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Lipidomics Profiling Reveals Serum Phospholipids Associated with Albuminuria in Early Type 2 Diabetic Kidney Disease

Shu Ye,[⊥] Ye-peng Hu,[⊥] Qiao Zhou, Hang Zhang, Zhe-zheng Xia, Shu-zhen Zhao, Zhe Wang, Sheng-yao Wang, Xin-yi Wang, Yi-kai Zhang, Zhi-da Chen, Guang-yun Mao, and Chao Zheng*

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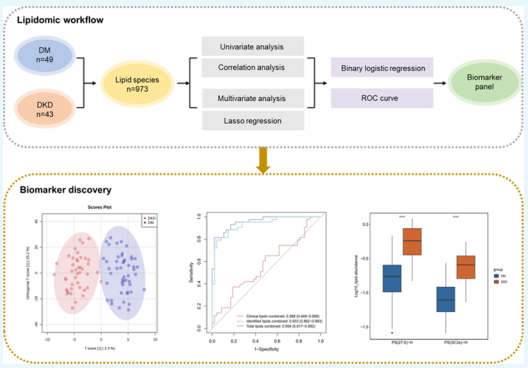
ABSTRACT: Early screening and administration of DKD are beneficial for renal outcomes of type 2 diabetic patients. However, the current early diagnosis using the albuminuria/creatine ratio (ACR) contains limitations. This study aimed to compare serum lipidome variation between type 2 diabetes and early DKD patients with increased albuminuria through an untargeted lipidomics method to explore the potential lipid biomarkers for DKD identification. 92 type 2 diabetic patients were enrolled and divided into two groups: DM group (ACR < 3 mg/mmol, n = 49) and early DKD group (3 mg/mmol ≤ ACR < 30 mg/mmol, n = 43). Fasting serum was analyzed through an ultraperformance liquid mass spectrometry tandem chromatography system (LC-MS). Orthogonal partial least-squares discriminant analysis (OPLS-DA) and univariate and multivariate analysis were performed to filter differentially depressed lipids. Receiver operating characteristic (ROC) curves were used to estimate the diagnostic capability of potential lipid biomarkers. We found that serum phospholipids including phosphatidylserine (PS), sphingomyelin (SM), and phosphatidylcholine (PC) were significantly upregulated in the DKD group and were highly correlated with the ACR. In addition, a panel of two phospholipids including PS(27:0)-H and PS(30:2e)-H showed good performance to help clinical lipids in early DKD identification, which increased the area under the curve (AUC) from 0.568 to 0.954. The study exhibited the serum lipidome variation in early DKD patients, and the increased phospholipids might participate in the development of albuminuria. The panel of PS(27:0)-H and PS(30:2e)-H could be a potential biomarker for DKD diagnosis.



1. INTRODUCTION

Diabetic kidney disease (DKD) is a major microvascular complication of type 2 diabetes and the leading cause of death from end-stage renal disease (ESRD).[1] The prognosis of diabetic kidney disease is irreversible, and its early identification is very important for diabetic patients.

The definition of DKD refers to diabetic patients with chronic kidney disease (CKD), which was mainly present as the increased albuminuria/creatine ratio (ACR) or declined estimated glomerular filtration rate (eGFR) for more than 3 months according to the KDGIO guideline.[2]

The ACR is widely regarded as an early biomarker of DKD but lacks specificity and sensitivity. Besides, proteinuria assessment has not been standardized around the world, which may hinder systematical screening in large cohorts at multiple centers.[1] Therefore, new methods were developed to seek more biomarkers for early detection of DKD.

The rapid development of various omics technologies facilitated the discovery of biological biomarkers. In proteomic studies, the CKD273 panel reported by Good et al. has been testified in multiple subsequent clinical studies to improve DKD diagnosis.[3−5] Metabolomic studies also reported that metabolites in serum or urine could help diagnose or predict DKD progression in type 2 diabetes mellitus.[6] In our previous study, a total of 11 serum metabolites were determined as potential biomarkers for DKD identification.[7] However, as a major branch of metabolomics, lipidomics has not been widely used in DKD research, although it was demonstrated that the pathogenesis of DKD including albuminuria is tightly related to dyslipidemia.[8]

Lipidomics represented a shift from individual lipid classes to whole lipid metabolites to understand the roles of lipids in the pathophysiological activities of diseases more comprehensively. Previous lipidomic findings focusing on diabetes have been reported.[9−11] However, the results of the current DKD lipidomic studies have not formed a unified conclusion, and the expression patterns of different lipid classes in early DKD are still not clear. More relevant studies are needed to

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determine and verify the potential lipid biomarkers of early diabetic kidney disease.

In this study, we conducted a widely untargeted lipidomics strategy in T2DM and DKD patients with a slightly increased ACR to elucidate the serum lipidome variation and its correlation with albuminuria. Moreover, we utilized regression analysis to identify a panel of potential lipid biomarkers for the auxiliary diagnosis of early DKD.

2. MATERIALS AND METHODS

2.1. Study Subjects. 92 T2DM patients were recruited from the Diabetes Center and Department of Endocrinology, Second Affiliated Hospital of Wenzhou Medical University. The study strictly followed the tenets of the Declaration of Helsinki and was approved by the institutional review boards of the hospital. All the patients were between 18 and 80 years old, consistent with the diagnosis of type 2 diabetes with eGFR $\geq$ 60 mL/(min·1.73 m^{-2}). Patients were further divided into two groups based on ACR levels: DM group with normal albuminuria (ACR < 3 mg/mmol, n = 49) and early DKD group with increased albuminuria for at least 3 months (3 mg/mmol $\leq$ ACR < 30 mg/mmol, n = 43)[2]. Patients were recruited into the DKD group for our study only if they had ACR $\geq$ 3 mg/mmol in at least two tests. The following exclusion conditions were as follows: (i) refractory hypertension or serious cardiovascular diseases; (ii) physiological or pathological conditions that may cause albuminuria include strenuous exercise, infections, and active kidney stones; (iii) recent use of nephrotoxic drugs; and (iv) definite diagnoses of other kidney disease. All patients gave informed written consent before participating with high compliance.

