We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Metabolomics in Neonatology

Mina H. Hanna and Patrick D. Brophy

Additional information is available at the end of the chapter

Abstract

Throughout recent decades, the incidence of preterm birth has risen worldwide, and although the majority of preterm neonates now survive infancy, many suffer from debilitating morbidities in the short term and/or increased disease risks in the long term. Traditional diagnostic biomarkers suffer from considerable confounders, limiting their use in the early identification of diseases. There is a need to develop novel biomarkers that can identify, in real time, the evolution of organ dysfunction in an early diagnostic, monitoring, and prognostic fashion. Use of "omics," particularly metabolomics, may provide valuable information regarding functional pathways underlying different pathologies and prediction of clinical outcomes. The emerging knowledge generated by the application of metabolomics in neonatology provides new insights that can help to identify markers of early diagnosis, disease progression, response to treatment, and new therapeutic targets. In this chapter, we review the current knowledge of different metabolomics technologies in neonatal-perinatal medicine, including biomarker discovery, defining as yet unrecognized biologic therapeutic targets, and linking of metabolomics to relevant standard indices and long-term outcomes.

Keywords: metabolomics, biomarkers, personalized healthcare

1. Introduction

"Omics" refers to the collective technologies used to explore the roles, relationships, and actions of the various types of molecules that make up the phenotype of an organism. Living systems complexity and adaptiveness can be read through self-organized highly interconnected networks whose interacting components are dynamically coordinated in hierarchical patterns. Systems biology is a scientific discipline that endeavors to quantify all of the molecular elements of a biological system to assess their interactions and to integrate that information into network models. Therefore, systems biology reflects the knowledge acquired by omics in a meaningful manner [1].

INTECH
open science | open minds

From genes to metabolites, the omics technologies have progressed significantly in the medical field over the last decade secondary to the remarkable advancement in laboratory methodologies and analytical tools. We discuss in this chapter the current knowledge of metabolomics technologies in neonatal-perinatal medicine including biomarker discovery, defining as yet unrecognized biologic therapeutic targets, and linking of metabolomics to relevant standard indices.

2. Metabolomics technologies

The two major methodologies applied in metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Both techniques can deliver high sensitivity, selectivity, and throughput data with high degree of reproducibility [2]. NMR spectroscopy is a quantitative, nondestructive, reproducible technique that provides detailed information on solution-state molecular structures, based on atom-centered nuclear interactions. The advantage of applying NMR is that it uses the magnetic properties of atomic nuclei, delivering simultaneous information on both the structure and molecular mobility of metabolites without the need for the preselection of analytical parameters or sample derivatization procedures. However, sensitivity is a limiting factor and often metabolite concentrations in the range of 1–10 μmol/L are required for detection and quantification by NMR [3]. Mass spectrometry analytical platforms tend to have much higher sensitivity, enabling extensive assessment of different metabolites in biological fluids or tissues samples. **Figure 1** illustrates the flow of the processes of metabolomics.

The first step is sample collection, consistency in collection and processing through standard operating procedures is important to avoid iatrogenic biases. Variables to consider in this step: (1) circadian variation and time of collection during the day, (2) nutritional impact, and (3) gestational age at birth and postnatal days of life. Following collection, samples may be stored for extended periods of time. However, metabolites stability over time should be a part of quality control measurements in conjunction with analytical variability. Prior to analysis, the samples have to be extracted into a suitable solvent using chromatography, commonly used method is either gas or liquid chromatography (GC or LC) followed by ionization in a fluid or matrix; and subsequently, metabolites are identified using a mass spectrometer on the basis of their mass-to-charge ratio (m/z) and their representation in the spectrum. Metabolite identification in MS is destructive based on fragmentation patterns either through the measurement of molecular mass (indicative of the molecular formula) or by collection of fragmentation mass spectra (indicative of molecular structure). Therefore, the application of this technology has the advantage of identifying novel metabolites not previously described in databases. On the other hand, ion suppression in complex biological samples limits the ability to quantify metabolites secondary to the interaction of multiple analytes that are present in the ionization source at the same time [4, 5].

