Positive

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters (continued)

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
MATE1	GGPEATLEVR.light	514.7722	516.314	380	14	5	Positive
MATE1	GGPEATLEVR.light	514.7722	457.7507	380	10	5	Positive
MATE1	GGPEATLEVR.light	514.7722	412.1827	380	14	5	Positive
MATE1	MEAPEEPAPVR.light	613.2977	894.468	380	21	5	Positive
MATE1	MEAPEEPAPVR.light	613.2977	539.33	380	25	5	Positive
MATE1	MEAPEEPAPVR.light	613.2977	483.2562	380	13	5	Positive
MATE1	MEAPEEPAPVR.light	613.2977	447.7376	380	17	5	Positive
MATE1	MEAPEEPAPVR.light	613.2977	332.1275	380	9	5	Positive
MATE1	MEAPEEPAPVR.light	613.2977	261.0904	380	17	5	Positive
MATE2-K	SFGSPNR.light	382.6879	530.2681	380	5	5	Positive
MATE2-K	SFGSPNR.light	382.6879	473.2467	380	9	5	Positive
MATE2-K	SFGSPNR.light	382.6879	386.2146	380	9	5	Positive
MATE2-K	SFGSPNR.light	382.6879	379.1612	380	5	5	Positive
MATE2-K	SFGSPNR.light	382.6879	339.1719	380	9	5	Positive
MATE2-K	SFGSPNR.light	382.6879	235.1077	380	5	5	Positive
MATE2-K	AAEEAKK.light	373.7058	675.3672	380	13	5	Positive
MATE2-K	AAEEAKK.light	373.7058	604.3301	380	9	5	Positive
MATE2-K	AAEEAKK.light	373.7058	475.2875	380	9	5	Positive
MATE2-K	AAEEAKK.light	373.7058	338.1872	380	9	5	Positive
MATE2-K	AAEEAKK.light	249.4729	475.2875	380	4	5	Positive
MATE2-K	AAEEAKK.light	249.4729	346.2449	380	4	5	Positive
MATE2-K	AAEEAKK.light	249.4729	338.1872	380	4	5	Positive
MATE2-K	AAEEAKK.light	249.4729	302.6687	380	0	5	Positive
MATE2-K	AAEEAKK.light	249.4729	201.087	380	4	5	Positive
MATE2-K	AAEEAKK.light	249.4729	200.7711	380	4	5	Positive
MATE2-K	FSIAVSR.light	390.2241	632.3726	380	13	5	Positive
MATE2-K	FSIAVSR.light	390.2241	545.3406	380	9	5	Positive
MATE2-K	FSIAVSR.light	390.2241	432.2565	380	9	5	Positive
MATE2-K	FSIAVSR.light	390.2241	361.2194	380	9	5	Positive
MATE2-K	FSIAVSR.light	390.2241	235.1077	380	9	5	Positive
MATE2-K	TPEEAHALSAPTSR.light	489.5793	683.3415	380	13	5	Positive
MATE2-K	TPEEAHALSAPTSR.light	489.5793	634.8151	380	9	5	Positive
MATE2-K	TPEEAHALSAPTSR.light	489.5793	618.3206	380	17	5	Positive
MATE2-K	TPEEAHALSAPTSR.light	489.5793	460.2514	380	13	5	Positive
MATE2-K	TPEEAHALSAPTSR.light	489.5793	455.8968	380	13	5	Positive
MATE2-K	VGMALGAADTVQAK.light	666.353	973.5313	380	19	5	Positive
MATE2-K	VGMALGAADTVQAK.light	666.353	860.4472	380	23	5	Positive
MATE2-K	VGMALGAADTVQAK.light	666.353	803.4258	380	15	5	Positive

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters (continued)