2.2. Sample Preparation and Lipid Extraction. 4 ml of fasting morning blood was collected from patients and sent for instant processing. After collection, blood samples were centrifuged at 3000 × rpm for 10 min to separate the serum. The supernatant was transferred and stored at −80 °C until analysis.

For lipidomic analysis, lipids were extracted according to the methyl *tert*-butyl ether (MTBE) method. Briefly, serum samples were first homogenized with 200 μL of water, 240 μL of methanol, and 800 μL of MTBE, and the mixture was subjected to ultrasound treatment for 20 min at 4 °C followed by sitting still for 30 min at room temperature. The solution was centrifuged at 14,000 *g* for 15 min at 10 °C, and the upper organic solvent layer was obtained and dried under nitrogen for liquid chromatography isolation and mass spectrometry analysis. Equal amounts of samples were extracted and mixed into eight quality control (QC) samples to determine the state of the instrument before sample detection and equilibrate the chromatography−mass spectrometry system during the entire experiment.

2.3. LC-MS/MS Analysis and Raw Data Processing. Reverse-phase chromatography was selected for liquid chromatography separation using a CSH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters). The lipid extracts were redissolved in 200 μL of 90% isopropanol/acetonitrile and centrifuged at 14,000 *g* for 15 min; finally, 3 μL of sample was injected. Solvent A was acetonitrile/water (6:4, v/v) with 0.1% formic acid and 0.1 mM ammonium formate, and solvent B was acetonitrile/isopropanol (1:9, v/v) with 0.1% formic acid and 0.1 mM ammonium formate. The initial mobile phase was 30% solvent B at a flow rate of 300 μL/min. It was held for 2 min and then linearly increased to 100% solvent B in 23 min, followed by equilibrating at 30% solvent B for 10 min.

For mass spectrometry detection, mass spectra were acquired using a Q-Exactive Plus instrument in positive and negative modes. ESI parameters were optimized and preset for all measurements as follows: source temperature: 300 °C; capillary temperature: 350 °C; ion spray voltage: 3000 V; S-Lens RF level: 50%; and scan range of the instruments: m/z 200−180. MS1 resolution at m/z 200:70,000 and MS2 resolution at m/z 200:17,500.

For lipid identification, raw data were searched using the "Lipid Search" engine based on MS/MS math, which contains more than 30 lipid classes and more than 1,500,000 fragment ions in the database. Mass tolerance for both the precursor and fragment was set at 5 ppm, and the product ion threshold was set at 5%.

2.4. Statistical Analysis. Data analysis was concluded with a data quality assessment, quantitative statistics, lipid composition, and difference analysis. Tools of MetaboAnalyst 5.0 (https://www.metaboanal.ca/) and R (version 4.2.1) were utilized. First, lipid abundance was log10-transformed for normalization, and the missing values were replaced by zero for subsequent analysis. Orthogonal partial least-squares discriminant analysis (OPLS-DA) with a permutation test for 1000 times was carried out to estimate the lipid difference between two groups. Differentially depressed lipids were filtered by Student's *t*-test (fold change of DKD/DM groups ≥1.2 or ≤0.8) with the false discovery rate (FDR) adjusted *p*-value and variable importance in the project (VIP) acquired by OPLS-DA analysis. For differential lipid filtration, the whole dataset was randomly sampled into training and testing groups at a ratio of 7:3 by R, and lasso regression analysis was performed to determine the degree of discrimination between DM and DKD groups. Binary logistic regression analysis and receiver operating characteristic (ROC) analysis were utilized to evaluate the diagnostic power of chosen lipids. p-value < 0.05 was regarded as significant.

2.5. Serum Phosphatidylserine Measurement. For biomarker validation, other 26 serum samples from DM (n = 13) and early DKD (n = 13) patients were used to test the PS levels using an ELISA kit (HM10702, Bioswamp). Briefly speaking, 40 μL of serum samples and 10 μL of biotin-labeled anti-PS antibody were added to each well of the plate. Then, 50 μL of enzyme labeling reagent was added to each well. After incubation for 30 min at 37 °C, the plate was washed five times and dried. The staining reagent was added for 10 min, and the PS concentration was tested under 450 nm using a microplate reader.

3. RESULTS

3.1. Demographic and Clinical Characteristics. Among the 92 enrolled type 2 diabetic patients, 49 were DM patients with normal albuminuria and the other 43 were early DKD patients with slightly increased albuminuria for more than 3 months. Detailed demographic clinical features were shown including age, gender, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), kidney functional indexes (urine acid, serum creatine, eGFR, ACR), and blood lipid levels triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) (Table 1). There was no difference in age, gender, BMI, blood pressure, and lipid levels between two groups. Among the kidney

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Table 1. Demographic and Clinical Characteristics of the DM and DKD Groups

indexes	DM (n = 49)	DKD (n = 43)	p
age (y)	59.78 ± 8.62	62 ± 9.95	0.254
male (%)	28, 57.1%	29, 67.4%	0.390
BMI (kg/m^2)	23.83 ± 2.34	23.69 ± 3.13	0.808
SBP (mmHg)	130.71 ± 15.27	137.26 ± 17.6	0.061
DBP (mmHg)	71.55 ± 10.54	73.5 ± 12.48	0.421
FBG (mmol/L)	7.1 ± 1.79	6.98 ± 2.33	0.783
urine acid (μmol/L)	306.59 ± 89.81	309.83 ± 77.39	0.856
serum creatine (μmol/L)	56.75 ± 14.11	60.18 ± 15.54	0.278
eGFR (mL/min·[1.73 m^2])	103.68 ± 11.94	100.91 ± 13.26	0.300
ACR (mg/mmol)	1.53 ± 0.64	9.78 ± 7.03	<0.01
triglyceride (mmol/L)	2.06 ± 1.63	1.67 ± 1.18	0.196
total cholesterol (mmol/L)	4.29 ± 1.11	4.27 ± 1.05	0.921
HDL-C (mmol/L)	1 ± 0.25	1.06 ± 0.34	0.374
LDL-C (mmol/L)	2.39 ± 0.91	2.5 ± 0.83	0.544

Abbreviations. SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; PBG: postprandial blood glucose; eGFR: estimated glomerular filtration rate; ACR: albumin/creatinine ratio; HDL-C: high-density lipoprotein cholesterol; and LDL-C: low-density lipoprotein cholesterol.

functional indexes, only the ACR was significantly increased in the DKD group, indicating the early stages of patients. Specific information on each sample is listed in the supplementary table (Table S1).

