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Urine myo-inositol, the novel prognostic biomarker for diabetic kidney disease: a targeted metabolomics study using NMR

NRM 기법의 대사체학으로 밝힌 요중 myo-inositol의 당뇨성 신병증 경과 예측인자로써의 효용성

2020 년 2 월

서울대학교 대학원

임 상 의 과 학 과

권 소 이

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지도 교수 임 춘 수

이 논문을 의학석사 학위논문으로 제출함

2019 년 10 월

서울대학교 대학원

임 상 의 과 학 과

권 소 이

권소이의 석사 학위논문을 인준함

2020 년 1월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

Abstract

Soie Kwon

Department of Clinical Medical Sciences, College of Medicine

The graduate school

Seoul National University

Diabetic kidney disease (DKD) is a leading cause of CKD and ESRD. Metabolomics has been increasingly applied to identify the cause of chronic kidney disease (CKD), as it can present epigenetics and suggest corresponding treatment options. Only a few metabolomics studies were conducted in DKD patients, and the results are inconclusive. I investigated the association between urine metabolites and renal disease progression in DKD, using 800 MHz NMR based targeted metabolomics profiling.

Prospectively stored urine samples from consecutive

patients with DKD stage 1 to 5 (n=208) and their healthy controls (n=26) were analyzed. Cross-sectional associations were evaluated between eGFR or UPCR (urine protein creatinine ratio) and 26 urinary metabolites. Multivariable adjusted Cox models were conducted for the risk prediction of ESRD and mortality. The receiver-operating characteristic (ROC) analyses were used to assess the additive effect of each metabolite to predict progression to ESRD.

ESRD occurred in 103 (44.0%) patients and 65 (27.8%) deaths occurred dring median 4.5 year (IQR, 2.06-6.58) follow-up period. The median fold change in 9 metabolites (glucose, mannose, myo-inositol, glycerol, lactate, fumarate, creatine, taurine and choline) in patient group revealed a trend corresponding to DKD stages. Linear regression showed that myo-inositol had a strongest association with eGFR. The relationship between the competitive metabolites and outcomes (ESRD and mortality) was investigated by

multivariate Cox models after adjusting for the baseline covariates. Of these, 4 metabolites (myo-inositol, glycerol, fumarate, oxoisocaproate) had predictive values for ESRD, and among them, only myo-inositol retained predictive significance in mortality (adjusted HR 1.004, 95% confidence interval 1.002-1.006, p-value <0.001). At ROC anaylsis, urinary myo-inositol had additive effect to serum creatinine concentration and UPCR in prediction for ESRD progression (NRI = 2.9%, P = 0.03; IDI = 35.1%, P = 0.02).

My results suggest that myo-inositol can be a predictive biomarker for the risk of ESRD progression in DKD. Further mechanistic studies are needed to elucidate the pathophysiological roles of myo-inositol in DKD.

Keywords : DKD, Metabolomics, Myo-inositol, ESRD, All-cause mortality

Student Number : 2018-28788

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Chapter 1. Introduction

The estimated glomerular filtration rate (eGFR) and urine proteinuria are the most concrete and frequently used predictive biomarker of chronic kidney disease (CKD) regardless of the cause (1). In clinical practice, GFR is usually calculated by serum creatinine or cystatin C concentration instead of an iothalamate or inulin clearance measurement based golden standard, because inconvenient (2). However, these methods have limitations, in that they are synthetized and influenced by the muscle in creatinine and the liver in cystatin C (3; 4).

As CKD is a collection of various kidney disease, its mechanisms are diverse depending on the cause. Kidney biopsy, an invasive procedure, is the only way to distinguish the cause because there are no confirmed noninvasive methods to differentiate CKD etiologies. The demand for safer and more precise methods for distinguish the pathophysiology and corresponding treatment is increasing. Metabolomics has been increasingly applied to identifying new biomarkers of diseased specific CKD (5–7). Urine

metabolomics, which includes metabolic breakdown products and reveals renal condition from the biological waste made by kidney, has received considerable attention (8; 9).

Currently, DKD is a most common cause of ESRD. Moreover, DKD continues to increase in the elderly population and among patients with diabetes (10). Considering the limited treatment choices, reduction in quality of life and increases in mortality among DKD and DM-ESRD patients, early detection of DKD and possible treatment choices for DKD is needed. Previous metabolomics studies were conducted based on this trend and reported changes in mitochondrial and fatty acid metabolites in DKD patients (11-15). However, the results are varying.

Previous well organized animal study did a serum and urinary analysis at early and late stages by untargeted metabolomics between *db/m* and *db/db* mice. Which demonstrated early phase increase of branched chain amino acid and homocysteine-methionine metabolism and late phase increase of ketone and fatty acid metabolism (12). Base on this study, I investigated the association between urine metabolites and ESRD progression in

DKD cohort.

Chapter 2. Materials and Methods

2.1 Study design and population

From June 2011 to June 2018, the urine of DKD patients and healthy controls was prospectively collected at Seoul National University Boramae Medical Center. Consecutive patients with DKD stages 1–5 (n=208) and their healthy controls (n=26) with normal kidney function and no diabetes were enrolled from the prospective cohort. DKD stages were divided based on the CKD epidemiology collaboration equation (CKD-EPI) eGFR using serum creatinine(16). To be eligible for the study, a patient had to be older than 18 years and was not on dialysis at the time of enrollment. Urine samples were collected at the time of enrollment.

This study was conducted under the approval of the Research Ethics Committee of the Seoul National University Boramae Medical Center. All procedures were followed the accordance with the ethical standards of the institutional and/or national research committee and with the 2013 Declaration of Helsinki and tis later amendments or comparable ethical standards. Informed consent was

obtained to human research participants for the use of urine.

2.2 Clinical Data Collection

The baseline clinical parameters such as age, sex, body mass index (BMI), comorbidities and laboratory findings, including serum level of complete blood cell counts, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, creatinine, cholesterol, uric acid, HbA1c (Hemoglobin A1c) and urinary protein-to-creatinine ratio (UPCR), were collected from electronic medical records. The eGFR was measured using the CKD-EPI.

2.3 Definition of Study Outcomes

The primary study outcome was the number of ESRD events (maintenance dialysis or kidney transplantation). Information regarding ESRD was obtained from the Korean Society of Nephrology database(17). All-cause mortality data were obtained from the National Database of Statistics Korea. Patients were

followed up until their death or August 2018.

2.4 ^{1}H NMR Experiment

Frozen urine samples were sent to laboratory. Before nuclear magnetic resonance (NMR) experiment, urine samples were thawed at room temperature. Urine samples were filtered through Amicon® Ultra centrifugal filters for 500 μL – 3K (Millipore, Billerica, MA, USA) at 12,000 rpm for 10 minutes at 4° C to remove protein. The resulting 300–μL supernatant from the urine sample was mixed with 300 μL of 0.2 M sodium phosphate buffer (pH 7.0) and 1 mM sodium azide in deuterium oxide (D_2O). After adjusting the pH to 7.0 ± 0.1, 540 μL of sample was mixed with 60 μL of 5 mM 3–(trimethylsilyl) propionic 2,2,3,3–acid (TSP) in D_2O, and the 600–μL samples were placed in 5–mm Bruker SampleJet NMR tubes (Z112273, Bruker BioSpin AG, Fällanden, Switzerland).

One–dimensional (1D) ^{1}H NMR spectra were acquired with an Ascend 800–MHz AVANCE III HD Bruker spectrometer (Bruker BioSpin AG) using a triple–resonance 5–mm CPTIC cryogenic

probe. To acquire 1D ^{1}H spectra of the urine samples, Bruker standard 1D nuclear Overhauser enhancement spectroscopy (NOESY)-presat (noesypr1d) pulse sequences were used as follows: relaxation delay (*RD*) - 90° - short delay - 90° - mixing - 90° - *Acq*, with *RD* = 4.0 s, short delay = 12.18 us, *n* = 128, dummy scans = 16, acquisition time (*Acq*) = 2.0 s, and mixing time (mixing) = 10 ms. The water signal was suppressed at the water peak during the *RD* and mixing time. Fourier domain points were acquired at 65,536 data points with a spectral width of 20 ppm.

The NMR data were processed using TopSpin (ver. 3.1, Bruker BioSpin, Rheinstetten, Germany). All spectra were baseline-corrected and phase-corrected manually. The processed NMR spectra were imported into Chenomx for identification and quantification, and the 800-MHz Chenomx library (ver. 7.1, Chenomx, Edmonton, AB, Canada) was used to identify individual compounds. The assignment of ambiguous peaks due to peak overlap was confirmed by spiking with standard compounds. Signal assignment for representative samples was facilitated by the acquisition of two-dimensional (2D) correlation spectroscopy

(COSY) and heteronuclear single quantum correlation (HSQC). The quantification of urinary metabolites was achieved using Chenomx, which used the concentration of TSP to determine the concentration of individual compounds. The urinary concentrations were normalized to the levels of creatinine (metabolite μM/creatinine mM).

