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Metabolic Signature of Chronic Kidney Disease: the search continues

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According to the recent United States Renal Data System (USRDS) report, Chronic kidney disease (CKD) afflicts approximately 15% of individuals in the United States and associated with substantial morbidity and economic costs with elevated risk of cardiovascular disease, end-stage renal disease (ESRD), and mortality, even with modest decreases in glomerular filtration rate (GFR; ref (1)). Current classification and treatment strategy of CKD primarily relies on a rather imprecise clinical phenotyping, including estimates of GFR and markers of kidney damage, such as albuminuria. Therefore, robust markers to detect patients at risk for CKD, predicting CKD trajectory and response to therapeutic interventions are needed. In the past two decades, a new field of science called “systems biology” has emerged. The goal of systems biology is to characterize a biological system on a gene (genomics), transcript (transcriptomics), protein (proteomics), or metabolite (metabolomics) level. Initial large-scale genome-wide association (GWAS) and transcriptional- based studies are more recently being followed by mass spectrometry (MS)–based proteomic and metabolomics. An advantage of MS analyses lies in the fact that such analysis can be carried out in readily available noninvasively collected biofluids (plasma and urine). As such, the MS-based platforms are more amenable to biomarker discovery and translation into clinical practice. The goal of metabolomics is to comprehensively identify and quantify all endogenous and exogenous small molecule metabolites (typically < 1 kDa in size) in a biological system. In essence it provides a snap shot of the metabolic status of the organism. To date >10,000 small molecules have been identified, providing a rich resource for biomarker discovery. One of the challenges of doing metabolomic studies is the complexity of the metabolome itself. The metabolome contains a wide variety of chemically diverse compounds such as lipids, organic acids, carbohydrates, amino acids, nucleotides and steroids among others (2). Another issue is that metabolites occur in a wide dynamic range of concentrations (nanomolar to millimolar) in the human body. Finally, the metabolome can be influenced by exogenous metabolites obtained from food or medications which also may not be uniform in each subject. Therefore, it is evident that comprehensive metabolomics is an analytical challenge. Research groups design metabolomic experiments with either an untargeted or targeted approach. In untargeted metabolomics, the goal is to detect as many metabolites as possible in a sample, in order to classify phenotypes based on a metabolite pattern. Meanwhile, in targeted metabolomics which is also known as “metabolic profiling”, the focus is limited to either a pre-determined set of metabolites or a specific chemical class of small molecules. Unequivocal identification and absolute quantitation can be performed in a targeted experiment and can provide both mechanistic information and new hypothesis in a given biological system. Indeed, such strategies have been used by several groups including ours to effectively to predict cancer, diabetes, obesity and cardiovascular disease phenotypes (3-7)

In this issue of AJKD, Goek et al examine the metabolic profile associated with decreased estimated GFR (eGFR) in a large general population using a targeted approach (8). The authors utilize two distinct cross-sectional study populations of ~ 4000 subjects (KORA F4 and TwinsUK study) and quantify 151 serum metabolites and relate these metabolites to the eGFR. Utilizing multiple robust statistical models, they identify 22 unique acyl carnitines and 516 metabolite ratios which are associated with eGFR with glutaryl carnitine and serine/ glutaryl carnitine ratio showing the highest statistical association. There are several strengths to this study. This is the first study that associates metabolic profiles with decreased GFR in a large cohort. An important strength of this work is validation of the initial metabolic signature in a second cohort. Second, the metabolic profile correlates with level of eGFR irrespective of etiology of CKD. This might implicate the metabolites in common pathways of disease progression or those cleared by the kidney. The results validate independent studies by other groups which implicate acyl carnitines in CKD (9, 10). However there are some limitations to this study. The cross-sectional nature of the study limits temporal association with eGFR level and the measured metabolites. Second, the eGFR estimates are less precise at values greater than 60 (11). Third, patients with CKD are likely to have different systemic and kidney diseases and prevalence of individual disease processes such as diabetes in the sampling pool could skew inferences made on the metabolism of the entire pool. Therefore, future studies are warranted in disease specific CKD cohorts with lower eGFR and longitudinal follow-up.

Several recent studies have used similar metabolomic strategies in CKD. We recently utilized an untargeted metabolomic strategy to explore underlying mechanisms of progressive murine diabetic nephropathy (DN; ref (12)) defining metabolic changes that accompany DN phenotype and reversal with therapy. Three clinical studies highlight potential application of metabolomics into DN research. The study from Zhang and colleagues utilized Ultra performance liquid chromatography (UPLC) coupled with orthogonal acceleration time-of-flight mass spectrometry (oaTOF-MS) to distinguish the global serum profiles from healthy volunteers, type 2 diabetics and DN patients (13). Significant changes in the serum levels of leucine, dihydrosphingosine and phytosphingosine were noted, indicating perturbations of amino acid metabolism and phospholipid metabolism. A second study used the FinnDiane Cohort in an attempt to find novel markers for DN. 52 type 1 diabetic patients were recruited by the FinnDiane study that had normal albumin excretion rate (AER). After an average of 5.5 years of follow-up, one-half of the subjects progressed from normal to abnormal AER (microalbuminuria or macroalbuminuria), and the other half remained normoalbuminuric. Metabolite profiles of baseline 24 hour urine samples were obtained by gas chromatography (GC) and liquid chromatography (LC) MS and multivariate logistic regression modeling revealed a profile of metabolites that separated those patients who progressed to abnormal albuminuria from those who did not with an accuracy of 75% and a precision of 73%. The discriminating metabolites included acyl-carnitines, acyl-glycines and metabolites related to tryptophan metabolism (14). In a related study, Xia et al analyzed plasma samples from 88 DN subjects by MS and noted differential regulation of four metabolites involved in nucleotide metabolism including adenosine, inosine, uric acid and xanthine (15). Other groups have investigated uremic toxin profiling in patients with kidney failure. The pattern of lipids was notable for a universal decrease in lower-molecular-weight triacylglycerols, and an increase in several intermediate-molecular-weight triacylglycerols in ESRD compared with controls using a LC/MS platform. These observations suggest disturbed triglyceride catabolism and/or beta-oxidation in ESRD and broad catabolic state including glycolysis, lipolysis, ketosis, and nucleotide breakdown (16). Finally, with the explosion of the other -omics platforms, integration of all the high throughput data becomes crucial. Several groups are investigating integration of GWAS data to other -omics platforms, e.g. transcriptomic or metabolomic data-sets (expression/metabolic Quantitative Trait Locus, eQTL/mQTL), from either normal

or diseased tissue (17). Recent work shows integration of metabolic and GWAS data supporting the feasibility of this approach (18-20) and should be a roadmap for future studies.

In conclusion, the search for new markers to better stratify patients with CKD according to the progression risk is ongoing. The ideal CKD marker should be readily measured in bio fluids like plasma and urine and be cost effective. Levels should be altered early, remain altered over wide range of disease, and be able to change with medications to monitor therapeutic response. The diagnostic tests should be highly specific and sensitive with basal levels allowing risk stratification for future progression. The ideal marker should be unaffected by demographics and clinical factors. While no single marker is likely to meet these criteria, panels of markers derived from multiple -omics platforms are likely to better prognosticate renal disease progression. It is important to determine whether the newly identified marker panels are purely associations or markers of underlying pathophysiological processes. Validating markers prospectively in large populations over extended follow-up periods with accurate clearance measurements and outcome measures such as the development of kidney failure is required before adaptation to real time clinical practice. Although advances in -omics technologies, and analysis have greatly improved, biomarker discovery and validation remain an expensive and daunting undertaking.

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