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Lipidomic Signature of Progression of Chronic Kidney Disease in the Chronic Renal Insufficiency Cohort

Farsad Afshinnia[1], Thekkelnaycke M. Rajendiran[2,3], Alla Karnovsky[3,4], Tanu Soni[3], Xue Wang[5], Dawei Xie[5], Wei Yang[5], Tariq Shafi[6], Matthew R. Weir[7], Jiang He[8], Carolyn S. Brecklin[9], Eugene P. Rhee[10], Jeffrey R. Schelling[11], Akinlolu Ojo[1], Harold Feldman[5], George Michailidis[12] and Subramaniam Pennathur[1,3,4], and the CRIC Study Investigators[13]

[1]Division of Nephrology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA; [2]Department of Pathology, University of Michigan, Ann Arbor, Michigan, USA; [3]Division of Bioinformatics, Michigan Regional Comprehensive Metabolomics Resource Core, Ann Arbor, Michigan, USA; [4]Department of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA; [5]Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [6]Department of Internal Medicine–Nephrology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [7]Division of Nephrology, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland, USA; [8]Department of Epidemiology, Tulane University School of Medicine, New Orleans, Louisiana, USA; [9]Division of Nephrology, University of Illinois at Chicago, Chicago, Illinois, USA; [10]Department of Medicine–Nephrology, Massachusetts General Hospital, Boston, Massachusetts, USA; [11]Division of Nephrology, MetroHealth Medical Center, Case Western Reserve University, Cleveland, Ohio, USA; and [12]Department of Statistics, University of Michigan, Ann Arbor, Michigan, USA

Introduction: Human studies report conflicting results on the predictive power of serum lipids on the progression of chronic kidney disease. We aimed to systematically identify the lipids that predict progression to end-stage kidney disease.

Methods: From the Chronic Renal Insufficiency Cohort, 79 patients with chronic kidney disease stages 2 to 3 who progressed to end-stage kidney disease over 6 years of follow-up were selected and frequency matched by age, sex, race, and diabetes with 121 nonprogressors with less than 25% decline in estimated glomerular filtration rate during the follow-up. The patients were randomly divided into training and test sets. We applied liquid chromatography-mass spectrometry-based lipidomics on visit year 1 samples.

Results: We identified 510 lipids, of which the top 10 coincided with false discovery threshold of 0.058 in the training set. From the top 10 lipids, the abundance of diacylglycerols and cholesteryl esters was lower, but that of phosphatidic acid 44:4 and monoacylglycerol 16:0 was significantly higher in progressors. Using logistic regression models, a multimarker panel consisting of diacylglycerols and monoacylglycerol independently predicted progression. The c-statistic of the multimarker panel added to the base model consisting of estimated glomerular filtration rate and urine protein-to-creatinine ratio as compared with that of the base model was 0.92 (95% confidence interval: 0.88–0.97) and 0.83 (95% confidence interval: 0.76–0.90, $P < 0.01$), respectively, an observation that was validated in the test subset.

Discussion: We conclude that a distinct panel of lipids may improve prediction of progression of chronic kidney disease beyond estimated glomerular filtration rate and urine protein-to-creatinine ratio when added to the base model.

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Correspondence: Farsad Afshinnia or Subramaniam Pennathur, University of Michigan, 1000 Wall St., Ann Arbor, Michigan 48105, USA. E-mail: fafshin@med.umich.edu (FA) or spennath@umich.edu (SP)

[13]CRIC Study Investigators: Lawrence J. Appel, Alan S. Go, John W. Kusek, James P. Lash, and Raymond R. Townsend.

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According to the Center for Disease Control and Prevention, there are currently more than 20 million people above the age of 20 with chronic kidney disease (CKD) in the United States.[1] In spite of its public health burden, the clinical care of the patients with CKD is largely dependent on the application of traditional biomarkers including serum creatinine, urine protein-to-creatinine ratio (UPCR), and estimated glomerular

filtration rate (eGFR), which are significantly limited by their precision, accuracy, and prognostic values especially early in the course of disease.[2,3] In CKD, metabolic derangements start at early stages where these inherent deficiencies are most prominent. Such limitations necessitate a shift of paradigm from exclusive reliance on traditional biomarkers to systematic approaches for the identification of prognostic markers.

