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Preterm Neonatal Urinary Renal Developmental and Acute Kidney Injury Metabolomic Profiling: An Exploratory Study

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Abstract

Introduction—AKI staging has been developed in the adult and pediatric populations, but these do not yet exist for the neonatal population. Metabolomics was utilized to uncover biomarkers of normal and AKI associated renal function in preterm infants. The study was comprised of 20 preterm infants with an AKI diagnosis who were matched by gestational age and gender to 20 infants without an AKI diagnosis.

Methods—Urine samples from pre-term newborn infants collected on day 2 of life were analyzed using broad spectrum NMR-metabolomics. Multivariate analysis methods were used to identify metabolite profiles that differentiated AKI and no AKI, and to identify a metabolomics profile correlating with gestational age in infants with and without AKI.

Results—There was a clear distinction between the AKI and no-AKI profiles. Two previously identified biomarkers of AKI, hippurate and homovanillate, differentiated AKI and no-AKI. Pathway analysis revealed similarities to cholinergic neurons, prenatal nicotine exposure on pancreatic β cells, and Amitraz-induced inhibition of insulin secretion. Additionally, a pH difference was noted. Both pH and the metabolites were found to be associated of AKI; however, only the metabolite was a significant predictor of AKI. Pathways for the no-AKI group that were uniquely correlated to gestational age included aminoacyl-t-RNA biosynthesis, whereas pathways in the AKI group yielded potential metabolite changes in pyruvate metabolism.

Conclusions—Metabolomics was able to differentiate the urinary profiles of neonates with and without an AKI diagnosis and metabolic developmental profiles were correlated with gestational age. Further studies in larger cohorts are needed to validate these results.

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Keywords

Neonatal; Acute Kidney Injury; Renal Development; Metabolomics; NMR Spectroscopy; Multivariate Analysis; Regression Analysis

Introduction

Premature infants are born with a paucity of nephrons which are underdeveloped, and at different levels of maturity at birth. Full nephrogenesis continues until 36 weeks gestations, and unfortunately, the extra-uterine environment is often not hospitable for proper nephrogenesis. Premature infants are at risk for short and long-term kidney disease. Acute kidney injury (AKI) among premature neonates is multifactorial and often due to infection, perinatal distress, and exposure to nephrotoxic medications[1]. The incidence of AKI in premature infants is estimated to be between 12.5 and 39.8 % during the first weeks of life. [2–4], and those with AKI have significantly worse outcomes in terms of mortality[5]. Most of these studies have used a rise in serum creatinine (SCr) from baseline level by 0.3 mg/dl to diagnose AKI[2]. Metabolomics may lend insight into the diagnosis of neonatal AKI, and the changing development of the newborn.

Urinary metabolomic studies have been used to uncover markers of disease [6–8], and are particularly promising and relevant in the neonatal community, as they utilize a biofluid readily available in most circumstances. Urine metabolomics have been used to document changes across developmental age; yet there is so much more to uncover. Metabolomics applications could be employed to evaluate the differences across neonatal developmental stages, the impact of AKI on different metabolomics pathways, the environmental exposures that lead to poor development and kidney injury, the nutritional demands of the growing premature infants, and to uncover urine biomarkers of many diseases[9]. All of these could improve the outcomes and understanding of the target pathways for certain diseases [8]. In addition, these urinary metabolites could provide real-time information to clinicians for earlier diagnosis and intervention [8].

To date no studies have fully described the developmental urinary metabolomic profiles of preterm neonates in the context of AKI. In addition to the disease state, samples derived at baseline and from well-matched controls are important to understanding normal developmental stages particularly in premature infants where renal development may be occurring *ex utero*.

We performed a prospective nested case-control study to improve our understanding of the metabolomics in premature infants. We evaluated the differences in the urine metabolome between infants with AKI and those without AKI (matched by gestational age and gender). Additionally, we evaluated the differences in the urine profiles across different gestational age categories in neonates with and without AKI.