2.5 Statistical Analysis

Since metabolite concentration for each value were not normally distriuted, they were used for analysis after natural logarithmic transformation. The analysis was conducted in cross-sectional and longitudinal manners. Cross-sectional analysis was used to compare metabolic difference among healthy control and CKD groups. We expressed categorical variables as percentage in whole patients, continuous variables which follow the normal distribution curve as means $\pm$ standard deviations. The trend of baseline characteristics according to CKD stage was compared using the Jonckheere-Terpstra test for continuous variables and the linear by linear association for categorical variables. The Mann-Whitney

post hoc analysis was applied. All tests were two-tailed and $P < 0.05$ was considered indicative of statistical significance. Correlation between metabolites and eGFR or UPCR was determined using Pearson correlation analysis.

Survival analysis was conducted in a longitudinal manner to investigate the relationship between urinary metabolites and hard outcomes (ESRD progression and all-cause mortality). The Kaplan-Meier survival analysis and the log-rank method were used to compare the outcomes among metabolic quantiles. Multivariate Cox regression adjusted for the effect of traditional risk factors was performed by backward conditional stepwise analysis. The contribution of several urinary metabolites levels to distinguish patients without ESRD who progressed to ESRD was examined by the area under the area under the receiver operating characteristic curve (AUROC) in simple and time dependent manners (18).

Statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and R version 3.5.1 (The Comprehensive R Archive Network: http://cran.r-project.org).

Results

3.1 Evaluation of Baseline Characteristics according to DKD Stages

DKD patients are older than healthy controls and have lower BMI (Table 1). A prevalence of hypertension is higher in advanced DKD groups. Serum creatinine and eGFR were significantly associated with DKD stages and UPCR distinguish DKD stage 1 patients from their healthy controls. Among DKD patients, there were no statistically significant differences in glycemic control, which can be estimated by serum glucose and HbA1c concertation.

3.2 Evaluation of the Correlation with Urinary Metabolites Trends and CKD Stages

In Table 2 and Figure 1, we show the median fold change in each urine metabolite in the patient group compared with those in the control group. A total of 19 metabolites showed a significant trend

across DKD stages, and 9 metabolites (glucose, mannose, myo-inositol, glycerol, lactate, fumarate, creatine, taurine, choline) maintained significance in the post-hoc analysis (Table 2). Myo-inositol (R^2= 0.442, $p-value$ < 0.001), choline (R^2= 0.293, $p-value$ <0.001) and citrate (R^2= 0.175, $p-value$ <0.001) were correlated with eGFR and choline (R^2= 0.281, $p-value$ < 0.001), mannose (R^2= 0.260, $p-value$ <0.001) and myo-inositol (R^2= 0.236, $p-2value$ <0.001) were correlated with UPCR. The metabolites were more strongly correlated with eGFR (Figure 2) than with the UPCR (Figure 3).

Figure 4 is a metabolic pathway of which significantly changed according to CKD progression based on urinary metabolites of present study. Urinary monosaccharide concentration and TCA intermediates tend to increase as kidney function worsen (more advanced CKD stages, decrement of eGFR and increment of albuminuria). Other pathways do not present constant correlation.

Table 1. Baseline characteristics of DKD patients and healthy controls. Categorical variables were presented as n (%), and continuous variables were shown as mean ± standard deviations.

	Total	Control	DKD1	DKD2	DKD3	DKD4	DKD5	P for trend
Patient number	N= 234	N= 26	N= 11	N= 22	N= 54	N= 63	N= 58	
Age (years)	58.5 ± 16.3	35.8 ± 15.2	39.0 ± 19.8	55.4 ± 14.5	63.7 ± 11.3	65.7 ± 11.9	60.9 ± 13.1	<0.001
Sex, male (n, [%])	147 (62.8%)	15 (57.7%)	8 (72.7%)	14 (63.6%)	38 (70.4%)	37 (58.7%)	35 (60.3%)	0.744
BMI (kg/m^2)	23.6 ± 3.8	24.0 ± 3.4	27.6 ± 4.2	24.9 ± 3.2	23.5 ± 3.9	23.6 ± 3.4	22.8 ± 4.3	0.002
HTN, (%)	150 (64.1%)	4 (15.4%)	4 (36.4%)	13 (59.1%)	39 (72.2%)	46 (73.0%)	44 (75.9%)	<0.001
Laboratory findings								
Creatinine (mg/dl)	2.8 ± 2.2	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	1.7 ± 0.3	2.8 ± 0.9	5.8 ± 2.1	<0.001
eGFR ($ml/min/1.73m^2$)	42.9 ± 36.7	112.5 ± 11.8	110.3 ± 17.1	79.9 ± 11.9	41.1 ± 7.5	21.6 ± 5.1	9.6 ± 2.7	<0.001
Hemoglobin (g/dl)	11.5 ± 2.3	14.4 ± 1.8	13.9 ± 1.6	12.6 ± 2.8	11.9 ± 1.8	10.9 ± 2.0	9.8 ± 1.2	<0.001
Calcium (mg/dl)	8.7 ± 2.1	9.0 ± 0.9	9.1 ± 0.6	8.9 ± 0.7	8.8 ± 0.5	9.2 ± 3.9	7.9 ± 0.9	<0.001
Phosphorus (mg/dl)	4.0 ± 0.9	3.5 ± 0.6	3.7 ± 0.6	3.7 ± 0.6	3.8 ± 0.6	3.9 ± 0.8	4.8 ± 0.9	<0.001
Albumin (g/dl)	3.8 ± 0.6	4.3 ± 0.4	4.2 ± 0.5	3.8 ± 0.6	3.9 ± 0.4	3.8 ± 0.7	3.5 ± 0.4	<0.001
AST	21.5 ± 10.4	25.1 ± 14.9	20.3 ± 5.1	25.3 ± 11.9	22.6 ± 11.6	20.3 ± 7.3	18.8 ± 8.9	<0.001
ALT	19.9 ± 14.7	28.9 ± 30.5	17.8 ± 9.3	26.7 ± 14.2	19.5 ± 11.5	17.0 ± 9.0	16.8 ± 8.5	0.007
Glucose	132.4 ± 53.4	109.5 ± 19.4	118.2 ± 25.0	132.4 ± 33.0	136.2 ± 54.3	135.6 ± 48.6	139.1 ± 72.6	0.281
Hemoglobin A1c	7.0 ± 1.3	6.0 ± 0.4	6.3 ± 0.9	7.7 ± 1.3	7.3 ± 1.2	6.8 ± 1.4	6.8 ± 1.4	0.057
Uric acid (mg/dl)	7.1 ± 2.3	5.0 ± 1.6	6.3 ± 1.2	5.9 ± 2.1	7.0 ± 2.3	8.0 ± 2.6	7.8 ± 1.6	<0.001

	Total	Control	DKD1	DKD2	DKD3	DKD4	DKD5	P for trend
Patient number	N= 234	N= 26	N= 11	N= 22	N= 54	N= 63	N= 58	
Total cholesterol (mg/dl)	161.9 ± 44.6	171.3 ± 35.3	162.8 ± 35.9	169.6 ± 45.4	158.5 ± 42.0	157.1 ± 44.3	162.4 ± 52.5	0.068
Triglyceride (mg/dl)	149.8 ± 81.7	155.7 ± 56.9	192.4 ± 132.9	127.2 ± 52.9	149.9 ± 75.6	139.1 ± 69.8	161.6 ± 100.3	0.799
HDL (mg/dl)	46.2 ± 35.0	50.1 ± 13.7	87.1 ± 129.3	46.7 ± 12.5	43.5 ± 11.5	50.8 ± 32.5	34.8 ± 10.7	<0.001
LDL (mg/dl)	91.3 ± 40.4	100.9 ± 30.2	72.4 ± 43.3	87.7 ± 30.8	88.8 ± 33.8	82.6 ± 34.0	103.7 ± 52.7	0.594
Urine PCR (mg/mg)	3.8 ± 4.6	0.1 ± 0.1	3.4 ± 3.3	2.9 ± 4.4	3.7 ± 5.4	2.9 ± 3.6	6.2 ± 4.5	<0.001

Abbreviation: CKD, chronic kidney disease; BMI, body mass index; HTN, hypertension; eGFR, estimated glomerular filtration rated (by CKD-EPI creatinine equation); AST, aspartate transaminase; ALT, alanine transaminase; HDL, high density lipoprotein; LDL, low density lipoprotein; PCR, protein-to-creatinine ratio

Table 2. Median fold change of urine metabolites according to DKD stage, compared with control group.