3.2. Data Quality and Lipidome Profiling Analysis. First, we comprehensively evaluated the instrument state through quality control samples. The mass spectrum of 8 QC samples in both positive and negative modes showed good instrument stability (Figure S1A). The range of lipid abundance also reflected the consistency of QC samples and showed no batch effect during sample detection (Figure S1B). In addition, lipid abundance was log10-transformed for normalization and subsequent analysis, and each sample was in normal distribution (Figure S1C). The entire workflow of the study is shown in Figure 1A. A total of 973 lipids were obtained; then, univariate and correlation analyses were performed to explore differentially expressed lipids in DKD. Multivariate and lasso regression analyses were made to filter the potential lipids in distinguishing early DKD from DM patients. Based on the results of lasso regression analysis and clinical lipid indexes, binary logistic analysis was used to further validate the auxiliary diagnostic capability of the selected lipids toward DKD. In total, 27 lipid classes were detected, and the top 10 classes were shown. Phosphatidylcholine (PC), triglyceride (TG), and sphingomyelin (SM) constituted the most serum lipid classes in diabetic patients (Figure 1B). The result of the OPLS-DA model indicated a clear difference in serum lipidome between DM and DKD groups, which passed through the 1000 permutation tests (R2Y = 85.6%, Q2 = 37.7%, p < 0.001) (Figure 1C,D).

3.3. Univariate Analysis of Differentially Expressed lipids. According to the FDR-adjusted p values and VIP scores based on OPLSA analysis (p < 0.05, VIP > 1), 60 differentially expressed lipids were obtained including 29 upregulated and

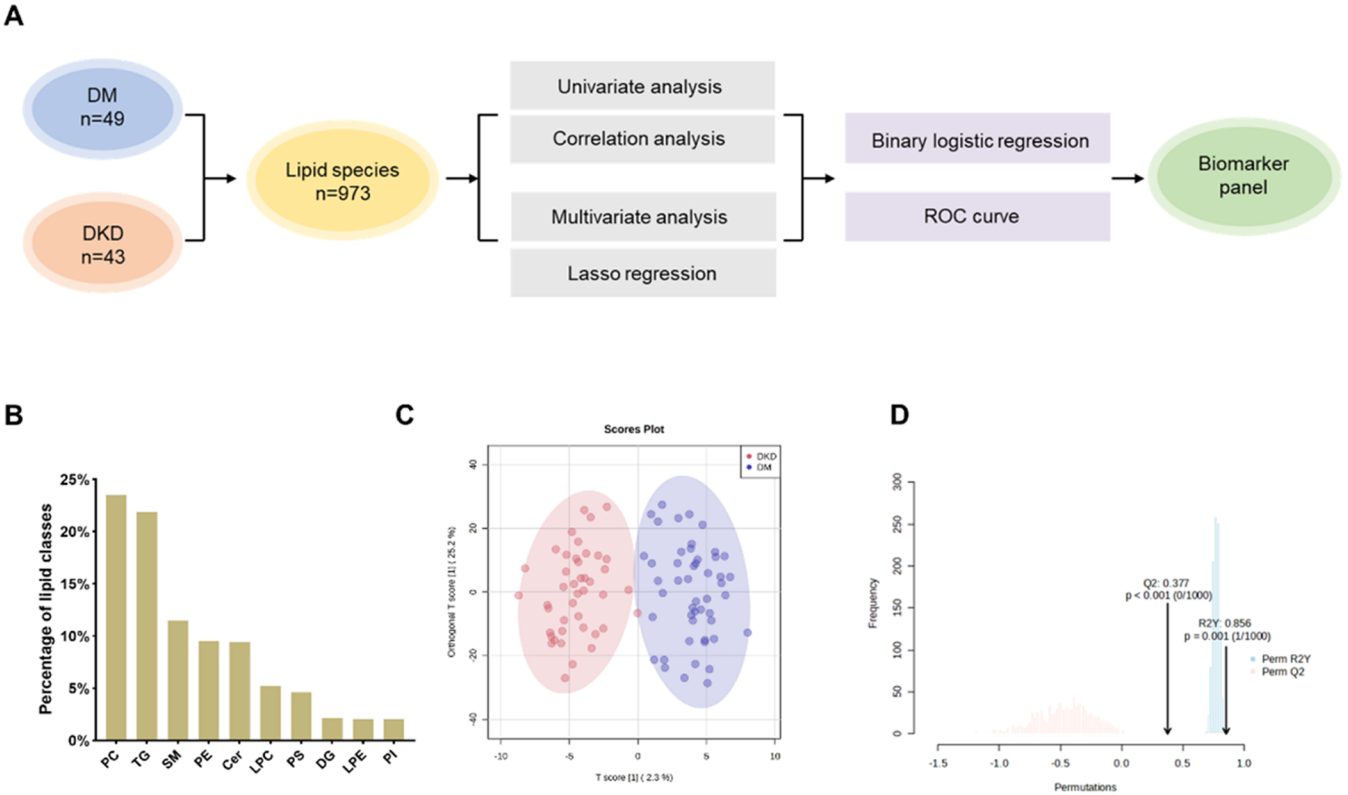

Figure 1. Study design and lipidome profiling. (A) Workflow of the study. (B) Identified lipid classes in total. PC: phosphatidylcholine; TG: triglyceride; SM: sphingomyelin; PE: phosphatidylethanolamine; Cer: ceramide; LPC: Lyso-PC; PS: phosphatidylserine; DG: diglyceride; LPE: Lyso-PE; and PI: phosphatidylinositol. (C) Orthogonal partial least-squares discriminant analysis (OPLS-DA) analysis of all the samples. (D) 1000 permutation tests of OPLS-DA analysis.