Methods

Human Subjects Study population

This prospective nested case-control study was performed on a cohort of very low birth weight (VLBW) infants admitted to the regional NICU between February 2012 and June 2013. Infants who met the inclusion criteria (birth weight (BW) $\geq$ 1200 grams and/or $\geq$ 31 weeks gestational age) and exclusion criteria (known congenital kidney disease or if they did not survive beyond the first 48 hours of life) were approached to participate in this study. Prospective data was collected on 122 infants. For this metabolomics investigation twenty (20) infants who met the diagnostic criteria for AKI, were selected as cases, and were matched by gestational age ($\pm$ 1 week) and gender. Twenty (20) similar infants without AKI were chosen as controls, as shown in Table 1. Cases and controls were selected to assure we had an equal representation of gestational categories (<27 weeks, 27–28 weeks and >28 weeks gestation). There was no significant difference in the AKI and no AKI patients as it relates to gestational age ($p = 0.84$), birth weight ($p=0.84$), and gender ($p=0.52$).

AKI was diagnosed in the context of this study if an infant had a rise in serum creatinine (SCr) of 0.3mg/dl from baseline as defined by the Kidney Disease Improving Global Outcomes Definition (KDIGO) during the first week of life. SCr was prospectively collected on days 1, 2, 3 and 4 of life in addition to any clinically measured SCr values.

This study was approved by University of Alabama at Birmingham (UAB) Institutional Review Board, informed parental consent was obtained in all infants. The clinical and research activities being reported are consistent with the Principles of the Declaration of Helsinki.

NMR Metabolomics

Urine was collected on day 2 of life using cuddle buns™ (Small Beginnings, Hesperia, CA, USA) diapers placed at the perineum. Urine was squeezed from the fibers of the diaper with a syringe onto a lab tube which was then centrifuged at 15,000 rpm for 10 min to remove any particulates. Samples were then stored at -80°C within 2–6 hours of collection until measurements by metabolomics analysis, similar to other metabolomics studies [10–13]. No preservatives were added to the urine samples as previous studies have shown urinary metabolites are stable for at least 4 weeks [14] and the use of preservatives when storing samples at -80°C have little to no impact on the resulting metabolomics profiles [15].

All 40 urine samples were analyzed using nuclear magnetic resonance (NMR) spectroscopy to analyze the metabolites that differentiated the urine from infants with and without AKI diagnosis. Sample preparation, data acquisition, data analysis, and pathway analysis followed procedures previously described [16–21]. Briefly, each of the 40 urine samples (200 μL) were prepared individually by addition of 25 μL D_2O and 25 μL Chenomx Internal Standard (Chenomx ISTD, Edmonton, Alberta, Canada). Phenotypic pools for AKI and no AKI were generated with 60 μL of each urine sample pooled, mixed, divided into 3 aliquots. Additionally, 400 μL from both phenotypic pools were mixed to form a total pooled sample and divided into 3 aliquots. These pooled samples were prepared identically to the individual

study samples. Metabolomics data were acquired for each of the individual study samples and for the pooled samples. ^1H NMR spectra of urine samples were acquired on a Bruker Avance III 950 MHz NMR spectrometer housed at the David H. Murdock Research Institute in Kannapolis, NC. Spectra were collected using the 1D NOESY presaturation pulse sequence (noesypr1d) and were pre-processed using ACD 1D NMR Processor 12.0 (ACD Labs, Toronto, CA). NMR bins (0.50–9.00 ppm) were made after excluding water (4.68–4.88 ppm), urea (5.60–6.00 ppm), and imidazole (7.20–7.28 ppm) using intelligent binning width of 0.04 ppm and 50% looseness factor. Integrals of each of the bins were normalized to total integral of each of the spectrum.

Multivariate and Statistical Analysis

Normalized binned NMR data were Pareto scaled and centered prior to multivariate analysis. The metabolomic profiles of the AKI and no AKI were analyzed to determine the urinary markers of AKI for all of the gestational ages. Then, the AKI and no AKI profiles were compared separately to understand the differences in development in the presence and absence of AKI. Multivariate data analysis methods (e.g. principal component analysis [PCA], orthogonal partial least squares [OPLS], and orthogonal partial least squares discriminant analysis [OPLS-DA]) were used to reduce the dimensionality and to enable the visualization of the separation of the study groups (SIMCA 14, Umetrics, Umeå, Sweden) [22, 23]. The PCA scores plots were inspected to ensure that the phenotypic pooled samples were tightly clustered in the center of phenotypic groups and that the pools of all individual study samples clustered in the center of all samples, a quality control method that is widely used in metabolomic studies [24]. Loadings plots and variable influence on projection (VIP) plots were inspected, and bins that had a VIP ≥ 1.0 with a jack-knife confidence interval that did not include 0 were determined to be important for differentiating the study groups. The VIP statistic summarizes the importance of the bin in differentiating the phenotypic groups [23]. All models used a 7-fold cross-validation to assess the predictive variation of the model (Q^2). Chenomx NMR Suite 7.7 Professional software (Edmonton, Alberta, Canada), which has a concentration library of approximately 350 compounds, and HMDB reference library was used to match the signals in the identified bins to metabolites [25–27]. Moreover, hippurate and homovanillate were fit for relative concentration determination using Chenomx. The pH of each spectrum was calculated based on the imidazole chemical shift compared to the residual water resonance [28].

Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc, Cary, NC). Statistical tests for continuous variables that were non-normally distributed were conducted using the Mann-Whitney U test; otherwise, a two-sided t-test with the Satterthwaite correction for unequal variances was used. The chi-square test was used to test for differences in categorical variables and Fishers exact test was used when cell counts were small. Logistic regression was used to predict the diagnosis of AKI using calculated pH (categorized at the mean pH level of 7.4) alone, the metabotype alone, and both pH and metabotype. The predictive component scores from the multivariate AKI OPLS-DA model were used to define the metabotype. For this exploratory study, p-values < 0.05 were considered to be statistically significant and were not adjusted for multiple testing. [29, 30]

Pathway Analysis

Using the results of the supervised multivariate analyses, library-matched metabolites were analyzed for pathway enrichment using the knowledge-based canonical pathways and endogenous metabolic pathways in the MetaCore, by Thomson Reuters (Philadelphia, PA). Ranking of relevant pathways was based on hypergeometric p-values.

Results

Metabolites that differentiate AKI from no AKI

Unsupervised (PCA, Fig 1a) and supervised (OPLS-DA, Fig 1b) multivariate analyses were used to determine the metabolites responsible for differentiating neonates diagnosed with AKI from those without an AKI diagnosis. While no clustering was observed as defined by the phenotypic anchors in the PCA, a clear distinction was observed by OPLS-DA between the AKI and non-AKI profiles (1 predictive and 2 orthogonal components, R^2X (cum) = 0.4, R^2Y =0.5, Q^2 (cum) = -0.189, 80% correct for the cases and 90% correct for the controls with a Fisher's probability = 8.3E-6). The receiver operating characteristic curve (ROC) is shown in Fig 1c, and the area under the curve (AUROC) was 0.93. Table 2 shows the library-matched bins that had a VIP ≥ 1 with a jack-knife confidence interval that did not include 0 and were determined to be important to separating the groups. One of the bins important to differentiating the AKI diagnosis groups contained hippurate and homovanillate (VIP=4.7, p =0.04, fold change=-2.1) which have been previously implicated in nephrotoxicity.[31–33] Both hippurate and homovanillate were fit for relative concentrations. The mean hippurate concentration was 82.3 μ M (SD = 85.4) for AKI and 59.7 μ M (SD = 28.5) for no-AKI (p = 0.27). The mean homovanillate concentration was 39.3 μ M (SD = 48.9) for AKI and 38.7 μ M (SD = 35.5) for no-AKI (p = 0.97). Pathway mapping suggests that the metabolic differences between groups could be related to cholinergic neurons, prenatal nicotine exposure on pancreatic β cells, and Amitraz-induced inhibition of insulin secretion, as shown in Figure 1D.

A shift in the imidazole resonances (pH indicator) in NMR spectra, as shown in Fig 2a, was observed during the inspection of the QC pooled samples. This shift indicates that the samples potentially have different pH values; therefore, the pH of each of the individual urine samples was calculated [28], and the pH was found to be statistically different (p =0.007) between infants with an AKI diagnosis (mean=7.5, SD=0.3) and those without an AKI diagnosis (mean=7.3, SD=0.2). Given the key contribution that the kidneys provide in maintaining acid–base balance and the existence of renal tubular acidosis of the newborn, this was a compelling finding [34]. The distribution of pH by AKI diagnosis is shown in Fig 2b.