Ln(Metabolites/Cr)	DKD1/Control	DKD2/Control	DKD3/Control	DKD4/Control	DKD5/Control	J-T test
Glucose	0.496*	1.504†	1.362†	1.562*	3.256†	<0.001
Mannose	0.597*	0.886†	0.901†	1.059†	2.143†	<0.001
Xylose	0.292	0.190	−0.158	−0.447†	−0.504†	<0.001
Myo-inositol	0.119	1.004†	1.705†	2.254†	2.966†	<0.001
Glycerol	−0.109	0.427	0.607†	0.594*	0.718†	0.002
Lactate	0.222	0.415†	0.583†	0.789†	1.460†	<0.001
Pyruvate	−0.070	0.193	−0.269	−0.301	−0.091	0.66
Citrate	−0.392	0.190	−0.602†	−1.623†	−1.262†	<0.001
2-Oxoglutarate	0.194	0.637	−0.101	−0.066	0.353	0.992
Succinate	0.638*	0.613†	1.159†	0.694†	0.789†	0.032
Fumarate	1.005	1.207†	1.133†	1.146†	1.731†	<0.001
Pyroglutamate	0.068	0.191	0.179	0.220*	0.506†	<0.001
Acetone	−0.386	0.336*	0.313	0.413	0.424*	0.012
O-Acetylcarnitine	−0.747	−0.735	−0.336	−0.504	−0.317	0.505
Isoleucine	−0.171	0.147	−0.056	−0.292	0.735†	0.007
Leucine	−0.111	0.122	−0.029	−0.083	0.662†	<0.001
Valine	0.013	0.236	−0.035	−0.139	0.763†	<0.001
Creatine	−0.737*	−0.701	−1.096†	−1.283†	−1.195†	<0.001
Taurine	−0.568†	−0.075	−0.296*	−0.315*	−0.606†	0.003
Threonine	−0.016	0.067	−0.212	−0.224	0.549	0.088
Carnitine	−0.681	−0.565	−0.238	−0.495	−0.536	0.291
2-Oxoisocaproate	−0.176	0.088	−0.200	0.015	0.085	0.068
Choline	0.232	0.661†	0.872†	1.550†	2.405†	<0.001
DMG	0.560	0.412*	0.144	−0.063	−0.175	<0.001
TMAO	−0.433	0.341	0.188	0.420	0.882†	<0.001
Betaine	0.164	0.887†	0.921†	0.862†	0.679†	0.126

Post-hoc analysis with Mann-Whitney test *P <0.05; †P <0.01; ‡P <0.001 (compared each DKD groups with Control).

Figure 1. The relative intensity of urine metabolite/creatinine across all five CKD stages and controls, presented by dot plot

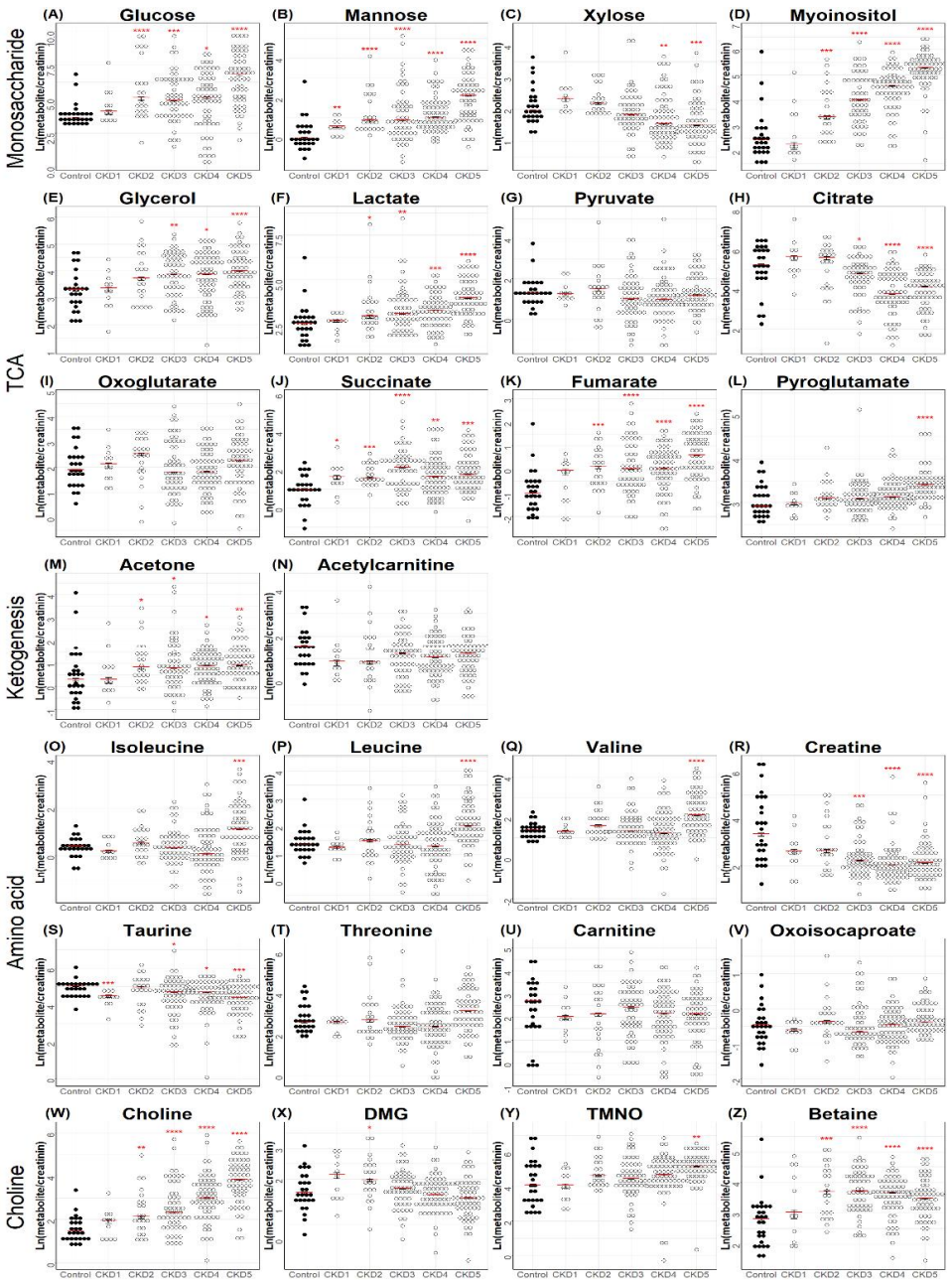


Figure 2. Correlation matrix for urine metabolites concentration with eGFR

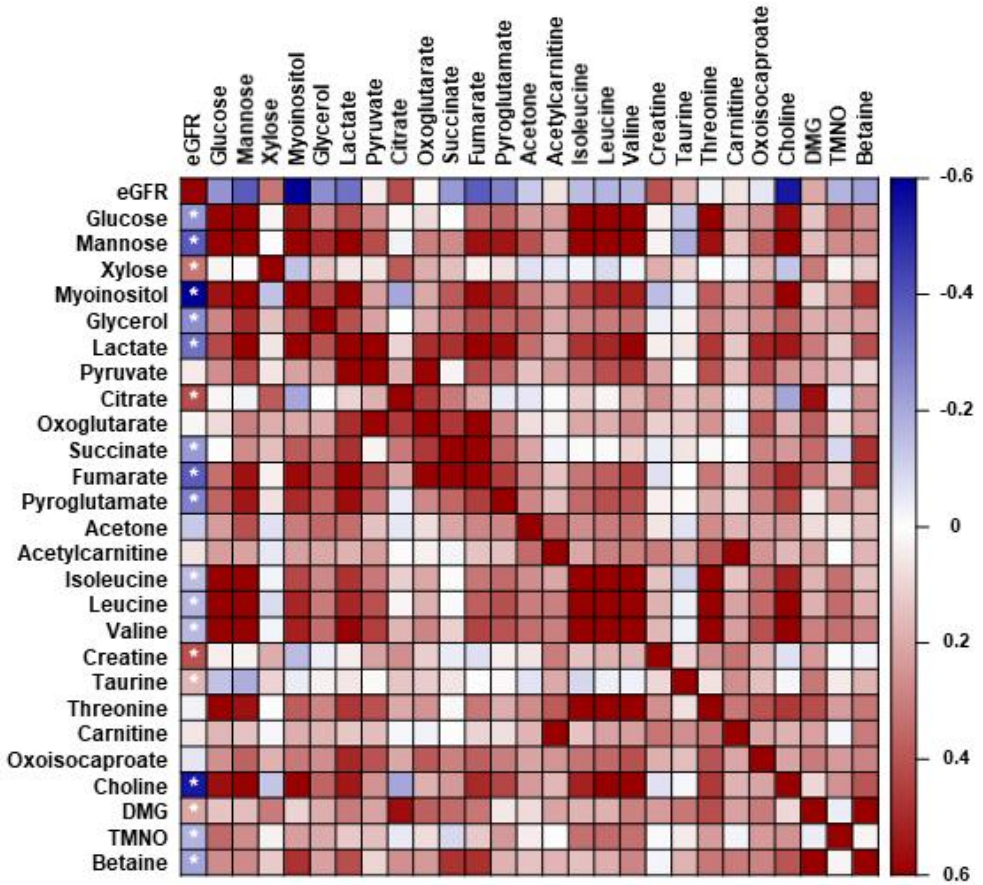


Figure 3. Correlation matrix for urine metabolites concentration with albumin creatinine ratio