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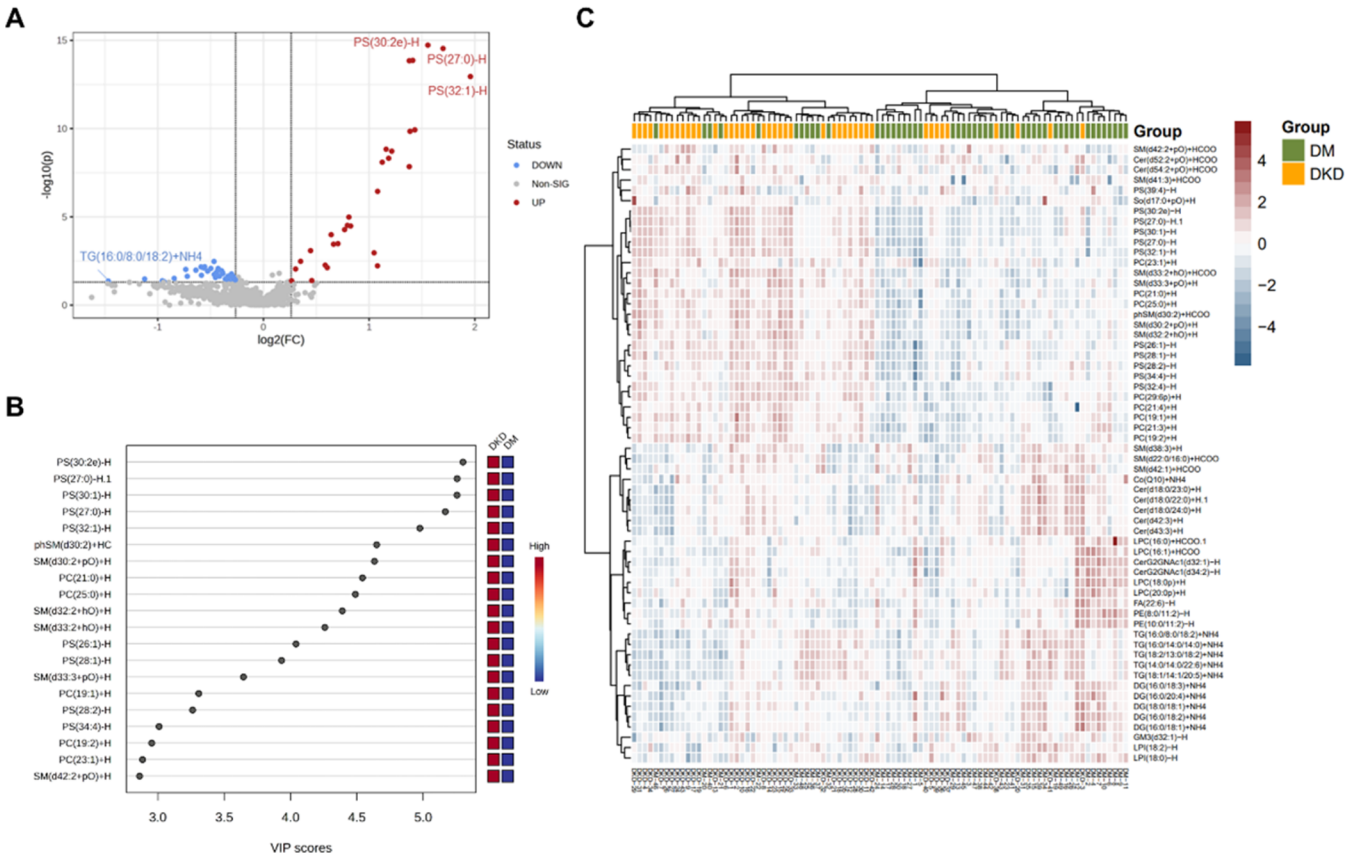

Figure 2. Filtration of differentially expressed lipids. (A) Volcano plot of the identified lipids. (B) VIP scores of the top 20 differentially expressed lipids from OPLS-DA analysis. (C) Unsupervised clustering heatmap of the 60 differentially expressed lipids.

31 downregulated lipids, suggesting the metabolic state variation of serum lipid associated with DKD (Table S2). The three most significantly increased lipids were PS(32:1)-H, PS(27:0)-H and PS(30:2e)-H, while TG(16:0/8:0/18:2) + NH4 was the most significantly decreased lipid (Figure 2A). The VIP scores of the top 20 important lipids were listed, which were mostly PS, PC, and SM, and PS(30:2e)-H and PS(27:0)-H also had the highest scores (Figure 2B). The heatmap of unsupervised clustering showed that the differential lipids in most samples of the same group could be clustered together, and lipids in the same class were clustered closely and expressed consistently from the DM to DKD group. The upregulated lipid classes were mainly PS and PC, while the downregulated lipid mainly belonged to TG, DG, LPC, and LPI. SM was present in both trends, reflecting more flexible functional changes in the development of proteinuria in DKD (Figure 2C).

3.4. Class Difference and Correlation Analysis of Differentially Expressed Lipids. To understand the lipid class variation between the two groups, the relative content percentage of 15 lipid classes consisting of 60 differential lipids was computed. It was found that downregulated classes, especially TG and DG, accounted for the largest proportion of the differential lipids, while the main upregulated classes such as PS and PC were less abundant (Figure S2). Since patients in the DKD group in our study mainly presented increased albuminuria, Spearman correlation among 60 differential lipids and ACR was performed. The results showed that the upregulated lipid class, especially PS, was highly positively correlated with ACR, while some downregulated lipid classes such as TG and DG were also statistically correlated with ACR, but the correlation was not as obvious as the upregulated lipids. PS(27:0)-H had the strongest correlations with ACR (coefficient = 0.72) (Table S3). In addition, most of the upregulated lipids were also more strongly correlated with each other than the downregulated lipids, further suggesting that PS, PC, and some SM lipids may play important synergistic roles in the occurrence of albuminuria (Figure 3A). To further filter the differential lipids for multivariate analysis, 12 lipids with an absolute value of correlation coefficient >0.5 were selected, which belonged to four lipid classes including PS, PC, SM, and phSM (Figure 3B,C). All 12 lipids were upregulated, and 7 of 12 were PS (Table S4). The relative abundance distribution of the 12 lipid classes was presented, further indicating that most lipids from the four classes were upregulated in the DKD group, and PS was the most significantly changed lipid class, although the abundance was not high in the differential lipids (Figures 3C and S2).