Metabolome characterization can be performed in a targeted manner, or in a nontargeted (pattern-recognition) manner. The target method implicates identification and quantification of specific metabolites in a given biofluid or tissue extract by comparing the spectrum of
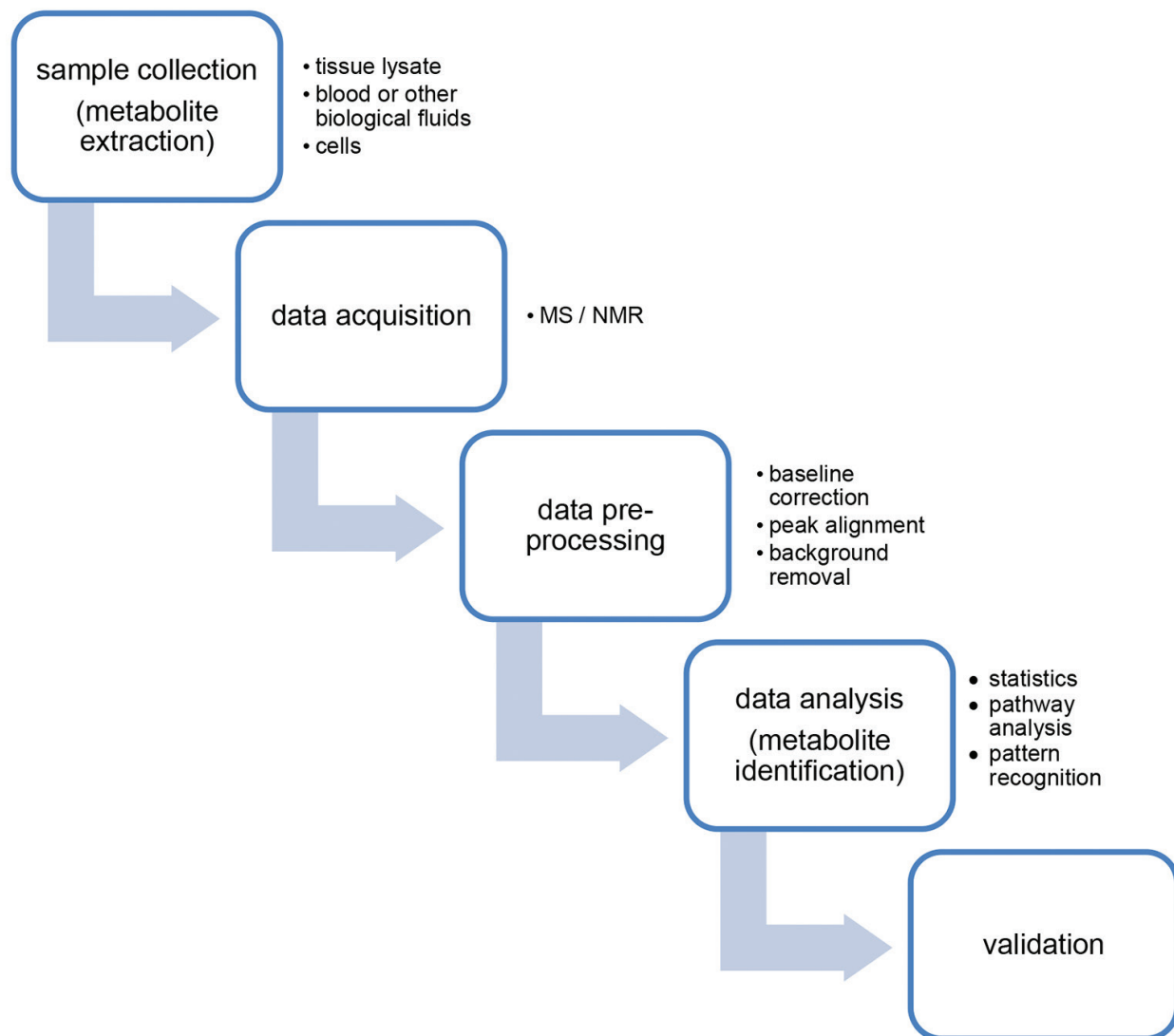
Figure 1. Metabolomics work flow.