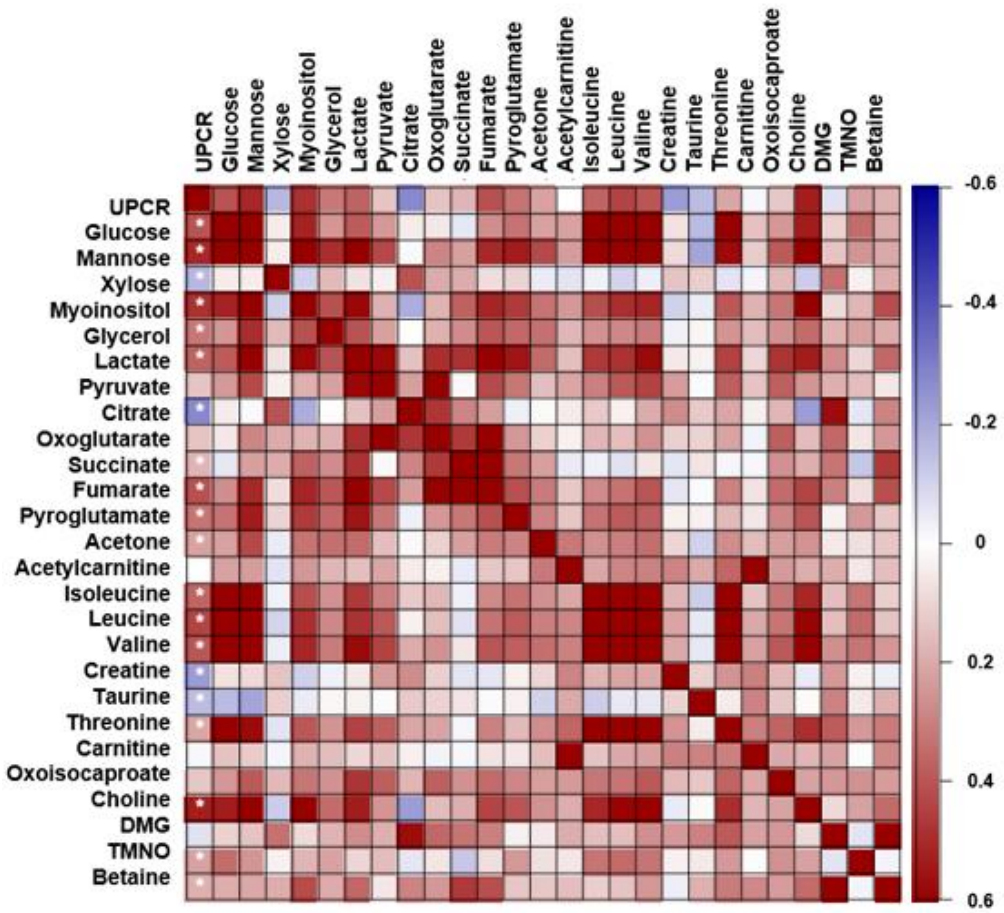
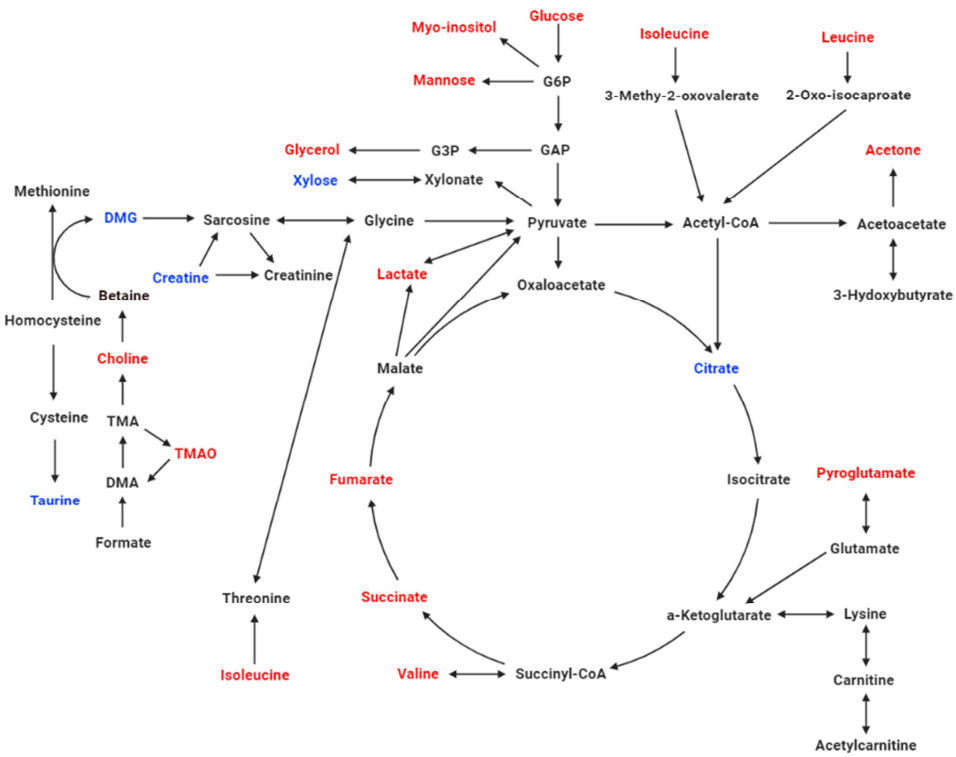


Figure 4. Metabolic pathways of which significantly changed according to CKD progression based on urinary metabolites of the present study.

(The red color indicates up-regulated metabolites, and the blue color indicates down-regulated metabolites)



3.3 Metabolites associated with ESRD Progression

The median fallow up period was 4.5 years (IQR, 2.06–6.58), during which 103 participants (44.0%) progressed to ESRD and 65 patients (27.8%) died. The composite outcome was achieved in 135 participants (57.7%) and no participant in the control group achieved thecomposite outcome. In Kaplan–Meier analysis, 17 metabolites were associated with ESRD progression (Figure 5) and 14 metabolites were associated with all–cause mortality (Figure 6). All of the outcomes showed high correlations with monosaccharides and low correlations with the ketogenic pathway in Kaplan–Meier analysis. After multivariate Cox analysis, 12 metabolites were still associated with ESRD progression (Table 3) and 11 metabolites were associated with all–cause mortality (Table 4). ESRD progression was more closely related to urine monosaccharide concentration and TCA cycle metabolite concentration.

Figure 5. Kaplan–Meier survival curves of targeted metabolites for ESRD progression.

Patients with first(solid), second(dashed), third(dotted) and forth (dot dash) quantile of each level of Ln(metabolites/Cr) were subjected to these analyses.

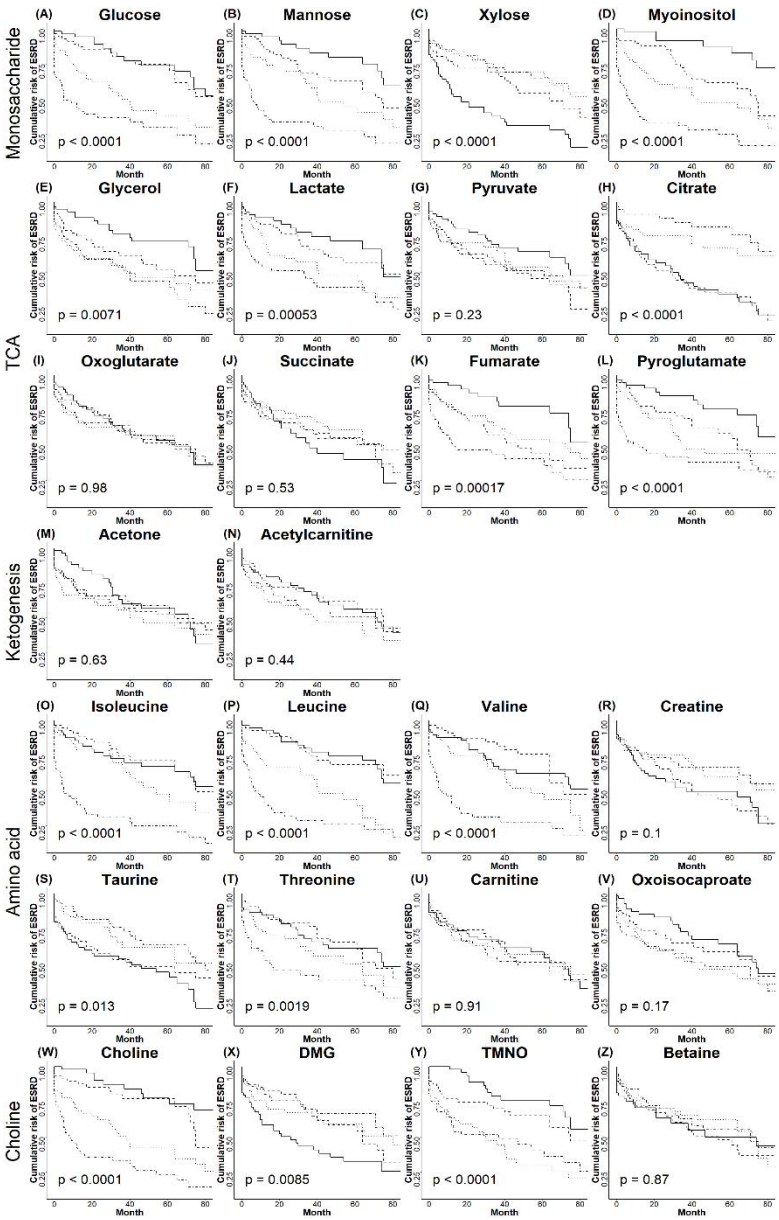


Figure 6. Kaplan–Meier survival curves of targeted metabolites for all–cause mortality.