To further explore the pH difference, logistic regression modeling was utilized to understand the predictive nature of the pH and metabolomics profiles. The results of the logistic regression modeling to predict the diagnosis of AKI showed that high pH (pH > 7.4) is predictive of an AKI diagnosis when it is the only predictor included in the model. Similarly, the metabolotype alone was also predictive of AKI. When both pH and the metabolotype were included in the model, only the metabolotype was a significant predictor of AKI. Fig 3 shows

the comparisons of the ROC curves for the three models, and Table 3 shows the 95% confidence interval for each model's AUROC. The AUROC for the models including the metabolotype are significantly higher (AUROC=0.9) than the AUROC for the pH only model (AUROC=0.7, $p < 0.001$).

Metabolites associated with gestational age categories

The urinary profiles of the neonates were investigated by orthogonal partial least squares (OPLS) regression using gestational age (measured in days) to correlate the metabolomics data with gestational age for infants diagnosed with AKI and those without an AKI diagnosis. Fig 4a and 4b show trajectory of the gestational ages according to AKI status in OPLS models, while Fig 4c and 4d demonstrate the prediction of gestational age in patients with no AKI through the metabolomics data. Tables 4 and 5 list the metabolites that were identified as being important to the prediction of gestational age with VIP, while Table 6 includes a comprehensive list of metabolites unique for AKI and No AKI, and common metabolites for both groups that correlated with gestational age. The functional ontology was created for AKI and no AKI correlated metabolites to understand gestational age. Aminoacyl-t-RNA biosynthesis in the mitochondria and cytoplasm were the top scoring probable pathway identified for the unique no-AKI metabolites (Fig 5a), while the unique AKI metabolites yielded potential changes in pyruvate metabolism (Fig 5b). The common metabolites for the gestational age prediction also show (Fig 5c) that the biochemical pathways important for describing the gestational ages of all the neonates is aminoacyl-tRNA biosynthesis

Discussion

Urinary metabolomic profiles of preterm neonates with AKI have not been examined prior to this metabolomics study. This study sought to understand the differences between the urinary metabolomic profiles of neonates with and without AKI, controlling for gender and gestational age. Additionally, it was hypothesized that the urine metabolome differs across different gestational age categories in neonates with and without AKI and can correlate with gestational age.

To understand the differences between infants with an AKI diagnosis and those without, metabolomics profiles were evaluated by unsupervised and supervised multivariate analysis. The infants with an AKI diagnosis could be differentiated from those without a diagnosis based on the supervised analysis (Figure 1B), and the metabolites important to the differentiating between AKI and non-AKI were determined with library matching. The highest VIP bin contained hippurate and homovallinate both of which have been previously implicated in nephrotoxicity [31–33]. Previously the homovallinate was noted to be elevated in the AKI patients, whereas in this cohort, the homovallinate was lower. It appears that the directionality of the NMR bin is driven by the hippurate change. Hippurate has been noted to be decreased in nephrotoxicology studies [32], and this corresponds with our findings. Plasma tyrosine metabolism has also been noted to be perturbed in previous AKI studies [35, 36]. Metabolites important to differentiating infants with and without an AKI diagnosis demonstrated that the process implicated in AKI, could be related to cholinergic neurons,

prenatal nicotine exposure on pancreatic β cells, and Amitraz-induced inhibition of insulin secretion, as shown in Fig 1D. Interestingly, cholinergic receptors are found in intercalated cells (ICCs) which regulate aquaporins [37]. These ICCs also help regulate the pH throughout the kidneys, making a compelling connection between the pathway analysis and the pH differences observed in samples from infants with an AKI diagnosis compared to infants without AKI. Animal investigations have also implicated a possible common toxic pathway response for AKI and Amitraz [38]. While the toxic effects from the Amitraz were reversed with alpha2-adrenergic antagonists, the patient suffered from AKI, demonstrated by the presence of severe, acute, multifocal, glomerular and tubular necrosis. Prenatal nicotine exposure has been linked with oxidative stress in the kidneys [39]. Pathways associated with oxidative stress have also observed that in cases such as contrast-induced acute kidney injury in diabetic patients [40].