interest to a library of reference spectra of pure compounds. This approach may suffer from an inherent bias as it captures only a part of the metabolome. Alternatively, the global nontargeted approach serves as a hypothesis-generating unbiased tool running as a first screening assay in clinical biomarker discovery studies, followed by targeted analysis for the metabolites that show significant differences or changes. The global pattern-recognition method can also screen for a multitude of key compounds in specific metabolic pathways which provide valuable information for metabolic fingerprinting.

The vast amount of data generated by metabolomics methods provides a unique opportunity to investigate alterations in metabolic pathways in response to changes in the cellular environment, and/or disease conditions. However, the high complexity of this data introduces a challenging aspect of data analysis that requires careful use of statistical methodologies and computational tools for efficient data visualization and analysis. Metabolic pathway analy-

sis implies integration of the identified metabolites into metabolic correlation networks in order to better understand the complex relationships among various metabolites. Therefore, it allows researchers to correlate observed chemometric changes to the underlying pathological mechanisms.

3. Metabolomics in neonatology

3.1. Preterm birth and postnatal maturation

Preterm birth represents the aggregation of heterogeneous phenotypes, it is a complex disorder caused by multifactorial influences and the interplay of numerous risk factors.

Metabolomic profiling of amniotic fluid was able to distinguish patients who delivered at term from patients who delivered preterm. A decrease in carbohydrates was associated with preterm delivery in the presence or absence of inflammation whereas an increase in amino acid metabolites was a unique feature of preterm labor with inflammation [6].

Wilson et al. examined the associations between the degree of prematurity and the levels of amino acids, enzymes, and endocrine markers in a large cohort of infants. They concluded that children at different stages of prematurity are metabolically distinct [7]. Similarly Atzori et al. found that metabolomic analysis revealed distinct urinary metabolic profiles in neonates of different gestational ages, suggesting that gestational age has a strong effect on the metabolic profile of neonates, and applying this technology may predict the post-maturation of preterm and term neonates [8]. Furthermore, metabolomic analysis showed significant alterations in three metabolic pathways: (1) arginine and proline; (2) urea cycle; and (3) glycine, serine, and threonine between neonates with intrauterine growth restriction (IUGR) and controls [9].

3.2. Maternal chorioamnionitis and preeclampsia

The application of metabolomics methods has shown a clear distinction between preterm infants born to mothers with histological chorioamnionitis (HCA) from those born to mothers without HCA. Metabolites discriminating were the following: mannitol, 4-hydroxyphenylacetate, p-cresol, myo-inositol, trimethylamine-N-oxide, and 1-methylnicotinamide [10]. Similarly, metabolomics has the potential to identify changes under clinical conditions, such as preeclampsia (PE), that are associated with placental molecular pathophysiology. Heazell et al. have demonstrated that placental tissue from uncomplicated pregnancies cultured in 1% oxygen (hypoxia) had metabolic similarities to explants from preeclampsia pregnancies cultured at 6% oxygen (normoxia). This group of metabolites includes prostaglandins, a number of long-chain fatty acids and several amino acids [11]. Metabolic footprinting offers a hypothesis-generating strategy to investigate factors absorbed by and released from the placenta. Horgan et al. analyzed the metabolic footprint of placental villous explants cultured at different oxygen tensions between women who deliver a small for gestational age (SGA) baby and those from normal controls. SGA explant media cultured under hypoxic conditions

was noted, on a univariate level, to exhibit the same metabolic signature as controls cultured under normoxic conditions for 49% of the metabolites of interest, suggesting that SGA tissue is acclimatized to hypoxic conditions *in vivo* [12].