3.5. Multivariate and Lasso Regression Analysis for Potential Lipid Biomarkers. Based on the results mentioned above, 12 lipids showed a strong correlation with albuminuria, suggesting their potential as lipid biomarkers for early diabetic kidney disease. Therefore, we performed multivariate analysis through lasso regression to find the most representative differential lipids. The whole dataset was randomly divided into a training set (n = 64) and a testing set (n = 28) at a ratio of 7:3, and the corresponding sample is listed in Table S1. The 12 lipids were filtered in the training set with gradually decreased deviance, and the optimal lasso model with minimal deviance was finally obtained when two lipids were chosen

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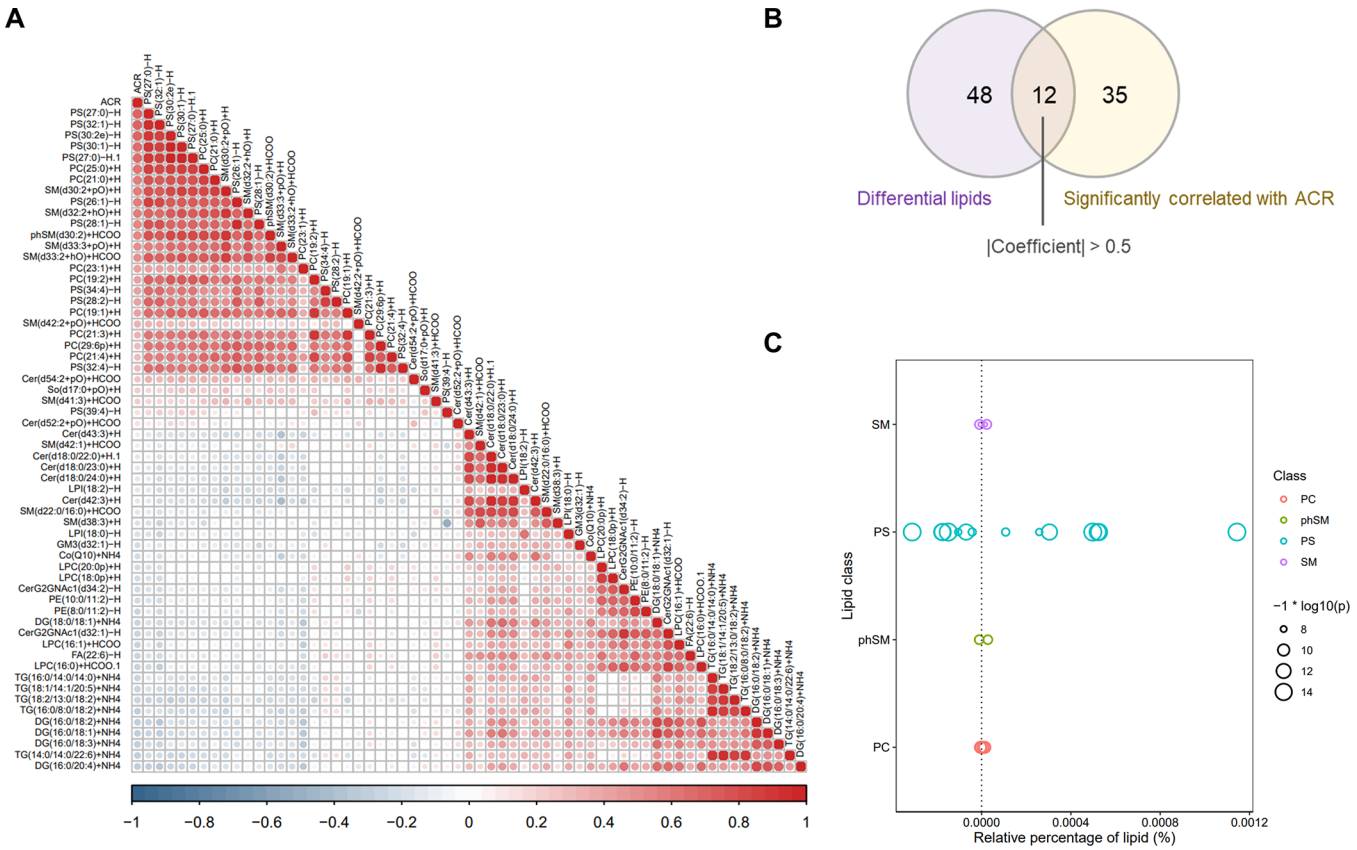

Figure 3. Correlation and class difference among differentially expressed lipids. (A) Spearman correlation analysis between ACR and differential lipids. (B) 12 differential lipids were chosen for lasso regression analysis. (C) Lipid class difference of 12 differential lipids between the two groups.

(Figure 4A,B). The discrimination degree of the regression model constructed by the three lipids for the DKD group in the training dataset was 0.945 (Figure 4C). The model was further validated in the testing dataset and found to be better at distinguishing the DKD group with the area under the curve (AUC) of 0.917, indicating that the two lipids screened by lasso regression analysis performed good diagnostic ability between DM and early DKD groups (Figure 4C). The two lipids were PS(27:0)-H and PS(30:2e)-H with a significant increase in DKD (Figure 4D).

3.6. Binary Logistic Regression Analysis of Potential Lipid Biomarkers. The differential lipids obtained by lasso regression showed a good diagnostic efficacy for DKD, which could be used as potential lipid biomarkers. Considering that blood lipid levels are frequently measured in diabetic patients including TG, TC, HDL-C, and LDL-C in clinical settings, we wondered whether the two differential lipids could help these common lipids to distinguish DKD from DM patients. Therefore, binary logistic regression analysis was performed on the entire data based on a combined model of clinical lipids including TG, TC, HDL-C, and LDL-C. The single AUC value of clinical TG, TC, HDL-C, and LDL-C was 0.472 (0.350−0.593), 0.474 (0.352−0.595), 0.537 (0.416−0.658), and 0.558 (0.436−0.679), respectively (Figure S3). The AUC of clinical lipids combined was 0.568 (0.448−0.688) and increased to 0.954 (0.917−0.992) after adding the two potential lipid biomarkers (Figure 5A). In addition, the AUC of the combined model of two lipids was 0.933 (0.882−0.983), and each of them performed better than single clinical lipids (>0.9) (Figure 5B), underlining that the panel of PS(27:0)-H and PS(30:2e)-H was a good diagnostic model to improve clinical lipids to identify early DKD from DM. To validate our lipidomic results, we further detected PS levels in other 26 serum samples from DM (n = 13) and early DKD (n = 13) patients using the ELISA method. The clinical information on patients for validation is shown in Table S5. Consistent with the lipidome results, the serum PS level was significantly increased in the DKD group and presented the same trend in different genders (male: n = 8; female: n = 5), further confirming that the serum phosphatidylserine was widely elevated in patients with diabetic kidney disease (Figure 5C).