Several factors, including infection, perinatal distress, and exposure to nephrotoxic medications, lead to AKI susceptibility in the neonate [1]. Understanding the biomarkers associated with and the impact of AKI on neonatal kidney function is important for early detection and intervention. In this study urinary metabolomic analysis was utilized to investigate the developing non-AKI and AKI kidneys with urinary profiles of neonatal infants between the gestational ages 23–31 weeks. The OPLS regression model and NMR library matching investigations found metabolites important in the correlation with changing gestational age. For infants without an AKI diagnosis, gestational age was correlated with metabolites of aminoacyl-t-RNA biosynthesis, suggesting that this was a process modulated during the observed gestational age range. This is a key pathway as it regulates amino acid synthesis, and the proximal tubules of the kidneys are responsible for hydrolyzing small proteins [41]. The degradation of β_2 microglobulin, in particular, has been correlated with gestational age, reflecting maturing proximal renal tubular function [42]. Gluconate was identified as one of the highest VIPs in this analysis. However, it is unclear if this was due to differences occurring in the neonates during the 23–31 gestational weeks or if it is due to differences in trophic feeding strategies [43, 44]. For infants diagnosed with AKI, gestational age was correlated with metabolites that suggest that pyruvate metabolism was a process modulated during this gestational age range. Pyruvate metabolism has previously been demonstrated to be downregulated in a kidney cancer metabolomics and proteomics study, as were the pathways closely associated with gluconeogenesis [45]. The specific nature of pyruvate metabolism in the presence of renal injury as yet to be fully understood. Both propylene glycol and ethylene glycol were found to be predictors of gestational age in premature neonates with AKI. Many external factors have been proposed to contribute to malfunction of neonatal kidneys, including maternal diet, stress, hypertension, and drugs administered to the mother or to the newborn [46, 47]. Drug formulations, in particular, contain propylene glycol and poly-ethylene glycol, which are co-solvents and could be a factor in the kidney injury [48–50] or a possible environmental contaminant.

For all infants in our cohort, threonine was important to the correlation with gestational age. We also found that for infants without an AKI diagnosis, glycine was important to the correlation with gestational age. Both metabolites have been discussed previously in urinary metabolomics of neonates as markers of insufficient growth *in utero* [51, 52]. It has been suggested that because both of these amino acids are glucogenic; an increase of glycine

would cause a reduction of amino acid oxidation or reduced glucogenesis as a means of conservation [53]. In addition Atzori and colleagues found amino acids to differentiate preterm (23–32 weeks GA) and term infants (33–36 weeks GA), including both hippurate and proline [10]. Atzori and colleagues also found that tryptophan, phenylalanine, malate, tyrosine, x-hydroxybutyrate, and N-acetylglutamate were important to differentiating the preterm and term infants, which were not found to predict gestational age in the no-AKI infants in this cohort. Additionally, in a study of infants with intrauterine growth retardation (IUGA) compared to adequate (AGA) and large (LGA) for gestational age, the two metabolites that best differentiated these urinary profiles were myo-inositol and creatinine, which the authors postulate related to a hypo-insulinemic condition from which the IUGA fetuses. This correlates well with the finding described here and elsewhere that threonine and glycine are descriptors of insufficient *in utero* growth. [13].

The AKI associated profiles in this gestational range cohort indicated that pyruvate metabolism was being modulated, so the important difference in the renal development of neonates with an AKI diagnosis compared to those without could very well be related to energy metabolism. It has been observed previously that accumulation of calcium and magnesium during energy metabolism causes damage in the renal mitochondria [54, 55].

In summary, this manuscript describes the developmental urinary metabolomic profiles of preterm neonates, with or without AKI. Using metabolomics, the profiles of AKI and no AKI neonatal cohorts, differentiated by metabolites related to aminoacyl-t-RNA biosynthesis. Additionally, the pH of the samples were significantly different, suggesting influence of the cholinergic receptors in the ICCs in regulating the acid-base balance in the disease state. Gestational age in the no-AKI profiles were best described by metabolites in the aminoacyl-t-RNA biosynthesis pathway, while the AKI subjects were better represented by changes in pyruvate metabolism.

A limitation of this exploratory study is that the sample size was small and did not include a validation sample; however, this study found a set of urinary metabolites that clearly differentiated neonates with and without AKI. The broad spectrum metabolomics and pH results warrant a future targeted metabolomics study in a larger cohort. Validation of the markers identified in this study may assist clinicians in better diagnosis and treatment of AKI in at risk population.