Lipids are diverse and abundant molecules with significant links to different metabolic pathways along with diverse cellular and biological functions.[4,5] In the past, lipid studies in CKD have largely been limited to studying the changes at class level of a limited number of lipids such as total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein with conflicting results in terms of the association between dyslipidemia and progression of CKD.[6–11] As a result of these limited approaches, the effect of diverse intraclass variation within these lipid classes as well as the alterations in various other classes of lipids on the progression of CKD has remained poorly understood. More recently, the use of conventional lipid measurements for the description of lipoprotein abnormalities in mild CKD has come into question.[12] On the other hand, the application of the lipidomics and/or metabolomics approach in a number of diseases such as diabetes,[13,14] cardiovascular diseases,[15] and other inflammatory processes[16] has provided characteristic lipid signatures and mechanistic insights to disease processes.[17] These studies provide proof-of-principle on the clinical applicability of the candidate metabolites for risk prediction, an approach that is rarely taken in CKD. In a recently published report, Reis *et al.*[18] have compared the lipid signature of LDL in patients at the advanced stage of CKD (stages 4 and 5) with the control group using the liquid chromatography-mass spectrometry-based lipidomics approach. To our knowledge, there is no study in CKD aimed at the identification of lipid signature predictive of incident end-stage kidney disease (ESKD) at early stages of CKD. Therefore, this study examines the systematic identification of prognostic serum lipid metabolites at CKD stages 2 and 3 to predict progression to ESKD using liquid chromatography-mass spectrometry-based lipidomics in the Chronic Renal Insufficiency Cohort (CRIC) patient population.

METHODS

Patients

This study is a case-control study nested in the core CRIC study. The design of CRIC is published previously.[19,20] CRIC is a multicenter cohort of patients with mild-to-moderate CKD, with recruitment starting in 2003 with the goals of examining risk factors for CKD and cardiovascular events, and developing predictive models that would identify high-risk subgroups. The core study has recruited 3939 subjects over a 5-year period through 2008. Inclusion criteria of the subcohort used for this study were eGFR $\geq$ 30 ml/min at visit year 1 and an age of 18 years or more with no racial or gender restriction. Cases were defined as patients who progressed to ESKD over the next 6 years of follow-up. ESKD is defined as needing chronic dialysis or having kidney transplantation. Controls were defined as patients who were frequency matched with cases by their baseline age, sex, race, and diabetes and had less than 25% decline in eGFR during the 6-year mean follow-up. One milliliter of fasting serum sample from visit year 1 as baseline was obtained from the selected subcohort. Demographic, clinical, and laboratory variables from baseline were retrieved from the corresponding patients. eGFR calculated by CKD Epidemiology Collaboration is used for multivariable adjustments.

Data Acquisition

Liquid chromatography-mass spectrometry-based shotgun lipidomics using a TripleTOF 5600 was applied for lipid identification (see the Supplementary Methods for details).

Statistical Analysis

After data acquisition, the missing values for lipids were imputed using the K nearest-neighbor method.[17,21] Then the data were log2 transformed followed by normalization using the cross-contribution compensating multiple internal standard normalization method.[22] The cohort was randomly divided into the training and test sets with a 2:1 ratio in an attempt to develop the probabilistic predictive model of multimarker panel predictive of progression in the training set followed by its validation in the test set. The compound-by-compound *t*-test was applied to identify the top differentially regulated lipids that passed the nominal threshold P value of <0.05, followed by the Benjamini-Hochberg procedure for false discovery rate (FDR) correction[23,24] accounting for multiple comparisons. In parallel, the partial least square-discriminant analysis (PLS-DA)[25,26] and Random Forest (RF)[27] classification methods were applied on the top lipids with nominal significance in the training set to generate the rank of the variable important in projection by each classification method separately (Figure 1). The rationale for using PLS-DA and RF classification methods besides the application of the Benjamini-Hochberg procedure for FDR correction was to assess concordance of the products of different classification methods and to compare if the proposed lipids by different methods differed. Then logistic regression models with