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
MATE2-K	VGMALGAADTVQAK.light	666.353	732.3886	380	15	5	Positive
MATE2-K	VGMALGAADTVQAK.light	666.353	472.2588	380	15	5	Positive
MATE2-K	VGMALGAADTVQAK.light	444.5711	616.8188	380	7	5	Positive
MATE2-K	VGMALGAADTVQAK.light	444.5711	588.3081	380	7	5	Positive
MATE2-K	VGMALGAADTVQAK.light	444.5711	546.3246	380	11	5	Positive
MATE2-K	VGMALGAADTVQAK.light	444.5711	392.5411	380	3	5	Positive
MATE2-K	VGMALGAADTVQAK.light	444.5711	218.1499	380	11	5	Positive
MDR1	EIIGVVSQEPVLFATTIAENIR.light	800.4442	917.5051	380	16	5	Positive
MDR1	EIIGVVSQEPVLFATTIAENIR.light	800.4442	816.4574	380	16	5	Positive
MDR1	EIIGVVSQEPVLFATTIAENIR.light	800.4442	715.4097	380	24	5	Positive
MDR1	EIIGVVSQEPVLFATTIAENIR.light	800.4442	531.2885	380	16	5	Positive
MDR1	EIIGVVSQEPVLFATTIAENIR.light	800.4442	512.3079	380	16	5	Positive
MDR1	EIIGVVSQEPVLFATTIAENIR.light	800.4442	955.5095	380	16	5	Positive
MDR1	AGAVAEEVLAAIR.light	635.3617	971.552	380	18	5	Positive
MDR1	AGAVAEEVLAAIR.light	635.3617	900.5149	380	18	5	Positive
MDR1	AGAVAEEVLAAIR.light	635.3617	430.2772	380	10	5	Positive
MDR1	AGAVAEEVLAAIR.light	635.3617	359.2401	380	10	5	Positive
MDR1	AGAVAEEVLAAIR.light	635.3617	299.1714	380	18	5	Positive
MDR1	AGAVAEEVLAAIR.light	423.9102	543.3613	380	6	5	Positive
MDR1	AGAVAEEVLAAIR.light	423.9102	430.2772	380	2	5	Positive
MDR1	AGAVAEEVLAAIR.light	423.9102	420.7267	380	2	5	Positive
MDR1	AGAVAEEVLAAIR.light	423.9102	359.2401	380	2	5	Positive
MDR1	AGAVAEEVLAAIR.light	423.9102	272.1843	380	2	5	Positive
MDR1	IATEAIENFR.light	582.3064	979.4843	380	16	5	Positive
MDR1	IATEAIENFR.light	582.3064	749.3941	380	16	5	Positive
MDR1	IATEAIENFR.light	582.3064	678.357	380	24	5	Positive
MDR1	IATEAIENFR.light	582.3064	565.2729	380	24	5	Positive
MDR1	IATEAIENFR.light	582.3064	490.2458	380	12	5	Positive
MDR1	IATEAIENFR.light	388.54	565.2729	380	1	5	Positive
MDR1	IATEAIENFR.light	388.54	486.2558	380	5	5	Positive
MDR1	IATEAIENFR.light	388.54	436.2303	380	5	5	Positive
MDR1	IATEAIENFR.light	388.54	283.1401	380	1	5	Positive
MDR1	STVVQLLER.light	522.806	856.5251	380	10	5	Positive
MDR1	STVVQLLER.light	522.806	757.4567	380	18	5	Positive
MDR1	STVVQLLER.light	522.806	658.3883	380	14	5	Positive
MDR1	STVVQLLER.light	522.806	530.3297	380	18	5	Positive
MDR1	STVVQLLER.light	522.806	428.7662	380	10	5	Positive
MDR1	TTIVIAHR.light	455.7771	708.4515	380	12	5	Positive

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters (continued)