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Figure 1.

The unsupervised PCA (a) and supervised multivariate analysis OPLS-DA (b for differentiating neonates with an AKI diagnosis from those without an AKI diagnosis. The supervised analysis had an $R^2X_{cum}=0.39$, $R^2Y_{cum}=0.54$, and a $Q^2_{cum}=-0.189$. The classification was 80% correct for the cases, 90% correct for the controls, and 85% correct for all samples with a Fisher probability score of $8.3E-6$. The OPLS-DA model had an area under the curve of 0.93 (c). (d) The curated pathways important for differentiating the AKI diagnosis.

Figure 2. The urinary pH was observed to be different in the no-AKI and AKI QC pools by the imidazole chemical shift (a) in the NMR spectra. Box plot of the calculated pH of no-AKI and AKI urinary profiles (b). The mean pH of the no-AKI was 7.29, while the AKI urinary profiles were 7.53, $p = 0.007$.

Figure 3.

ROC curves for the three logistic regression models of the urinary profiles to predict AKI. Model (a) included pH as the only predictor of AKI had an area of the curve (AUC) of 0.68. Model (b) included the metabotype only had an AUC of 0.93. Model (c) which included both the calculated pH and the metabotype had an AUC of 0.93.

Figure 4.

The predicted vs observed OPLS models of gestational age of no AKI profiles (a) and AKI profiles (b). Each point represents one urinary profile, and the goodness of fit (R^2) is noted in the upper left corner. The metabolites that model the gestational age prediction are listed in tables 4 and 5.

Figure 5. Curated pathways important to the correlation with gestational age that are unique to neonates without an AKI diagnosis (a), with an AKI diagnosis (b), and common to all neonates regardless of AKI diagnosis (c).

Table 1

Comparison of subject characteristics between controls and acute kidney injury cases

	No AKI (N = 20)	AKI (N = 20)	p-value *
Infant Demographics			
Birth weight (grams)	834.0 ± 291.9	815.8 ± 288.2	0.84
GA weeks (weeks)	26.3 ± 2.3	26.1 ± 2.3	0.84
Apgar 1 minute *	2.0 (1.0, 6.0)	3.5 (1.5, 6.0)	0.32
Apgar 5 minute *	7.0 (4.0, 7.5)	7.0, (4.0, 8.0)	0.73
Length (cm) *	31.3 (29.5, 37.3)	31.5 (28.5, 34.8)	0.80
Head Circumference (cm) *	22.3 (21.3, 25.5)	23 (21.5, 25.0)	1.0
Male	9 (45%)	7 (35%)	0.52
Race			0.75
White	10 (50%)	9 (45%)	
Black	10 (50%)	11 (55%)	
Maternal Demographics			
Prenatal Care **	20 (100%)	19 (95%)	1.0
Diabetes **	1 (5%)	2 (10%)	1.0
Hypertension **	4 (20%)	7 (35%)	0.48
Preeclampsia **	6 (30%)	1 (5%)	0.09
Smoking **	2 (10%)	3 (15%)	1.0
Multi-parity **	4 (20%)	12 (60%)	0.02
Steroids **	20 (100%)	19 (95%)	1.0
Indomethacin **	3 (15%)	5 (25%)	0.69
Chorioamnionitis	10 (50%)	10 (50%)	1.0
Interventions			
Umbilical Artery Catheter	12 (60%)	13 (65%)	0.74
Indomethacin	9 (45%)	11 (55%)	0.53
Surfactants	14 (70%)	12 (60%)	0.51

* Non-normally distributed continuous data expressed as median (25%,75%) and evaluated using the Mann-Whitney U test. Otherwise, the t-test with the Satterthwaite correction was used.

** Fisher's exact test was used when cell counts < 5. Otherwise the chi-square test was used.

Table 2

Metabolites that differentiated no-AKI and AKI urinary profiles. The positive fold change means the peak intensity of AKI is higher than non-AKI. Two bins were not identified through the Chenomx library matching; because they were deemed significant and were high VIP; future studies will involve identifying these metabolites by 2D NMR techniques.