4. DISCUSSION

DKD is one of the major microvascular complications of diabetes, causing irreversible kidney injury in patients. As the earliest sign, microalbuminuria is affected easily by multiple physiological and pathological factors. Moreover, its underlying pathophysiological mechanism is still not fully understood, which brings limitations to improving the specificity and sensitivity of early DKD diagnosis or prediction.[12] The fast development of high-throughput omics technology has made the discovery of disease biomarkers much easier. In this study, we purposed using LC-MS-based lipidomics to elucidate serum lipid variation between DM and DKD patients and to explore potential lipid biomarkers helpful to diagnose DKD.

A total of 92 type 2 diabetic patients were enrolled, and 43 were early DKD patients with moderately increased albuminuria for at least 3 months. Among the clinical indexes of renal function, only the ACR level was statistically significant between DM and DKD groups; thus, patients in the DKD group could be regarded in the early stage. As a result, 973

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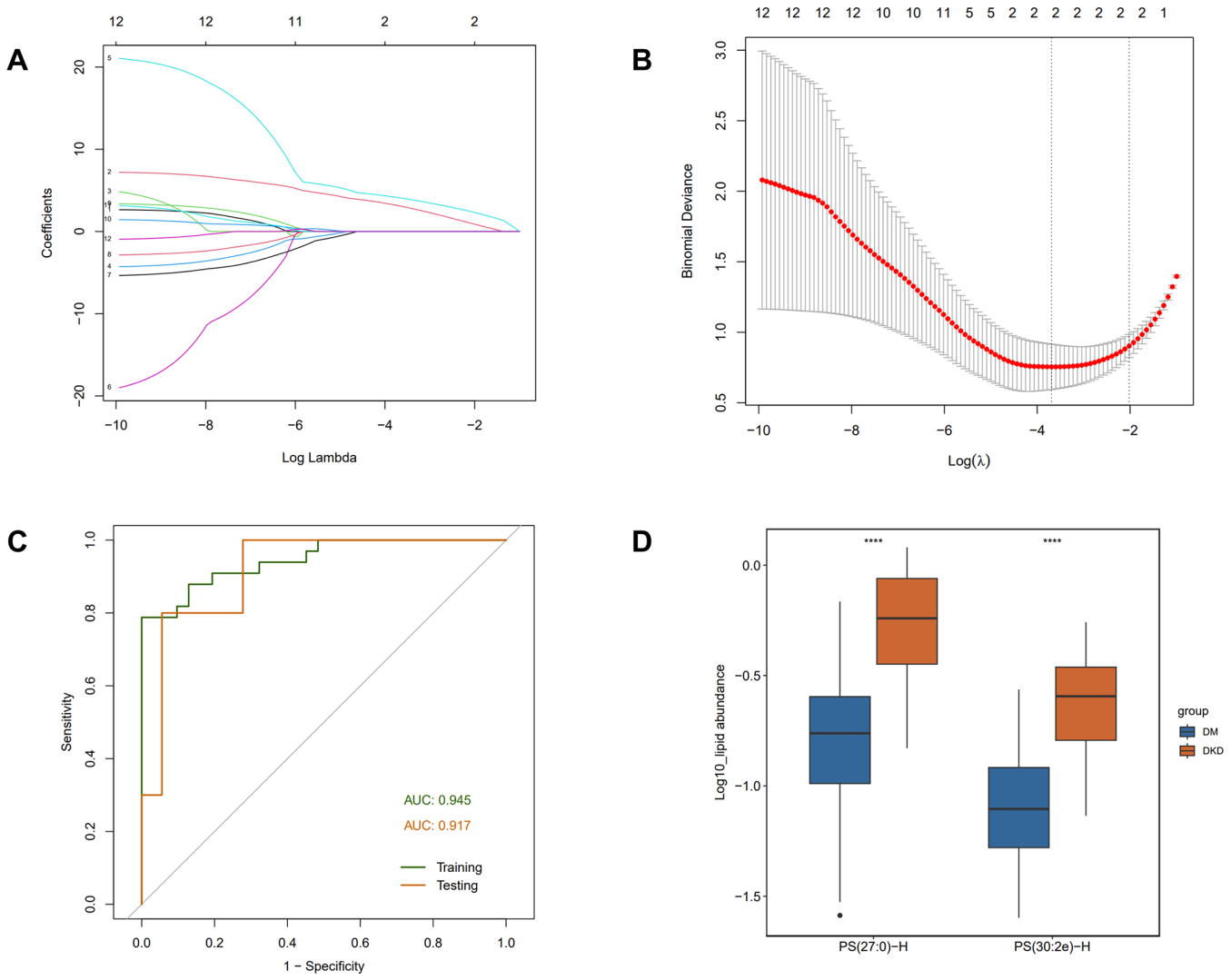

Figure 4. Lasso regression analysis for potential lipid biomarkers. (A) Diagram of 12 lipid selection. (B) Plot of model error variation during lipid selection. (C) Discrimination degree of the panel of PS(27:0)-H and PS(30:2e)-H for DKD in the training and testing datasets. (D) Lipid abundance of PS(27:0)-H and PS(30:2e)-H in DM and DKD groups.

serum lipids were identified, belonging to 27 lipid classes. OPLS-DA analysis showed clear isolation of two groups with permutation tests for 1000 times with $p < 0.001$.

60 differentially expressed lipids were acquired, and the clustering heatmap showed that the upregulated lipids were mainly classified into PS, PC, and SM, while TG and DG were the major types of downregulated lipids. The correlation among lipid molecules from the same class was strong, and the ACR was tightly related with the upregulated lipids, especially for PS, indicating that the increase of PS lipids may be linked to the occurrence of albuminuria in early DKD. Twelve differential lipids highly correlated with the ACR belonged to SM, PS, phSM, and PC classes despite low intensities.