3.3. Respiratory distress syndrome and bronchopulmonary dysplasia

Respiratory distress syndrome (RDS), formerly also known as hyaline membrane disease, is a common problem in preterm newborn infants. Surfactant deficiency or inactivation is a major contributing factor for the development of RDS. Metabolic profiling of bronchoalveolar lavage fluid (BALF) is a promising tool for assessing novel biomarkers of RDS in preterm infants. Applying GC-MS based metabolomic analysis revealed 10 metabolites that are over-expressed in BALF collected during mechanical ventilation following surfactant administration [13].

Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease in infants with a multifactorial pathogenesis arising from a complex interaction between genetic and environment factors. Comparing the urinary metabolic profiles at birth of preterm neonates, Fanos et al. found five discriminant metabolites: lactate, taurine, trimethylamine-N-oxide (TMAO), myo-inositol (which increased in BPD patients), and gluconate (which was decreased) [14]. The increase in urinary lactate in the BPD group may represent a process of anaerobic respiration. Taurine and TMAO have anessential biological role for osmoregulation and membrane stabilization. Additionally, taurine has essential roles in calcium homeostasis, renal cell cycle and apoptosis, nerve cell activity and detoxification [15]. The data emerging from this study provide better insights into the pathophysiological mechanisms of BPD development.

3.4. Hypoxic ischemic encephalopathy

Hypoxic ischemic encephalopathy (HIE) is a complex neurological injury, characterized by biphasic depletion in high energy phosphates, with an estimated incidence of two per 1000 deliveries. Walsh et al. performed metabolomic analysis on umbilical cord blood from newborns that were divided into three groups: those with confirmed HIE ($n = 31$), asphyxiated infants without encephalopathy ($n = 40$) and matched controls ($n = 71$). Targeted metabolomic analysis showed a significant alteration between study groups in 29 metabolites from 3 distinct classes (amino acids, acylcarnitines, and glycerophospholipids). A logistic regression model using five metabolites clearly delineates severity of asphyxia andclassifies HIE infants with area under the curve (AUC) = 0.92 [16].

3.5. Necrotizing enterocolitis/late onset sepsis

Necrotizing enterocolitis (NEC) and late onset sepsis (LOS) are the leading causes of death among preterm infants. Stewart et al. compared the serum proteomic and metabolomic profiles longitudinally in preterm infants with NEC or LOS, matched to controls. While no single protein or metabolite was detectedin all NEC or LOS cases which was absent in controls; several proteins were identified which were associated with disease status. The expression of these proteins generally varied between diseased infants, potentially relating to differing pathophysiology ofdisease [17]. Similarly, Wilcock et al. found metabolomic

differences in preterm babies at risk of NEC. However, sample sizes were insufficient to confidently identify a biomarker. Additionally, network modeling of preterm and term metabolomes suggested possible nutritional deficiency and altered pro-insulin action in preterm babies [18].

3.6. Neonatal kidney injury

Acute Kidney Injury (AKI) is common in neonates undergoing cardiac surgery, and is associated with increased mortality and ICU length of stay [19]. Mass spectrometry-based metabolomics was used in a prospective cohort of pediatric cardiac surgery patients ($n = 40$). Twenty-one of these children developed acute kidney injury defined as an increase in serum creatinine concentrations 50% or greater from baseline after 48–72 h. Homovanillic acid sulfate (HVA-SO$_4$), a dopamine metabolite was identified as a marker indicating AKI with 90% sensitivity and 95% specificity using a cutoff value of 24 ng/ml at 12 h after surgery [20]. Atzori et al. showed a correlation between urinary metabolic profiles and neutrophil gelatinase-associated lipocalin (NGAL) concentration in a cohort of young adults born with extremely low-birth weight (ELBW), using partial least-squares discriminant analysis [21].