Patients with first(solid), second(dashed), third(dotted) and forth (dot dash) quantile of each level of Ln(metabolites/Cr) were subjected to these analyses.

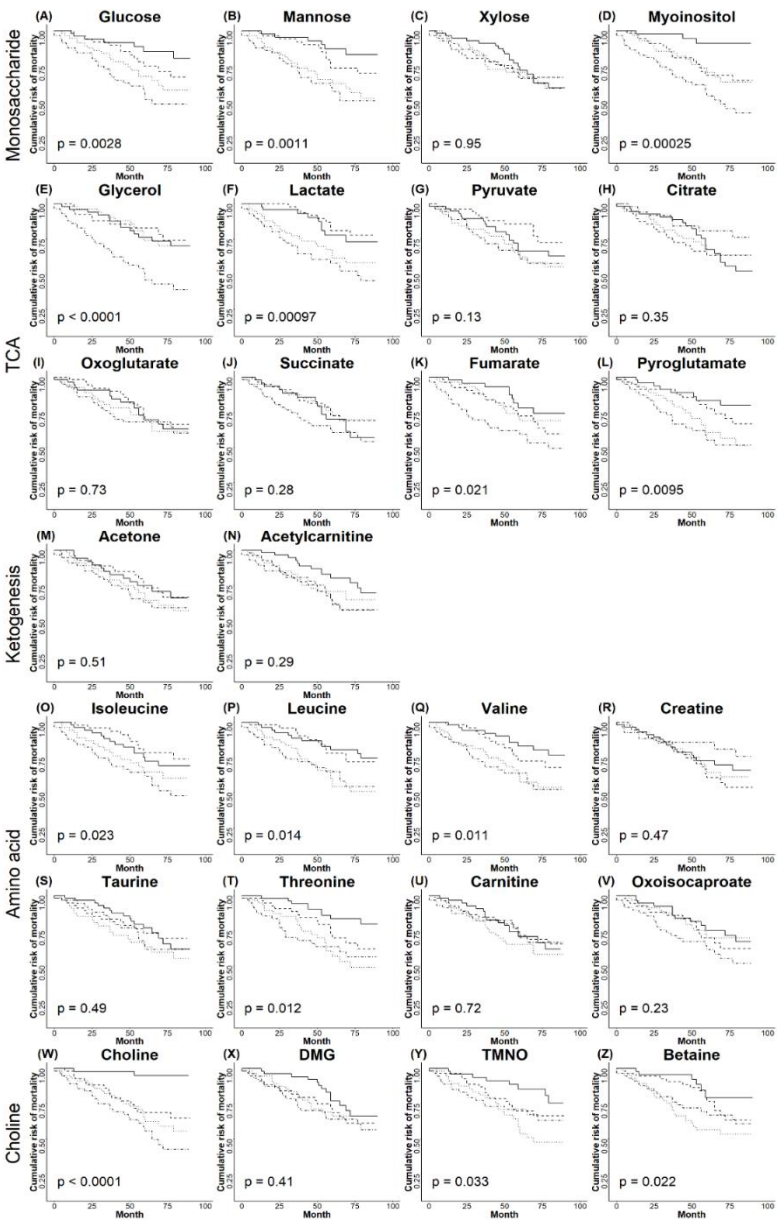


Table 3. Association of urine metabolites/creatinine with renal outcome, using backward stepwise multivariate Cox model.

Ln(Metabolites/Cr)	Unadjusted			Model 1			Model 2			Model 3		
	HR	CI	P value	HR	CI	P value	HR	CI	P value	HR	CI	P value
Glucose	1.345	1.22–1.483	<0.001	1.227	1.111–1.355	<0.001	1.299	1.155–1.460	<0.001	1.223	1.081–1.383	0.001
Mannose	1.692	1.454–1.968	<0.001	1.445	1.225–1.705	<0.001	1.437	1.194–1.729	<0.001	1.338	1.088–1.645	0.006
Xylose	0.501	0.366–0.686	<0.001									
Myoinositol	2.129	1.711–2.648	<0.001	1.453	1.121–1.883	0.005	1.542	1.163–2.046	0.003	1.441	1.063–1.954	0.019
Glycerol	1.489	1.188–1.866	0.001	1.524	1.193–1.948	0.001	1.445	1.117–1.868	0.005	1.519	1.124–2.053	0.007
Lactate	1.406	1.201–1.647	<0.001	1.433	1.168–1.758	0.001	1.454	1.186–1.783	<0.001	1.326	1.070–1.642	0.010
Pyruvate	1.15	0.954–1.387	0.142	1.520	1.232–1.876	<0.001	1.544	1.232–1.936	<0.001	1.338	1.073–1.669	0.010
Citrate	0.668	0.575–0.776	<0.001									
Oxoglutarate	1.039	0.849–1.272	0.708	1.332	1.067–1.664	0.011	1.507	1.187–1.914	0.001	1.326	1.047–1.678	0.019
Succinate	1.062	0.87–1.295	0.556									
Fumarate	1.396	1.166–1.672	<0.001	1.373	1.098–1.717	0.005	1.449	1.145–1.833	0.002	1.413	1.119–1.784	0.004
Pyroglutamate	3.981	2.519–6.29	<0.001	2.817	1.723–4.604	<0.001	3.847	2.262–6.544	<0.001	3.229	1.707–6.107	<0.001
Acetone	1.13	0.918–1.391	0.249									
Acetylcarnitine	1.083	0.88–1.334	0.452	1.364	1.075–1.731	0.010	1.368	1.039–1.801	0.026			
Isoleucine	1.888	1.553–2.295	<0.001	1.489	1.225–1.810	<0.001	1.474	1.191–1.824	<0.001	1.226	0.985–1.526	0.068
Leucine	2.232	1.766–2.821	<0.001	1.631	1.297–2.050	<0.001	1.616	1.262–2.069	<0.001	1.427	1.091–1.865	0.009
Valine	1.775	1.449–2.174	<0.001	1.419	1.171–1.719	<0.001	1.566	1.294–1.894	<0.001	1.276	1.021–1.595	0.032
Creatine	0.791	0.628–0.997	0.047	1.322	0.999–1.750	0.051						

Ln(Metabolites/Cr)	Unadjusted			Model 1			Model 2			Model 3		
	HR	CI	P value	HR	CI	P value	HR	CI	P value	HR	CI	P value
Taurine	0.741	0.611–0.899	0.002									
Threonine	1.379	1.15–1.653	0.001	1.241	1.038–1.483	0.018	1.241	1.001–1.539	0.049			
Carnitine	1.034	0.868–1.231	0.711	1.237	1.011–1.514	0.039						
Oxoisocaproate	1.599	1.131–2.261	0.008	2.668	1.795–3.965	<0.001	2.634	1.732–4.006	<0.001	2.063	1.337–3.184	0.001
Choline	1.776	1.529–2.063	<0.001	1.330	1.097–1.612	0.004	1.235	0.999–1.526	0.051	1.211	0.982–1.495	0.074
DMG	0.599	0.445–0.806	0.001									
TMNO	1.318	1.098–1.581	0.003	1.207	1.009–1.444	0.039	1.276	1.044–1.558	0.017			
Betaine	0.945	0.737–1.213	0.659									

Abbreviations: HR, hazard ratio; CI, confidence interval

Model 1: adjusted for age, sex, hypertension and eGFR.
Model 2: adjusted for model 1 variables, plus hemoglobin, albumin, AST, ALT, cholesterol and uric acid.
Model 3: adjusted for model 2 variables, plus spot urine protein–albumin ratio and hemoglobin A1c

Table 4. Association of urine metabolites/creatinine with all-cause mortality, using backward stepwise multivariate Cox model.

