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ORIGINAL ARTICLE

Optimization of kidney dysfunction prediction in diabetic kidney disease using targeted metabolomics

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Abstract

Aims Metabolomics have been used to evaluate the role of small molecules in human disease. However, the cost and complexity of the methodology and interpretation of findings have limited the transference of knowledge to clinical practice. Here, we apply a targeted metabolomics approach using samples blotted in filter paper to develop clinical-metabolomics models to detect kidney dysfunction in diabetic kidney disease (DKD).

Methods We included healthy controls and subjects with type 2 diabetes (T2D) with and without DKD and investigated the association between metabolite concentrations in blood and urine with eGFR and albuminuria. We also evaluated performance of clinical, biochemical and metabolomic models to improve kidney dysfunction prediction in DKD.

Results Using clinical-metabolomics models, we identified associations of decreased eGFR with body mass index (BMI), uric acid and C10:2 levels; albuminuria was associated to years of T2D duration, A1C, uric acid, creatinine, protein intake and serum C0, C10:2 and urinary C12:1 levels. DKD was associated with age, A1C, uric acid, BMI, serum C0, C10:2, C8:1 and urinary C12:1. Inclusion of metabolomics increased the predictive and informative capacity of models composed of clinical variables by decreasing Akaike's information criterion, and was replicated both in training and validation datasets.

Conclusions Targeted metabolomics using blotted samples in filter paper is a simple, low-cost approach to identify outcomes associated with DKD; the inclusion of metabolomics improves predictive capacity of clinical models to identify kidney dysfunction and DKD-related outcomes.

Keywords Metabolomics · Type 2 diabetes · Diabetic kidney disease · Filter paper · Amino acids · Acylcarnitines

Abbreviations

DKD	Diabetic kidney disease
DBS	Dried blood samples
T2D	Type 2 diabetes
eGFR	Estimated glomerular filtration rate
A1C	Glycosylated hemoglobin
ACEI/ARB	Angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
U/B ratio	Ratio or urinary divided by blood concentration of measured metabolites
ARG	Arginine
CIT	Citrulline
GLY	Glycine
ALA	Alanine
LEU	Leucine + isoleucine
MET	Methionine
PHE	Phenylalanine
TYR	Tyrosine
VAL	Valine
ORN	Ornithine

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PRO	Proline
SA	Succinylacetone
C0	Free carnitine
C2	Acetylcarnitine
C3	Propionylcarnitine
C4OH\C3DC	3-Hydroxybutyryl + malonyl carnitine
C5OH\C4DC	3-Hydroxyisovaleril + methylmalonyl carnitine
C5DC\C6OH	Glutaryl + 3-hydroxyhexanoyl carnitine
C6DC	Adipylcarnitine
C4	Butyrylcarnitine
C5	Isovalerylcarnitine
C5:1	Tiglylcarnitine
C6	Hexanoylcarnitine
C8	Octanoylcarnitine
C8:1	Octenoylcarnitine
C16	Decanoylcarnitine
C16:1	Decenoylcarnitine
C16:1OH	Decadienoylcarnitine
C16OH	Dodecanoylcarnitine
C10	Dedecenoylcarnitine
C10:1	Tetradecanoylcarnitine
C10:2	Tetradecenoylcarnitine
C12	Tetradecadyenylcarnitine
C12:1	3-Hydroxy-tetradecanoylcarnitine
C14	Hexadecenoylcarnitine
C14:1	Hexadecenoylcarnitine
C14:2	3-Hydroxy-hexadecanoylcarnitine
C14OH	3-Hydroxy-hexadecenoylcarnitinae
C18	Octadecanoylcarnitine
C18:1	Octadecenoylcarnitine
C18:1OH	Octadecenoylcarnitine
C18:2	3-Hydroxy-octadecanoylcarnitine
C18OH	3-Hydroxy-octadecanoylcarnitine

Introduction

Diabetic kidney disease (DKD) is a diabetes complication whose clinical diagnosis is made based on the presence of albuminuria and/or reduced estimated glomerular filtration rate (eGFR) in the absence of signs or symptoms of other primary causes of kidney damage [1]. DKD imposes significant burden in patients by increasing mortality risk and posing barriers for allotransplantation in advanced stages [2, 3]. Early DKD alterations include glomerular hypertrophy, mesangial expansion and basal membrane thickening, with late changes characterized by nodular sclerosis. Unfortunately, identification of histological alterations is costly, invasive and has not shown independent correlation with clinical outcomes [4, 5]. This has led to development of biomarkers to improve identification and screening of DKD. Existing kidney dysfunction indicators in DKD include serum creatinine and albuminuria, both of which have major limitations [6–8]. Serum creatinine concentration changes until there is an important loss of renal function. In addition, renal function is overestimated by the amount of tubular secretion of creatinine and varies per age, gender, muscle mass and metabolism, body weight, protein and water intake [5]. Albuminuria onset demonstrates established glomerular dysfunction but is not exclusive of DKD, despite being required for diagnosis [2, 7]. Additional kidney function markers in DKD include cystatin-C and the neutrophil gelatinase-associated lipocalin (NGAL), which have been shown to correlate with kidney dysfunction in T2D; nevertheless, their clinical usefulness remains to be further studied [7, 8]. Therefore, development of novel biomarkers that correlate with kidney dysfunction and improve identification of DKD at earlier stages is still an unmet necessity [2, 8].