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
MDR1	TTIVIAHR.light	455.7771	595.3675	380	12	5	Positive
MDR1	TTIVIAHR.light	455.7771	496.299	380	16	5	Positive
MDR1	TTIVIAHR.light	455.7771	354.7294	380	12	5	Positive
MDR1	TTIVIAHR.light	455.7771	298.1874	380	12	5	Positive
MDR1	TTIVIAHR.light	304.1871	496.299	380	8	5	Positive
MDR1	TTIVIAHR.light	304.1871	405.2532	380	4	5	Positive
MDR1	TTIVIAHR.light	304.1871	354.7294	380	0	5	Positive
MDR1	TTIVIAHR.light	304.1871	298.1874	380	0	5	Positive
MDR1	TTIVIAHR.light	304.1871	203.1026	380	0	5	Positive
BCRP	SSLLDVLAAR.light	522.806	757.4567	380	18	5	Positive
BCRP	SSLLDVLAAR.light	522.806	644.3726	380	10	5	Positive
BCRP	SSLLDVLAAR.light	522.806	435.774	380	14	5	Positive
BCRP	SSLLDVLAAR.light	522.806	430.2772	380	14	5	Positive
BCRP	SSLLDVLAAR.light	522.806	317.1932	380	6	5	Positive
BCRP	LAEIYVNSSFYK.light	717.3692	1007.483	380	21	5	Positive
BCRP	LAEIYVNSSFYK.light	717.3692	844.4199	380	17	5	Positive
BCRP	LAEIYVNSSFYK.light	717.3692	745.3515	380	13	5	Positive
BCRP	LAEIYVNSSFYK.light	717.3692	427.2551	380	17	5	Positive
BCRP	LAEIYVNSSFYK.light	717.3692	314.171	380	21	5	Positive
BCRP	LFDSLTLLASGR.light	646.8641	1032.568	380	18	5	Positive
BCRP	LFDSLTLLASGR.light	646.8641	717.4254	380	14	5	Positive
BCRP	LFDSLTLLASGR.light	646.8641	503.2936	380	14	5	Positive
BCRP	LFDSLTLLASGR.light	646.8641	390.2096	380	18	5	Positive
BCRP	LFDSLTLLASGR.light	646.8641	319.1724	380	10	5	Positive
BCRP	TIIFSIHQPR.light	404.568	737.4053	380	10	5	Positive
BCRP	TIIFSIHQPR.light	404.568	499.2825	380	2	5	Positive
BCRP	TIIFSIHQPR.light	404.568	442.7405	380	10	5	Positive
BCRP	TIIFSIHQPR.light	404.568	369.2063	380	10	5	Positive
BCRP	TIIFSIHQPR.light	404.568	215.139	380	2	5	Positive
BCRP	VIQELGLDK.light	507.7951	802.4305	380	9	5	Positive
BCRP	VIQELGLDK.light	507.7951	674.3719	380	9	5	Positive
BCRP	VIQELGLDK.light	507.7951	432.2453	380	17	5	Positive
BCRP	VIQELGLDK.light	507.7951	401.7189	380	9	5	Positive
BCRP	VIQELGLDK.light	507.7951	213.1598	380	9	5	Positive
URAT1	AFSELLDLVGGLGR.light	723.9012	899.5309	380	25	5	Positive
URAT1	AFSELLDLVGGLGR.light	723.9012	786.4468	380	25	5	Positive
URAT1	AFSELLDLVGGLGR.light	723.9012	459.2674	380	21	5	Positive
URAT1	AFSELLDLVGGLGR.light	723.9012	402.2459	380	13	5	Positive

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters (continued)

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
URAT1	AFSELLDLVGGLGR.light	723.9012	232.1404	380	13	5	Positive
URAT1	AFSELLDLVGGLGR.light	482.9366	889.4666	380	5	5	Positive
URAT1	AFSELLDLVGGLGR.light	482.9366	558.3358	380	5	5	Positive
URAT1	AFSELLDLVGGLGR.light	482.9366	459.2674	380	5	5	Positive
URAT1	AFSELLDLVGGLGR.light	482.9366	459.2575	380	5	5	Positive
URAT1	AFSELLDLVGGLGR.light	482.9366	402.2459	380	5	5	Positive
URAT1	AFSELLDLVGGLGR.light	482.9366	279.6715	380	5	5	Positive
URAT1	GAVQDTLTPEVLLSAMR.light	600.989	836.9506	380	9	5	Positive
URAT1	GAVQDTLTPEVLLSAMR.light	600.989	787.4163	380	13	5	Positive
URAT1	GAVQDTLTPEVLLSAMR.light	600.989	786.3992	380	9	5	Positive
URAT1	GAVQDTLTPEVLLSAMR.light	600.989	723.3871	380	9	5	Positive
URAT1	GAVQDTLTPEVLLSAMR.light	600.989	558.3028	380	9	5	Positive
URAT1	MGALLLLSHLGR.light	640.879	908.5676	380	26	5	Positive
URAT1	MGALLLLSHLGR.light	640.879	795.4835	380	22	5	Positive
URAT1	MGALLLLSHLGR.light	640.879	682.3995	380	22	5	Positive
URAT1	MGALLLLSHLGR.light	640.879	569.3154	380	18	5	Positive
URAT1	MGALLLLSHLGR.light	640.879	260.1063	380	26	5	Positive
URAT1	GGAILGPLVR.light	476.8006	654.4297	380	16	5	Positive
URAT1	GGAILGPLVR.light	476.8006	541.3457	380	8	5	Positive
URAT1	GGAILGPLVR.light	476.8006	484.3242	380	12	5	Positive
URAT1	GGAILGPLVR.light	476.8006	412.2554	380	4	5	Positive
URAT1	GGAILGPLVR.light	476.8006	299.1714	380	12	5	Positive
URAT1	GGAILGPLVR.light	476.8006	271.1765	380	20	5	Positive
URAT1	GGAILGPLVR.light	318.2028	484.3242	380	4	5	Positive
URAT1	GGAILGPLVR.light	318.2028	387.2714	380	8	5	Positive
URAT1	GGAILGPLVR.light	318.2028	274.1874	380	4	5	Positive
URAT1	GGAILGPLVR.light	318.2028	271.1765	380	0	5	Positive
URAT1	GGAILGPLVR.light	318.2028	242.6657	380	4	5	Positive
GLUT2	SFEEIAAEFQK.light	649.8168	1064.526	380	15	5	Positive
GLUT2	SFEEIAAEFQK.light	649.8168	693.3566	380	15	5	Positive
GLUT2	SFEEIAAEFQK.light	649.8168	622.3195	380	11	5	Positive
GLUT2	SFEEIAAEFQK.light	649.8168	235.1077	380	27	5	Positive
GLUT2	SFEEIAAEFQK.light	433.547	1211.594	380	15	5	Positive
GLUT2	SFEEIAAEFQK.light	433.547	1064.526	380	15	5	Positive
GLUT2	SFEEIAAEFQK.light	433.547	622.3195	380	11	5	Positive
GLUT2	SFEEIAAEFQK.light	433.547	606.277	380	3	5	Positive
GLUT2	SFEEIAAEFQK.light	433.547	551.2824	380	7	5	Positive
GLUT2	SFEEIAAEFQK.light	433.547	339.1607	380	3	5	Positive