Ln(Metabolites/Cr)	Unadjusted			Model 1			Model 2			Model 3		
	HR	CI	P value	HR	CI	P value	HR	CI	P value	HR	CI	P value
Glucose	1.256	1.110-1.422	<0.001	1.249	1.101-1.416	0.001	1.265	1.111-1.439	<0.001	1.248	1.090-1.429	0.001
Mannose	1.502	1.232-1.831	<0.001	1.467	1.178-1.828	0.001	1.548	1.254-1.910	<0.001	1.510	1.214-1.879	<0.001
Xylose	1.086	0.776-1.52	0.631									
Myo-inositol	1.730	1.315-2.276	<0.001	1.529	1.106-2.114	0.010	1.675	1.256-2.234	<0.001	1.573	1.164-2.126	0.003
Glycerol	1.584	1.170-2.145	0.003	1.474	1.064-2.040	0.020	1.368	0.980-1.909	0.066	1.392	0.975-1.986	0.069
Lactate	1.527	1.251-1.864	<0.001	1.433	1.154-1.780	0.001	1.343	1.073-1.682	0.010	1.348	1.068-1.701	0.012
Pyruvate	1.144	0.904-1.448	0.262	1.246	0.994-1.562	0.057						
Citrate	0.886	0.728-1.080	0.231				1.277	0.992-1.643	0.058	1.256	0.972-1.623	0.081
Oxoglutarate	1.068	0.830-1.375	0.609	1.277	0.993-1.642	0.057	1.361	1.053-1.758	0.019	1.245	0.966-1.605	0.091
Succinate	1.125	0.889-1.424	0.327									
Fumarate	1.294	1.028-1.629	0.028	1.282	1.009-1.629	0.042	1.303	1.020-1.666	0.034	1.239	0.966-1.591	0.092
Pyroglutamate	2.705	1.558-4.696	<0.001	1.800	0.976-3.319	0.060	2.072	1.110-3.870	0.022	2.105	1.061-4.176	0.033
Acetone	1.225	0.938-1.599	0.136									
Acetylcarnitine	1.186	0.914-1.538	0.2									
Isoleucine	1.387	1.096-1.754	0.006	1.480	1.162-1.886	0.001	1.441	1.135-1.829	0.003	1.395	1.085-1.793	0.009
Leucine	1.567	1.195-2.057	0.001	1.626	1.220-2.166	0.001	1.710	1.305-2.241	<0.001	1.567	1.160-2.117	0.003
Valine	1.520	1.190-1.940	0.001	1.560	1.225-1.987	<0.001	1.533	1.220-1.925	<0.001	1.496	1.167-1.919	0.001
Creatine	0.819	0.606-1.106	0.193									
Taurine	1.125	0.836-1.513	0.437									

Ln(Metabolites/Cr)	Unadjusted			Model 1			Model 2			Model 3		
	HR	CI	P value	HR	CI	P value	HR	CI	P value	HR	CI	P value
Threonine	1.483	1.188–1.852	0.001	1.483	1.195–1.841	<0.001	1.445	1.164–1.794	0.001	1.405	1.123–1.757	0.003
Carnitine	1.079	0.868–1.342	0.491									
Oxoisocaproate	1.743	1.117–2.720	0.014	1.696	1.050–2.738	0.031						
Choline	1.637	1.335–2.007	<0.001	1.629	1.304–2.035	<0.001	1.638	1.317–2.037	<0.001	1.624	1.279–2.061	<0.001
DMG	1.244	0.861–1.796	0.244	1.566	1.043–2.350	0.031	1.494	0.977–2.283	0.064	1.578	1.008–2.470	0.046
TMNO	1.189	0.944–1.497	0.141									
Betaine	1.387	0.989–1.944	0.058	1.428	0.978–2.084	0.065						

Abbreviations: HR, hazard ratio; CI, confidence interval
Model 1: adjusted for age, sex, hypertension and eGFR.
Model 2: adjusted for model 1 variables, plus hemoglobin, albumin, AST, ALT, cholesterol and uric acid.
Model 3: adjusted for model 2 variables, plus spot urine protein–albumin ratio and hemoglobin A1c

3.4 ROC Analysis

I compared the serum creatinine concentration and UPCR with each urinary metabolite by receiver-operating characteristic (ROC) analysis to determine predictability for progression to ESRD. Choline, myo-inositol and citrate were the most predictive urine metabolites, although they were not superior to serum creatinine and UPCR alone (Table 5). To assess additive effect of each urinary metabolite to serum creatinine concentration and UPCR in prediction for ESRD progression, the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) was used (Table 6). Only myo-inositol improved prediction (NRI = 2.9%, P = 0.03; IDI = 35.1%, P = 0.02).

The time-dependent ROC curve analyses for censored ESRD progression data were additionally applicate to three most predictive urine metabolites. In time-dependent ROC analysis, serum creatinine was the best predictive biomarker in the

shorter follow-up period, and UPCR was the best predictive biomarker in longer follow-up period. Choline and myo-inositol were more predictive than UPCR in the 12month ESRD progression prediction (Table 7, Figure 7).

Table 5. The area under the curve(AUC) of each urine metabolites/creatinine compared with serum creatinine and urine protein creatinine ratio

Ln(Metabolite/Cr)	AUC (95% CI)
Serum creatinine	0.88 (0.836-0.923)
Urine protein creatine ratio	0.842 (0.786-0.897)
Choline	0.77 (0.708-0.832)
Myo-inositol	0.758 (0.697-0.818)
Citrate	0.732 (0.668-0.796)
Mannose	0.712 (0.645-0.779)
Leucine	0.697 (0.628-0.766)
Glucose	0.693 (0.624-0.762)
Xylose	0.683 (0.612-0.753)
Isoleucine	0.659 (0.586-0.732)
Lactate	0.655 (0.585-0.725)
Pyroglutamate	0.655 (0.585-0.725)
Valine	0.647 (0.573-0.721)
Taurine	0.634 (0.562-0.706)
Fumarate	0.632 (0.561-0.703)
TMNO	0.626 (0.554-0.698)
Creatine	0.62 (0.548-0.692)
Glycerol	0.616 (0.543-0.688)
DMG	0.616 (0.544-0.689)
Oxoisocaproate	0.594 (0.521-0.667)
Threonine	0.577 (0.501-0.653)

Ln(Metabolite/Cr)	AUC (95% CI)
Betaine	0.527 (0.453−0.601)
Acetone	0.523 (0.448−0.597)
Carnitine	0.504 (0.43−0.579)
Acetylcarnitine	0.499 (0.424−0.574)
Oxoglutarate	0.498 (0.423−0.573)
Succinate	0.485 (0.409−0.56)
Pyruvate	0.472 (0.397−0.546)

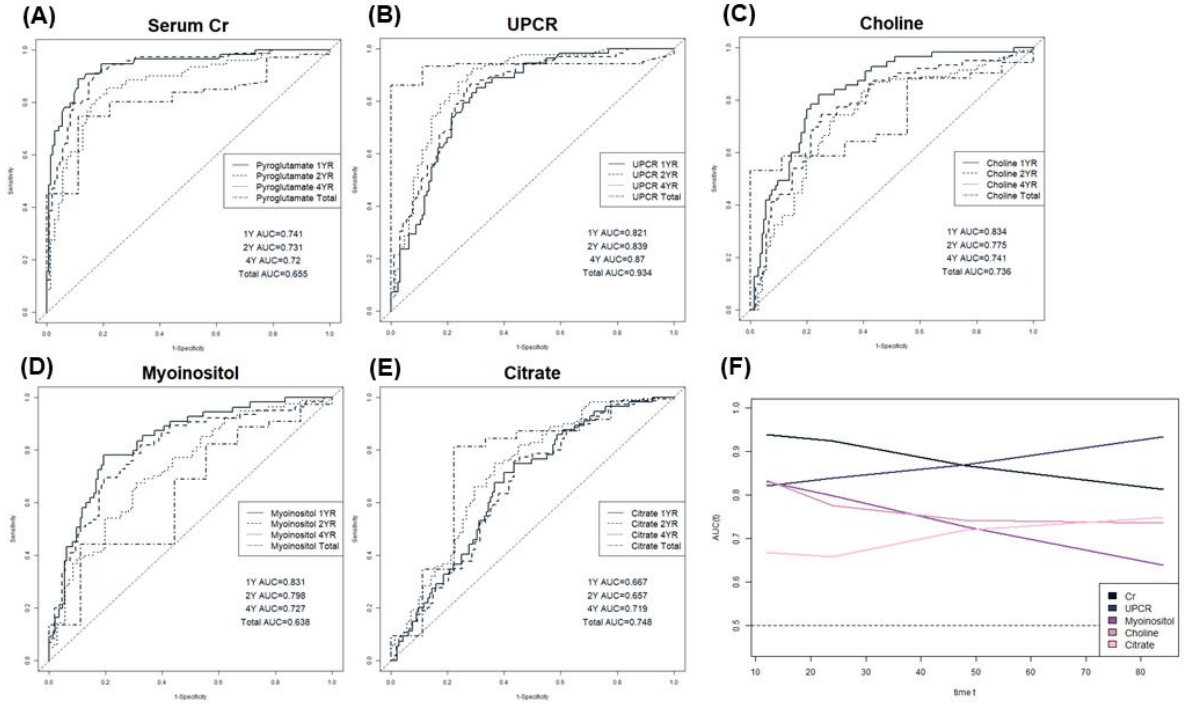
Table 6. Additive effect of urinary metabolites to predict ESRD progression, analyzed by net reclassification improvement (NRI) and integrated discrimination improvement (IDI)