3.6.1. Drug-induced nephrotoxicity

Nephrotoxic-medications are becoming increasingly recognized as a common and potentially modifiable cause of AKI in neonates. In a single center retrospective cohort 87% of very low birth weight infants (VLBW) were exposed to at least one nephrotoxic medication and on average these neonates were exposed to 14 days of nephrotoxic medications during their NICU stay [22]. Early identification of renal injury through omics technologies implicates defining different biomarkers that rely on the mechanisms of toxicity of each drug or drug class [23]. In our experimental study, gentamicin-induced acute kidney injury in newborn rats resulted in a distinct urinary metabolic profile characterized by glucosuria, phosphaturia, and aminoaciduria that preceded changes in serum creatinine. Additionally, lower levels of kynurenic acid were noted in the urine of gentamicin injected rats, coinciding with higher levels of tryptophan, suggesting a degrading effect of gentamicin toxicity on tryptophan metabolism pathway [24]. Xu et al. applied integrated pathway analysis and metabolite-transcript correlation analysis to define perturbed biochemical pathways and molecular functions that may be relevant to the mechanisms of nephrotoxicity. They concluded that transcriptional downregulation of luminal sodium-dependent transporters SLC5A1, SLC5A2, SLC6A18, and SLC16A7 might be the central mediators of drug-induced kidney injury and adaptive response pathways. The integrated pathway analysis performed on these studies indicates that cisplatin- or gentamicin-induced renal Fanconi-like syndromes manifested by glucosuria, hyperaminoaciduria, lactic aciduria, and ketonuria might be better explained by the reduction of functional proximal tubule transporters rather than by the perturbation of metabolic pathways inside kidney cells [25].

An alternative approach implicatesdiscoveryof a limited number of biomarkers that identify injury specific to primary sites in the kidney, such as the glomerulus or the proximal tubule. A prospective observational trial showed that the urinary excretion of biomarkers that signify

proximal tubular damage was higher in the gentamicin group compared with control and preceded the peak of SCr and urine output decrease [26]. The application of different omics technologies *in vitro* systems and preclinical models to predict nephrotoxicity allows testing of the safety and efficacy of novel therapies and enhances the development and implementation of new drugs.

Askenazi et al. demonstrated that urinary biomarkers can predict AKI and mortality in very low birth weight infants independent of gestational age and birth weight [27]. We found that urinary NGAL, osteopontin(OPN) and cystatin C (Cys C) increased significantly in infants who developed AKI, in contrast, urinary epidermal growth factor (EGF) and uromodulin (UMOD) decreased significantly in this group. Urinary biomarkers demonstrated a significant change 24 h prior to contemporary creatinine-based neonatal AKI definition [28]. It is particularly important to recognize the differences in omics biomarkers across different gestational ages, postnatal days, and fluid balance status when designing future validation studies [29–32].

4. Future directions

The application of metabolomics approaches in neonatology is currently experimented on different platforms due to its unique ability to generate functional readouts of systems biology, setting the ground for future personalized prenatal, neonatal, and pediatric care. Yet the clinical translation of this unprecedented large amount of data into clinical practices for neonatal health care requires addressing of the inherent interindividual variability [33]. Metabolomics has the greatest potential in the field of biomarker discovery because this technique defines the signature of the actual processes that are occurring within the body rather than just merely examining compounds (such as untranscribed DNA or pre- or post-translationally modified proteins) that may be redundant to these processes. Although currently omics studies are mainly descriptive in nature, the goal is that through integration of experimental approaches and computational modelling, better models for personalized health care delivery will be generated. The following stages delineate how to translate the biomarker(s) discovery association studies into clinical applications in a stepwise approach:

1. Defining a clear clinical question

2. Selection of appropriate patient group(s), samples, and clinical data collection

3. Identification of specific biomarkers

4. Validation in a separate group of patients/samples

5. Validation on a large scale

6. Delineation of the interaction of nutrition, gene expression, and metabolism throughintegration of pathophysiology using pathway analysis tools

Experimental work in model systems and integration with other omics approaches are essential steps to provide insight into the pathophysiologic interactions between selected

biomarkers and disease pathogenesis. Finally, large epidemiological cohort studies are needed to assess whether metabolomic biomarkers improve upon existing disease markers and to determine the validity of their application in different clinical settings.