To further filter representative lipids for DKD, lasso regression analysis was utilized in the 12 lipids chosen above and an optional model was built by PS(27:0)-H and PS(30:2e)-H. The panel performed well in both training and testing datasets, with the AUC of 0.945 and 0.917, respectively. In addition, it could also improve the discrimination of clinical lipids including TG, TC, HDL-C and LDL-C for DKD, suggesting its auxiliary diagnosis potential.

The lipids PS(27:0)-H and PS(30:2e)-H both belong to phosphatidylserine (PS), which is the most abundant negatively charged glycerophospholipid in eukaryotic membranes. Physiologically, PS mostly exists in the interior of the plasma membrane and in a small amount in the exterior; it would be externalized to the extracellular surface during cell apoptosis induction under pathological factors such as infection, autoimmune attack, mitochondrial function, and so on.[13−15] It has been reported that PS is more exposed to red blood cells in hypertensive and diabetic patients than in healthy cohorts, which may be attributed to tubulin inhibition of flipping enzyme activity due to the possible inhibition of flippase activity by tubulin.[16] Current studies reported that PS also externalizes to microparticles (MPs) in DKD, causing membrane remodeling. The most possible pathological mechanism is MPs formed under high glucose levels, inflammation, lipotoxicity, hypoxia, and uremic toxins. The exposed PS on MPs creates a catalytic surface for blood-clotting factors, facilitating coagulation activation. Bergen et al. explored the elevated expression of PS and proinflammatory high mobility group box-1 protein (HMGB1) in microvesicles (MVs) from patients with type 1 diabetes compared to healthy

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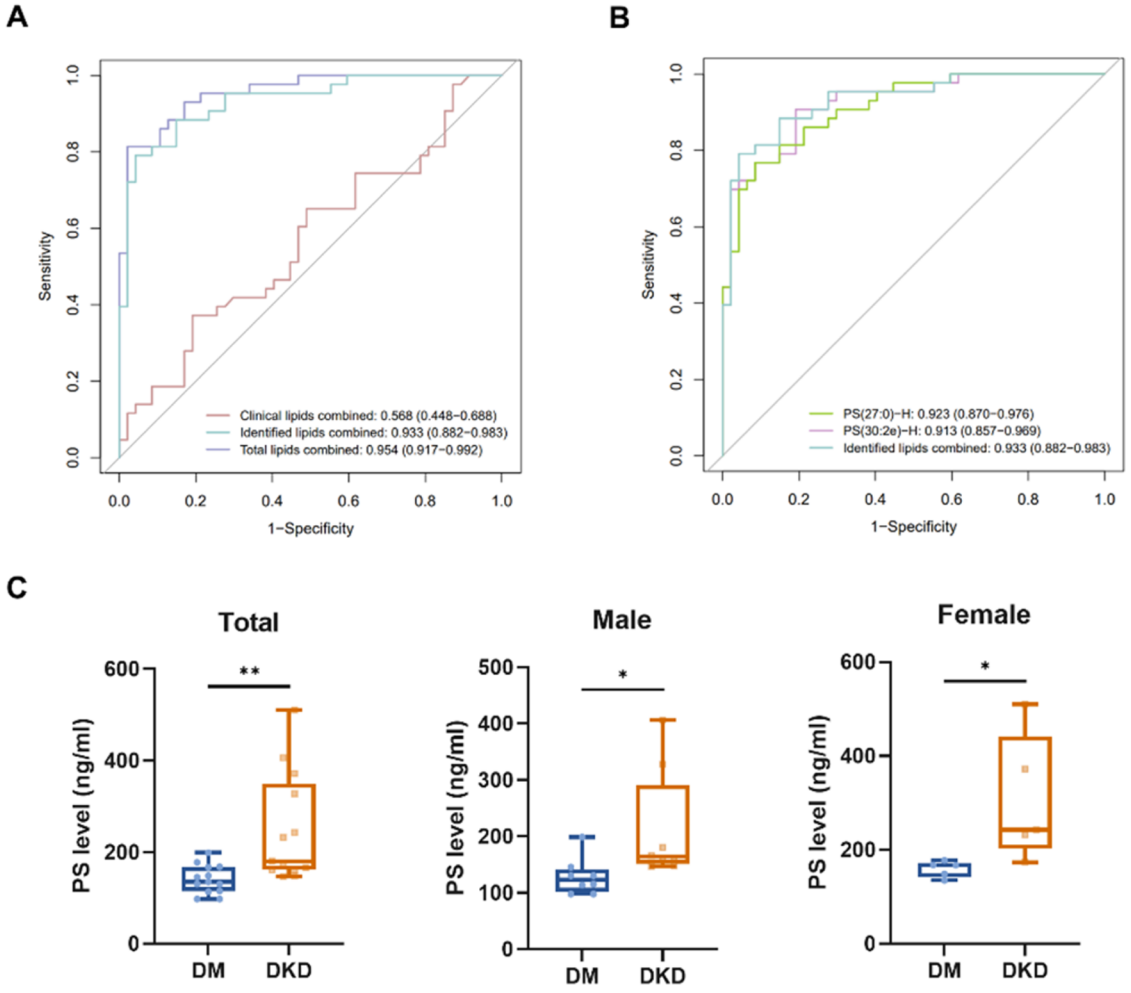

Figure 5. Binary logistic regression analysis of potential lipid biomarkers and clinical lipids. (A) ROC curves of combined clinical lipids (TG, TC, HDL-C, LDL-C), combined identified lipids (PS(27:0)-H, PS(30:2e)-H), and their total combined model. (B) ROC curves of single lipid biomarkers and the combined panel of PS(27:0)-H and PS(30:2e)-H. (C) PS level validation in other DM and DKD serum samples.

controls.[17] In addition, similar study also observed markedly increased PS exposure on human umbilical vein endothelial cells cultured with serum from diabetic patients with macroalbuminuria, contributing to the vascular endothelial cell injury and the occurrence of DKD.[18] Therefore, levels of PS+ blood cells and MPs were positively correlated with proteinuria, which could explain our results of the significantly elevated serum PS levels in the DKD group.