		DeLong test	NRI		IDI	
	AUC (95% CI)		P value	95% CI	P value	95% CI
Creatinine + UPCR	0.905 (0.865−0.945)	Reference	Reference		Reference	
Creatinine + UPCR + Choline	0.904 (0.864−0.945)	0.437	0.318	1.2% (−1.6%, 4.7%)	0.229	24.6% (−67.8%, 54.7%)
Creatinine + UPCR + Myoinositol	0.904 (0.864−0.945)	0.430	0.03	2.9% (0.1%, 8.8%)	0.02	35.1% (5.0%, 51.2%)
Creatinine + UPCR + Citrate	0.911 (0.873−0.949)	0.436	0.627	0.1% (−1.3%, 2.7%)	0.159	10.0% (−11.3%, 25.2%)
Creatinine +UPCR+ 3 metabolite (Choline + Myoinositol + Citrate)	0.888 (0.847−9.300)	0.264	0.03	3.6% (0.0%, 9.2%)	0.02	25.2% (3.4%, 44.1%)

Table 7. Time dependent receiver operating characteristics results of most predictive urinary metabolites on simple receiver operating characteristics

AUC (95% CI)	Serum creatinine	urine PCR	Choline	Myo-inositol	Citrate
12 month	0.939 (0.901-0.978)	0.821 (0.759-0.883)	0.834 (0.774-0.894)	0.831 (0.77-0.893)	0.667 (0.589-0.746)
24 month	0.924 (0.884-0.964)	0.839 (0.777-0.901)	0.775 (0.701-0.849)	0.798 (0.727-0.868)	0.657 (0.577-0.737)
48 month	0.867 (0.808-0.927)	0.87 (0.808-0.932)	0.741 (0.66-0.823)	0.727 (0.648-0.807)	0.719 (0.637-0.800)
Total	0.812 (0.697-0.928)	0.934 (0.875-0.993)	0.736 (0.613-0.859)	0.638 (0.451-0.824)	0.748 (0.550-0.946)

Figure 7. Time-dependent ROC curve of most predictive urinary metabolites compared with serum creatinine concentration and UPCR



Discussion

Given the innovative development, omics technologies are now widely used to identify biomarkers and mechanisms of disease (19). The metabolomics and proteomics approaches has been actively used in CKD and have recently been studied in accordance with the cause as the underlying mechanism differ (5; 20). This study, which involved a large prospective DKD cohort in the Korean population, suggests the predictive value of urinary myo-inositol concentration in ESRD progression by a targeted NMR-based metabolomics

As DKD is a leading cause of ESRD, some previous human and animal studies have been conducted and reported that mitochondrial function is dysregulated and bioenergy metabolism is reduced in DKD (5; 13; 21-24). However, the significant metabolites reported in each study were different, and there were no specific biomarkers identified. To identify

them, I conducted targeted metabolomics based on previous studies. By including all stages of DKD patients and controls, it was able to investigate the trends of each metabolite according to ESRD progression. I also analyzed the association between each metabolites and long term outcome (ESRD progression, all-cause mortality and composite outcome). Based on the multidisciplinary analysis, myo-inositol was found to be the most important metabolite.

Myo-inositol, the most stable and dominant mesocompound of inositol, is defined as sugar alcohol and was previously designated as vitamin B8. In the form of inositol derivatives, inositol triphosphates (IP_3), phosphatidylinositol phosphate lipids (PIP_2/PIP_3) and inositol glycan, inositol composes the eukaryotic cell membrane and works as a secondary messenger in the insulin signaling cascade and insulin resistance (25-27). Furthermore, myo-inositol is associated with the kidney. 80% of myo-inositol is synthesized in the

kidney, and the kidney is the sole organ for myo-inositol catabolism by myo-inositol oxygenase (MIOX) which is a renal tubular specific enzyme (27; 28). Some studies reported intracellular depletion of myo-inositol in DM patients and excessive urinary myo-inositol excretion in human and animal studies (29-31). A cell study suggested an association of MIOX overexpression with renal tubular injury.

As the myo-inositol thought to have insulin-sensitizing effect, myo-inositol supplementation is considered to be putative treatment for PCOS (32; 33). Because of the limitation choice of oral hypoglycemic, many studies investigate the dietary supplementation with myo-inositol in gestational diabetes mellitus (GDM)(34; 35). Furthermore, an in vitro and an in vivo study showed a benefit of myo-inositol supplementation on nephropathy (36; 37). In accordance with the results of this study, myo-inositol may have effects of glycemic control and renoprotection in DKD patients.

Although this results are informative, this study has some limitations. First, even though potential precedent mechanisms are discussed above, we need more detailed mechanical and experimental study. Second, since I obtained only one test per patient, demonstrating the differences between individuals over time may be difficult. In conclusion, urinary myo-inositol concentration can predict ESRD progression in DKD.

Reference

1. Andrassy KM: Comments on 'KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease'. Kidney Int 2013;84:622–623

2. Salgado JV, Neves FA, Bastos MG, Franca AK, Brito DJ, Santos EM, Salgado Filho N: Monitoring renal function: measured and estimated glomerular filtration rates – a review. Braz J Med Biol Res 2010;43:528–536

3. Ferguson TW, Komenda P, Tangri N: Cystatin C as a biomarker for estimating glomerular filtration rate. Curr Opin Nephrol Hypertens 2015;24:295–300

4. Shlipak MG, Mattes MD, Peralta CA: Update on cystatin C: incorporation into clinical practice. Am J Kidney Dis 2013;62:595–603

5. Hocher B, Adamski J: Metabolomics for clinical use and research in chronic kidney disease. Nat Rev Nephrol 2017;13:269–284

6. Kalim S, Rhee EP: An overview of renal metabolomics. Kidney Int 2017;91:61–69

7. Rhee EP: Metabolomics and renal disease. Curr Opin Nephrol

Hypertens 2015;24:371-379

8. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P, Dame ZT, Poelzer J, Huynh J, Yallou FS, Psychogios N, Dong E, Bogumil R, Roehring C, Wishart DS: The human urine metabolome. PLoS One 2013;8:e73076

9. Zhang A, Sun H, Wu X, Wang X: Urine metabolomics. Clin Chim Acta 2012;414:65-69

10. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bragg-Gresham J, Balkrishnan R, Bhave N, Dietrich X, Ding Z, Eggers PW, Gaipov A, Gillen D, Gipson D, Gu H, Guro P, Haggerty D, Han Y, He K, Herman W, Heung M, Hirth RA, Hsiung JT, Hutton D, Inoue A, Jacobsen SJ, Jin Y, Kalantar-Zadeh K, Kapke A, Kleine CE, Kovesdy CP, Krueter W, Kurtz V, Li Y, Liu S, Marroquin MV, McCullough K, Molnar MZ, Modi Z, Montez-Rath M, Moradi H, Morgenstern H, Mukhopadhyay P, Nallamothu B, Nguyen DV, Norris KC, O'Hare AM, Obi Y, Park C, Pearson J, Pisoni R, Potukuchi PK, Repeck K, Rhee CM, Schaubel DE, Schrager J, Selewski DT, Shamraj R, Shaw SF, Shi JM, Shieu M, Sim JJ, Soohoo M, Steffick D, Streja E, Sumida K, Kurella Tamura

M, Tilea A, Turf M, Wang D, Weng W, Woodside KJ, Wyncott A, Xiang J, Xin X, Yin M, You AS, Zhang X, Zhou H, Shahinian V: US Renal Data System 2018 Annual Data Report: Epidemiology of Kidney Disease in the United States. Am J Kidney Dis 2019;73:A7-A8

11. Darshi M, Van Espen B, Sharma K: Metabolomics in Diabetic Kidney Disease: Unraveling the Biochemistry of a Silent Killer. Am J Nephrol 2016;44:92-103

12. Kim NH, Hyeon JS, Kim NH, Cho A, Lee G, Jang SY, Kim MK, Lee EY, Chung CH, Ha H, Hwang GS: Metabolic changes in urine and serum during progression of diabetic kidney disease in a mouse model. Arch Biochem Biophys 2018;646:90-97

13. Lee J, Choi JY, Kwon YK, Lee D, Jung HY, Ryu HM, Cho JH, Ryu DH, Kim YL, Hwang GS: Changes in serum metabolites with the stage of chronic kidney disease: Comparison of diabetes and non-diabetes. Clin Chim Acta 2016;459:123-131