5. Summary

The rapidly expanding field of metabolomics has been driven in recent years byadvances in the analytical methods. Metabolomics will have major implications in the field of personalized health care in the future. After establishing metabolomic profiles in the neonatal population, the next step is metabolic fingerprinting. In such metabolomic investigations, the intention is not to identify each observed compound but to compare patterns or fingerprints of metabolites that change in response to disease or drug exposure. The combination of metabolic profiling and fingerprinting will lead to the maximum utilization of metabolomics. In one approach, changes in fingerprints correlating with metabolite profiles may be linked to a physiological or pathological state. As more quantitative metabolomic databases evolve, they can be integrated with data sets from the other "omics" technologies to enhance the data value and provide greater biological insight than anyone "omics" technique alone can offer. The promise of this emerging technology is focusing on translational metabolomics for the identification of biomarkers, monitoring postnatal metabolic maturation, and the implementation of a tailored management of neonatal disorders.

Author details

Mina H. Hanna[1]* and Patrick D. Brophy[2]

*Address all correspondence to: mina.hanna@uky.edu

1 Department of Pediatrics, University of Kentucky, Lexington, KY, USA

2 Department of Pediatrics, University of Iowa, Iowa City, IA, USA

References

[1] Pesce F, Pathan S, Schena FP. From -omics to personalized medicine in nephrology: integration is the key. Nephrol Dial Transplant 2013;28:24–8.

[2] Lindon JC, Nicholson JK. Spectroscopic and statistical techniques for information recovery in metabonomics and metabolomics. Annu Rev Anal Chem (Palo Alto Calif) 2008;1:45–69.

[3] Schnackenberg LK, Beger RD. Monitoring the health to disease continuum with global metabolic profiling and systems biology. Pharmacogenomics 2006;7:1077–86.

[4] Pan Z, Raftery D. Comparing and combining NMR spectroscopy and mass spectrometry in metabolomics. Anal Bioanal Chem 2007;387:525–7.

[5] Feng X, Liu X, Luo Q, Liu BF. Mass spectrometry in systems biology: an overview. Mass Spectrom Rev 2008;27:635–60.

[6] Romero R, Mazaki-Tovi S, Vaisbuch E, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med 2010;23:1344–59.

[7] Wilson K, Hawken S, Ducharme R, et al. Metabolomics of prematurity: analysis of patterns of amino acids, enzymes, and endocrine markers by categories of gestational age. Pediatr Res 2014;75:367–73.

[8] Atzori L, Antonucci R, Barberini L, et al. 1H NMR-based metabolomic analysis of urine from preterm and term neonates. Front Biosci (Elite Ed) 2011;3:1005–12.

[9] Dessi A, Atzori L, Noto A, et al. Metabolomics in newborns with intrauterine growth retardation (IUGR): urine reveals markers of metabolic syndrome. J Matern Fetal Neonatal Med 2011;24 Suppl 2:35–9.

[10] Syggelou A, Iacovidou N, Atzori L, Xanthos T, Fanos V. Metabolomics in the developing human being. Pediatr Clin North Am 2012;59:1039–58.

[11] Heazell AE, Brown M, Worton SA, Dunn WB. Review: the effects of oxygen on normal and pre-eclamptic placental tissue--insights from metabolomics. Placenta 2011;32 Suppl 2:S119–24.

[12] Horgan RP, Broadhurst DI, Dunn WB, et al. Changes in the metabolic footprint of placental explant-conditioned medium cultured in different oxygen tensions from placentas of small for gestational age and normal pregnancies. Placenta 2010;31:893–901.

[13] Fabiano A, Gazzolo D, Zimmermann LJ, et al. Metabolomic analysis of bronchoalveolar lavage fluid in preterm infants complicated by respiratory distress syndrome: preliminary results. J Matern Fetal Neonatal Med 2011;24 Suppl 2:55–8.

[14] Fanos V, Pintus MC, Lussu M, et al. Urinary metabolomics of bronchopulmonary dysplasia (BPD): preliminary data at birth suggest it is a congenital disease. J Matern Fetal Neonatal Med 2014;27 Suppl 2:39–45.

[15] Piersigilli F, Bhandari V. Biomarkers in neonatology: the new "omics" of bronchopulmonary dysplasia. J Matern Fetal Neonatal Med 2016;29:1758–64.