Table S7 Surrogate peptides for each of the renal membrane transporters studied and their corresponding MS/MS parameters (continued)

Transporter	Surrogate peptide	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
GLUT2	VSIIQLFTNSSYR.light	764.4119	1115.548	380	19	5	Positive
GLUT2	VSIIQLFTNSSYR.light	764.4119	987.4894	380	23	5	Positive
GLUT2	VSIIQLFTNSSYR.light	764.4119	874.4054	380	19	5	Positive
GLUT2	VSIIQLFTNSSYR.light	764.4119	727.3369	380	15	5	Positive
GLUT2	VSIIQLFTNSSYR.light	764.4119	413.2758	380	15	5	Positive
GLUT2	VSIIQLFTNSSYR.light	509.9437	727.3369	380	10	5	Positive
GLUT2	VSIIQLFTNSSYR.light	509.9437	654.4185	380	6	5	Positive
GLUT2	VSIIQLFTNSSYR.light	509.9437	626.2893	380	10	5	Positive
GLUT2	VSIIQLFTNSSYR.light	509.9437	512.2463	380	14	5	Positive
GLUT2	VSIIQLFTNSSYR.light	509.9437	437.7063	380	6	5	Positive
GLUT2	VSIIQLFTNSSYR.light	509.9437	364.1721	380	6	5	Positive
GLUT2	HVLGVPLDDR.light	374.2085	615.3097	380	5	5	Positive
GLUT2	HVLGVPLDDR.light	374.2085	506.3085	380	1	5	Positive
GLUT2	HVLGVPLDDR.light	374.2085	407.2401	380	9	5	Positive
GLUT2	HVLGVPLDDR.light	374.2085	405.1728	380	9	5	Positive
GLUT2	HVLGVPLDDR.light	374.2085	308.1585	380	1	5	Positive
GLUT2	HVLGVPLDDRK.light	416.9068	533.2678	380	10	5	Positive
GLUT2	HVLGVPLDDRK.light	416.9068	506.3085	380	10	5	Positive
GLUT2	HVLGVPLDDRK.light	416.9068	416.2398	380	2	5	Positive
GLUT2	HVLGVPLDDRK.light	416.9068	407.2401	380	10	5	Positive
GLUT2	HVLGVPLDDRK.light	416.9068	372.206	380	10	5	Positive
GLUT2	HVLGVPLDDRK.light	416.9068	237.1346	380	14	5	Positive
GLUT2	LGPSHILIIAGR.light	416.2592	567.3431	380	10	5	Positive
GLUT2	LGPSHILIIAGR.light	416.2592	538.8324	380	6	5	Positive
GLUT2	LGPSHILIIAGR.light	416.2592	359.716	380	6	5	Positive
GLUT2	LGPSHILIIAGR.light	416.2592	359.5573	380	6	5	Positive
GLUT2	LGPSHILIIAGR.light	416.2592	303.1775	380	10	5	Positive
GLUT2	LGPSHILIIAGR.light	416.2592	303.1739	380	10	5	Positive