14. Li L, Wang C, Yang H, Liu S, Lu Y, Fu P, Liu J: Metabolomics reveal mitochondrial and fatty acid metabolism disorders that contribute to the development of DKD in T2DM patients. Mol

Biosyst 2017;13:2392-2400

15. Posada-Ayala M, Zubiri I, Martin-Lorenzo M, Sanz-Maroto A, Molero D, Gonzalez-Calero L, Fernandez-Fernandez B, de la Cuesta F, Laborde CM, Barderas MG, Ortiz A, Vivanco F, Alvarez-Llamas G: Identification of a urine metabolomic signature in patients with advanced-stage chronic kidney disease. Kidney Int 2014;85:103-111

16. Levey AS, Stevens LA: Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. Am J Kidney Dis 2010;55:622-627

17. Yoon UA, Kim YC, Lee H, Kwon S, An JN, Kim DK, Kim YS, Lim CS, Lee JP, Kim H: The impact of sunlight exposure on mortality of patients with end stage renal disease. Sci Rep 2019;9:2230

18. Heagerty PJ, Lumley T, Pepe MS: Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 2000;56:337-344

19. Johnson CH, Ivanisevic J, Siuzdak G: Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol

2016;17:451-459

20. Canadas-Garre M, Anderson K, McGoldrick J, Maxwell AP, McKnight AJ: Proteomic and metabolomic approaches in the search for biomarkers in chronic kidney disease. J Proteomics 2019;193:93-122

21. Sharma K, Karl B, Mathew AV, Gangoiti JA, Wassel CL, Saito R, Pu M, Sharma S, You YH, Wang L, Diamond-Stanic M, Lindenmeyer MT, Forsblom C, Wu W, Ix JH, Ideker T, Kopp JB, Nigam SK, Cohen CD, Groop PH, Barshop BA, Natarajan L, Nyhan WL, Naviaux RK: Metabolomics reveals signature of mitochondrial dysfunction in diabetic kidney disease. J Am Soc Nephrol 2013;24:1901-1912

22. Pena MJ, Lambers Heerspink HJ, Hellemons ME, Friedrich T, Dallmann G, Lajer M, Bakker SJ, Gansevoort RT, Rossing P, de Zeeuw D, Roscioni SS: Urine and plasma metabolites predict the development of diabetic nephropathy in individuals with Type 2 diabetes mellitus. Diabet Med 2014;31:1138-1147

23. Ibarra-Gonzalez I, Cruz-Bautista I, Bello-Chavolla OY, Vela-Amieva M, Pallares-Mendez R, Ruiz de Santiago YND, Salas-Tapia MF, Rosas-Flota X, Gonzalez-Acevedo M, Palacios-Penaloza A,

Morales-Esponda M, Aguilar-Salinas CA, Del Bosque-Plata L: Optimization of kidney dysfunction prediction in diabetic kidney disease using targeted metabolomics. Acta Diabetol 2018;55:1151-1161

24. Chang HH, Choong B, Phillips AR, Loomes KM: The diabetic rat kidney mediates inosituria and selective urinary partitioning of D-chiro-inositol. Exp Biol Med (Maywood) 2015;240:8-14

25. Manning BD: Insulin signaling: inositol phosphates get into the Akt. Cell 2010;143:861-863

26. Larner J, Brautigan DL, Thorner MO: D-chiro-inositol glycans in insulin signaling and insulin resistance. Mol Med 2010;16:543-552

27. Croze ML, Soulage CO: Potential role and therapeutic interests of myo-inositol in metabolic diseases. Biochimie 2013;95:1811-1827

28. Hauser G, Finelli VN: The Biosynthesis of Free and Phosphatide Myo-Inositol from Glucose by Mammalian Tissue Slices. J Biol Chem 1963;238:3224-3228

29. Kennington AS, Hill CR, Craig J, Bogardus C, Raz I, Ortmeyer

HK, Hansen BC, Romero G, Larner J: Low urinary chiro-inositol excretion in non-insulin-dependent diabetes mellitus. N Engl J Med 1990;323:373-378

30. Kawa JM, Przybylski R, Taylor CG: Urinary chiro-inositol and myo-inositol excretion is elevated in the diabetic db/db mouse and streptozotocin diabetic rat. Exp Biol Med (Maywood) 2003;228:907-914

31. Haneda M, Kikkawa R, Arimura T, Ebata K, Togawa M, Maeda S, Sawada T, Horide N, Shigeta Y: Glucose inhibits myo-inositol uptake and reduces myo-inositol content in cultured rat glomerular mesangial cells. Metabolism 1990;39:40-45

32. Unfer V, Carlomagno G, Dante G, Facchinetti F: Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecol Endocrinol 2012;28:509-515

33. Regidor PA, Schindler AE, Lesoine B, Druckman R: Management of women with PCOS using myo-inositol and folic acid. New clinical data and review of the literature. Horm Mol Biol Clin Investig 2018;34

34. Brown J, Crawford TJ, Alsweiler J, Crowther CA: Dietary

supplementation with myo-inositol in women during pregnancy for treating gestational diabetes. Cochrane Database Syst Rev 2016;9:CD012048

35. American Diabetes A: 14. Management of Diabetes in Pregnancy: Standards of Medical Care in Diabetes-2019. Diabetes Care 2019;42:S165-S172

36. Cohen AM, Wald H, Popovtzer M, Rosenmann E: Effect of myo-inositol supplementation on the development of renal pathological changes in the Cohen diabetic (type 2) rat. Diabetologia 1995;38:899-905

37. Ziyadeh FN, Simmons DA, Snipes ER, Goldfarb S: Effect of myo-inositol on cell proliferation and collagen transcription and secretion in proximal tubule cells cultured in elevated glucose. J Am Soc Nephrol 1991;1:1220-1229

요약 (국문초록)

당뇨성 신장 질환은 만성 신장 질환과 말기 신장 질환의 주요 원인이다. 대사체학은 세포 대사를 분석하는 학문으로써, 세포 대사의 변화를 토대로 새로운 치료법을 제시할 수 있을 것이란 기대로 만성 신장 질환 환자를 대상으로 한 연구가 증가하고 있다. 당뇨성 신장 질환 환자에서 대사체학적 분석이 과거 수차례 시도되었으나 아직 명확한 결론이 나지 않았다. 이에 당뇨성 신병증 환자의 소변에서 800 HMz NMR 기법의 표적 대사체학을 시행, 표적 대사체와 당뇨성 말기 신장 질환 진행 간의 연관성을 분석하였다.

당뇨성 신장 질환 제1기부터 5기까지 총 208명의 환자와 신장 질환이 없는 건강한 대조군 26명 소변으로 전향적으로 모아 분석하였다. 우선, 소변에서 과거 연구를 통해 알려진 26개의 대사체를 측정하여 각 대사체의 측정값과 대사체 측정 시기에 시행한 사구체 여과율 그리고 소변 단백 크레아티닌 비의 연관성을 평가하였다. 각 대사체의 측정값과 말기 신장 질환 이행 및 사망과의 연관성을 분석하기 위해 콕스 모델을 사용하였다. 또한, 대사체를 추가로 고려하였을 때 말기 신장 질환 이행 예측력이 개선되는지 평가하기 위해 C 통계량을 이용하였다.

전체 환자에서 총 103명(44.0%)이 말기 신장 질환으로 진행하였으며 65명(27.8%)이 사망하였다. 대조군과 비교하였을 때 당뇨성 신병증 환자에서 9개의 대사체 (포도당, 만노오스, 마이오-이노지톨, 글리세롤, 락테이트, 푸마레이트, 크레이틴, 타우린 및 콜린)의 중간값이 신장 질환의 정도에 따른 경향성을 보였다. 선형 회귀 분석에서 마이오-이노지톨이 말기 신장 질환 진행 예측의 주요 인자인 사구체 여과율과 관련성이 가장 높았다.

소변 대사체와 말기 신장 질환 이행 및 사망 간의 연관성을 기본 공변량을 보정하여 콕스 비례 위험 모델로 분석하였다. 4가지 대사체 (마이오-이노지톨, 글라이세롤, 푸마레이트, 옥소 이소 카프로에이트)가 말기 신장 질환 이행 예측력이 있었으며, 이중 마이오-이노지톨만이 유의하게 사망을 예측하였다. C 통계에서도 마이오-이노지톨만이 말기 신장 질환 예측력을 높였다. (NRI = 2.9%, P = 0.03; IDI = 35.1%, p=0.02).

이 연구는 과거 비타민 B8으로 알려진 마이오-이노지톨이 당뇨성 환자에서 말기 신장 질환으로 진행할 위험을 예측하는 데 도움이 될 수 있음을 제시한다. 이를 기반으로 인슐린의 세포 내 이차 전달 물질인 마이오-이노지톨이 당뇨성 신질환과 기전적 연관성이 있을지 추가 기전 연구가 필요하다.

주요어: 당뇨병성 신장 질환, 대사체학, 마이오-이노지톨, 말기 신장 질환, 사망률

학 번 : 2018-28788