[16] Walsh BH, Broadhurst DI, Mandal R, et al. The metabolomic profile of umbilical cord blood in neonatal hypoxic ischaemic encephalopathy. PLoS One 2012;7:e50520.

[17] Stewart CJ, Nelson A, Treumann A, et al. Metabolomic and proteomic analysis of serum from preterm infants with necrotising entercolitis and late-onset sepsis. Pediatr Res 2016;79:425–31.

[18] Wilcock A, Begley P, Stevens A, Whatmore A, Victor S. The metabolomics of necrotising enterocolitis in preterm babies: an exploratory study. J Matern Fetal Neonatal Med 2016;29:758–62.

[19] Alabbas A, Campbell A, Skippen P, Human D, Matsell D, Mammen C. Epidemiology of cardiac surgery-associated acute kidney injury in neonates: a retrospective study. Pediatr Nephrol 2013;28:1127–34.

[20] Beger RD, Holland RD, Sun J, et al. Metabonomics of acute kidney injury in children after cardiac surgery. Pediatr Nephrol 2008;23:977–84.

[21] Atzori L, Mussap M, Noto A, et al. Clinical metabolomics and urinary NGAL for the early prediction of chronic kidney disease in healthy adults born ELBW. J Matern Fetal Neonatal Med 2011;24 Suppl 2:40–3.

[22] Rhone ET, Carmody JB, Swanson JR, Charlton JR. Nephrotoxic medication exposure in very low birth weight infants. J Matern Fetal Neonatal Med 2014;27:1485–90.

[23] Bonventre JV, Vaidya VS, Schmouder R, Feig P, Dieterle F. Next-generation biomarkers for detecting kidney toxicity. Nat Biotechnol 2010;28:436–40.

[24] Hanna MH, Segar JL, Teesch LM, Kasper DC, Schaefer FS, Brophy PD. Urinary metabolomic markers of aminoglycoside nephrotoxicity in newborn rats. Pediatr Res 2013;73:585–91.

[25] Xu EY, Perlina A, Vu H, et al. Integrated pathway analysis of rat urine metabolic profiles and kidney transcriptomic profiles to elucidate the systems toxicology of model nephrotoxicants. Chem Res Toxicol 2008;21:1548–61.

[26] Jansen D, Peters E, Heemskerk S, et al. Tubular injury biomarkers to detect gentamicin-induced acute kidney injury in the neonatal intensive care unit. Am J Perinatol 2016;33:180–7.

[27] Askenazi DJ, Montesanti A, Hunley H, et al. Urine biomarkers predict acute kidney injury and mortality in very low birth weight infants. J Pediatr 2011;159:907–12 e1.

[28] Hanna M, Brophy PD, Giannone PJ, Joshi MS, Bauer JA, RamachandraRao S. Early urinary biomarkers of acute kidney injury in preterm infants. Pediatr Res 2016;80(2):218–23.

[29] Saeidi B, Koralkar R, Griffin RL, Halloran B, Ambalavanan N, Askenazi DJ. Impact of gestational age, sex, and postnatal age on urine biomarkers in premature neonates. Pediatr Nephrol 2015;30:2037–44.

[30] Askenazi DJ, Koralkar R, Levitan EB, et al. Baseline values of candidate urine acute kidney injury biomarkers vary by gestational age in premature infants. Pediatr Res 2011;70:302–6.

[31] Askenazi D, Saeidi B, Koralkar R, Ambalavanan N, Griffin RL. Acute changes in fluid status affect the incidence, associative clinical outcomes, and urine biomarker performance in premature infants with acute kidney injury. Pediatr Nephrol 2016;31:843–51.

[32] Tsukahara H, Sugaya T, Hayakawa K, et al. Quantification of L-type fatty acid binding protein in the urine of preterm neonates. Early Hum Dev 2005;81:643–6.

[33] Hanna MH, Dalla Gassa A, Mayer G, et al. The nephrologist of tomorrow: towards a kidney-omic future. Pediatr Nephrol 2016.