Sphingomyelin (SM) is an important sphingolipid involved in multiple biological activities including lipoprotein uptake, cell survival, and apoptosis.[19] It was reported that serum sphingomyelin emerged as a significant covariate of albuminuria in human type 1 diabetes and the strongest lipid regressor for kidney disease.[20] Another study utilized NPLC−TOF/MS to characterize human plasma phospholipids in T2DM and DKD patients and found that the SM level was upregulated in T2DM and DKD, possibly due to increased glucocorticoids toward SM metabolism.[9] Recently, the same group further demonstrated that SM was associated with the progression to ESRD in type 1 diabetes.[21] The molecular mechanism of the aberrant sphingomyelin metabolic pathway was also elucidated in specific renal cell types such as podocytes or mesangial cells and found to be linked with early diabetic kidney disease.[22−24] In addition, the conversion between sphingomyelin and ceramide, another important sphingolipid, was reported to independently associate only with albuminuria but not with eGFR in T2DM.[25]

In our results, phosphatidylcholine (PC) was another lipid class upregulated in the DKD group, which is the richest class of phospholipids in whole mammalian cell types and subcellular organelles, accounting for 40−50% of total cellular phospholipids. In all nucleated mammalian cells including renal cells, PC is synthesized primarily by the CDP-choline pathway or methylated PE under three sequential methylation reactions in the liver.[26] An earlier study indicated that renal hypertrophy in diabetes is linked to stimulated renal PC biosynthesis presumably caused by enhanced uptake of choline and stimulation of choline kinase.[27]

Above all, the role of phospholipids in DKD has been investigated in multiple metabolomics studies.[28−30] Our results of a strong correlation between the ACR and upregulated lipid classes including PS, SM, or PC demonstrated that the increase of serum phospholipids may be a crucial metabolic variation in the occurrence of albuminuria in early DKD, which could be explained by that abnormal phospholipids activate the sorbitol pathway, oxidative stress, and activation of protein kinase C to induce renal cell injury. Moreover, the accumulated lipids stimulated the expression of the vascular endothelial growth factor and transforming growth factor-β to cause proteinuria.[31,32] Finally, we found a panel of two significantly regulated

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lipids including PS(27:0)-H and PS(30:2e)-H showing good performance to distinguish early DKD from the DM group, which could act as potential biomarkers for the auxiliary diagnosis.

Our study has some limitations. The patients used for lipidomics were all enrolled from a single center. In addition, it would be better if the detected lipids could be testified in larger prospective cohorts, and the specific roles of phospholipids, especially for PS, in DKD need further exploration.

5. CONCLUSIONS

In summary, we applied an untargeted lipidomics strategy based on LC-MS to compare lipid expression between T2DM and early DKD patients; 60 differentially expressed lipids were obtained and filtered using lasso regression analysis. We found that the increased phospholipids were highly correlated with the ACR, and the combined model of two phospholipids including PS(27:0)-H and PS(30:2e)-H showed a good performance in DKD identification. The results indicated that phospholipid variation may participate in the development of albuminuria in early DKD, and further research was needed to deeply understand the molecular mechanisms of increased phospholipids in DKD and validate the diagnostic capability of the biomarker panel.

■ ASSOCIATED CONTENT

Data Availability Statement

The data used to support the conclusions of the study can be accessed from the corresponding author upon request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05504.

Data quality and distribution, class comparison of differential lipids, single ROC curves of clinical TG, TC, HDL-C, and LDL-C (PDF)

Clinical information on type 2 diabetic patients, differentially expressed lipids between DM and DKD groups, correlation matrix of differentially expressed lipids, detailed information on 12 differential lipids filtered for lasso regression analysis, and clinical information on type 2 diabetic patients for serum PS validation (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Chao Zheng − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China; Diabetes Center and Department of Endocrinology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, China*; Email: chao_zheng@zju.edu.cn

Authors

Shu Ye − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*; orcid.org/0000-0003-2248-4168

Ye-peng Hu − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*

Qiao Zhou − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*

Hang Zhang − *Diabetes Center and Department of Endocrinology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, China*

Zhe-zheng Xia − *Center on Evidence-Based Medicine & Clinical Epidemiological Research, School of Public Health, Wenzhou Medical University, Wenzhou 325035, China*

Shu-zhen Zhao − *Center on Evidence-Based Medicine & Clinical Epidemiological Research, School of Public Health, Wenzhou Medical University, Wenzhou 325035, China*

Zhe Wang − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*

Sheng-yao Wang − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*

Xin-yi Wang − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*

Yi-kai Zhang − *Department of Endocrinology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*

Zhi-da Chen − *Department of Nephrology, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310027, China*

Guang-yun Mao − *Center on Evidence-Based Medicine & Clinical Epidemiological Research, School of Public Health, Wenzhou Medical University, Wenzhou 325035, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c05504

Author Contributions

⊥S.Y. and Y.-p.H. contributed equally to this work. S.Y. conducted the experiment, processed data, and wrote the article. H.Z. and Z.-d.C. collected the samples. Z.-z.X. and S.-z.Z. supported the data analysis. Y.-p.H., Z.W., and S.-y.W. supported the experimental setup. Q.Z., X.-y.W., and Y.-k.Z. edited and corrected the figures and tables. G.-y.M. edited the article and provided guidance in data analysis. C.Z. designed the study, revised the article, and supervised the project. All authors contributed to the article.

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Notes

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■ ABBREVIATIONS

DKD:diabetic kidney disease
CKD:chronic kidney disease
ESRD:end-stage renal disease
SBP:systolic blood pressure
DBP:diastolic blood pressure

FBG:fasting blood glucose
TG:triglyceride
TC:total cholesterol
HDL-C:high-density lipoprotein cholesterol
LDL-C:low-density lipoprotein cholesterol
BMI:body Mass Index
ACR:albuminuria/creatine ratio
eGFR:estimated glomerular filtration rate
VIP:variable importance in the project
LC-MS:liquid mass spectrometry tandem chromatography system
NPLC-TOF/MS:normal-phase liquid chromatography coupled with time-of-flight mass spectrometry
OPLS-DA:orthogonal partial least-squares discriminant analysis
ROC:receiver operating characteristic
AUC:area under the curve
MTBE:methyl *tert*-butyl ether
QC:quality control
PS:phosphatidylserine
SM:sphingomyelin
PC:phosphatidylcholine
NEG:negative mode
POS:positive mode
FC:fold change
FDR:false discovery rate-adjusted *p* value

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