Library Matched Bins	VIP	P-value	Fold Change*
Hippurate, Homovanillate	4.7	0.04	-2.1
Unknowns (2 bins)	1.5 – 3.7	0.07 – 0.22	(-9.0) – (-1.4)
Acetate, Lysine	3.2	0.14	-1.3
N-acetyltyrosine (4 bins)	1.6 – 2.0	0.07 – 0.17	(-1.5) – (-1.2)
Cholate	1.7	0.06	2.6
Benzoate	1.6	0.06	-4.8
Myo-inositol	1.4	0.28	-1.1
Xanthine	1.2	0.06	2.9
Choline	1.1	0.08	1.2
1,7-Dimethylxanthine, Hippurate	1.1	0.11	1.7
Sugars	1.1	0.11	-1.6
Tyrosine	1.0	0.10	1.4

Table 3

The area under the ROC Curve and the 95% confidence intervals for the logistic regression models predicting AKI diagnosis

	Area Under the ROC Curve (95% CI)
Model	
pH Only	0.68 (0.53, 0.82)
Metabotype Only	0.93 (0.85, 1.0)
pH+ Metabotype	0.93 (0.85, 1.0)

Table 4

Library matched metabolites that are important to the correlation (VIP = 1.0) of gestational age (days) for infants without an AKI diagnosis OPLS regression.

VIP	Library Matched Bins
3.62	Gluconate, Unknown
3.47	Creatine, Glycolate, Hippurate, Lactose, Unknown
2.88	Glycine, myo-Inositol
2.71	Creatine, Creatinine, Lysine, 4-Hydroxyphenyllactate
2.33	Proline
1.87	Methionine, Glutamine, Glutamate
1.73	Threonate, Unknown
1.71	Taurine, TMAO, Betaine, Arginine, Glucose
1.67	myo-Inositol
1.60	Lactose, Threonine
1.52	Lysine
1.42	Succinylacetone
1.38	Glutamine, Glutathione
1.38	Dimethylamine, Unknown
1.34	Arginine, Unknown
1.34	Unknown, Lactose
1.27	Unknown
1.26	trans-4-Hydroxy-L-proline
1.23	Leucine, Isoleucine
1.15	Lysine
1.04	Ureidopropionic acid
1.03	Unknown
1.02	Proline
2.00 – 3.06	Unknown (4 bins)

Table 5

Library matched metabolites that are important to the correlation of gestational age (days) for infants with an AKI diagnosis based on OPLS regression.

VIP	Library Matched Bins
5.86	Propylene glycol, Unknown
4.80	Propylene glycol, Glucose
3.52	Betaine, Propylene glycol, Glucose
3.45	Glucose, Proline
2.49	4-Hydroxyphenyllactate, N-Acetyltyrosine
2.22	Ethylene glycol, Gluconate
2.18	Glucose
2.06	Acetic acid, Acetylphosphate
1.87	Taurine, TMAO, Betaine, Arginine, Glucose
1.83	Glucose, Lactose, Glucuronate
1.57	Threonate, Unknown
1.41	1-Methylnicotinamide, lactose
1.24 – 1.26	4-Hydroxyphenyllactate (2 bins)
1.23	2-Hydroxybutyrate, Unknown
1.15	Dimethylamine, Unknown
1.11	Lactose, Threonine
1.03	N-Acetyltyrosine
1.01	Ethanolamine
1.00	N-Acetyltyrosine

Table 6

Library matched metabolites that were correlated to gestational age for all neonates, unique to neonates with an AKI diagnosis, and unique to neonates without an AKI diagnosis.

All Neonates	Unique to AKI	Unique to No AKI
Lactose	1-Methylnicotinamide	Ureidopropionic acid
Threonine	2-Hydroxybutyrate	Creatine
Lysine	4-Hydroxyphenyllactate	Dimethylamine
N-Acetyltyrosine	Acetic acid	Glutamine
Taurine	Ethanolamine	Glycine,
TMAO	Ethylene glycol	Leucine,
Betaine	N-Acetyltyrosine	Methionine
Arginine	Acetylphosphate	myo-Inositol
Glucose	Propylene glycol	Succinylacetone
Threonate	Dimethylamine	trans-4-Hydroxy-L-proline
Proline	Glucuronate	4-Hydroxyphenyllactate
Gluconate		Creatinine
		Hippurate
		Glycolate
		Glutathione
		myo-Inositol
		Isoleucine
		Glutamate