The study of the metabolome aims to identify small-molecule profiles of complex biological samples instead of individual metabolites to improve the informative capacity of biochemical analyses [9–12]. The utility of metabolomics in DKD has been proved by different groups, who have reported abnormal plasma concentrations of amino acids and acylcarnitines associated with risk of progression of kidney disease [13–15]. Targeted metabolomics is an alternative for the study of relatively high number of samples, with the advantage that it can be performed on dry biological samples blotted in filter paper, which is a low-cost approach to store and handle biological samples [15]. To the best of our knowledge, this approach has not been applied in studies of DKD. This work aims to propose the complementary use of metabolomics to improve kidney dysfunction prediction in DKD.

Materials and methods

Subjects and study setting

We performed a cross-sectional evaluation of 200 T2D individuals who were recruited from our Diabetes Clinic and healthy subjects from the Metabolic syndrome cohort of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), which is a cohort study that includes a large number of subjects with and without T2D. We divided the study population into three groups: (1) Healthy normotensive, non-obese subjects with normal kidney function, (2) T2D subjects without DKD (T2Dnon-DKD) with diabetes diagnosis duration ≥ 10 years and (3) T2D with DKD (T2DDKD) with ≥ 10 years diabetes duration and any grade of diabetic retinopathy to confirm microvascular disease. We excluded subjects with other causes of kidney disease, previous acute ischemic heart disease or any condition that may alter albumin excretion or creatinine

clearance. T2D was diagnosed according to standardized ADA recommendations. Albuminuria was defined by urinary albumin > 30 mg/24 h and DKD by the presence of either albuminuria and/or eGFR < 60 mL/min/1.73 m^2. The Human Research Ethics Committee of the INCMNSZ approved all proceedings in the study.

Biochemical and anthropometric measurements

Twenty-four hour urine sample recollection was performed the day prior to biochemical evaluation; patients received a container with 200 µl of protease inhibitor (aprotinin protease inhibitor 500 KIU/mg, Thermo Fisher Scientific) and were instructed to collect urine during a 24 h period. After recollection, blood was drawn between 8:00–9:00 am after an overnight fast of 8–12 h. Glucose, total cholesterol, triglycerides, uric acid, serum and urine creatinine, HDL cholesterol, and albuminuria were determined by enzymatic colorimetric commercially available reagents using the Synchron CX Delta (Beckman Coulter); A1C levels were measured by high-performance liquid chromatography. For GFR estimation (eGFR), we calculated creatinine clearance using a 24 h creatinine measurement [16, 17].

In the same visit, a complete medical and family history was obtained from all subjects, including evaluation of dietary protein intake from 1 month and the day prior to urine recollection. Patients were weighed on calibrated scales and height was determined with a floor scale stadiometer. We consigned all medications used by patients; none of the subjects were receiving carnitine or fatty acid supplements during the evaluation.

Metabolomic analyses

Fasting capillary blood and 24 h urine samples obtained the day of biochemical evaluation were collected in filter paper cards (Protein Saver 903 cotton cards, Whatman-GE, USA), dried and conserved in refrigeration until analysis, performed as previously described [18, 19]. Eleven amino acids, free carnitine and 30 acylcarnitines were measured with a commercial kit (NeoBase Non-derivatized MS/MS kit; PerkinElmer Waltham Massachusetts). From each sample, a 3 mm diameter disk was punched with an automatic device (Dried blood spot punch Wallac 1296-071) into a 96-well sample plate and 190 µL of extraction solution containing a mixture of 22 stable isotope-labeled internal standards were added. The plate was covered with aluminum foil, incubated with agitation (30 °C at 650×*g* for 30 min). 30 µL of sample extracts were directly injected by a 2777 C Waters auto-sampler (Waters Corp., Milford, MA and HPLC pump Waters 1525 µ) to the electrospray tandem mass spectrometry equipment (Quattro Micro API tandem MS using multiple reaction monitoring (MRM) mode), with a flow rate of 1.5 mL/min and an analysis time of 1.5 min. Metabolites were quantified by reference to appropriate internal standards with the MassLynx® software. Low and high analytical controls were included in each plate in triplicate; additionally, a blank sample (extraction solution with internal standards) was included in each plate. Intra- and inter-plate variation coefficients were calculated based on repetitive measurements of the analytical control sample. Inter- and intra-assay variation coefficients ranged from 5 to 9%.

Statistical analysis

Inter-group comparisons

We used Kolmogorov–Smirnov test to explore distribution of each variable. Log and inverse transformations were applied to approximate normality in variables showing non-parametric distribution. Data are presented as mean ± SD or as median and interquartile range, where appropriate. Categorical variables are reported as frequencies and percentages; frequency distribution of categorical variables between groups was compared using chi-squared tests. To evaluate inter-group differences in individual metabolites, we compared metabolite concentrations across groups using ANOVA and Fisher's LSD for multiple post hoc comparisons. To evaluate the association between metabolites, eGFR and albuminuria, we performed partial correlation analysis adjusted for BMI, A1C, gender, ACRI/ARB use, SBP, DBP and dietary protein intake.

Linear clinical-metabolomics models

We developed manual step-wise multiple linear regression models using eGFR and albuminuria as dependent variables to evaluate the association of clinical, biochemical and metabolomic variables adjusted for age, sex, A1C, ACEI/ARB use, protein consumption, SBP and DBP. Variables were removed from the model until the best fitting model with the maximum adjusted r^2 was achieved; to confirm the improvement in the informative capacity of the model we used the Akaike information criterion (AIC) in both models. Models were also tested for multicollinearity using both tolerance and variance inflation factor (VIF). The models were validated using cross-validation derived from a training and validation samples randomly split from the original cohort to correct for over-optimism. Variables selected to enter regression analyses were those correlated significantly with albuminuria and eGFR.

Binary logistic clinical-metabolomics models

Variables associated with eGFR and albuminuria in linear regression analyses were included in binary logistic

regression models to detect decreased eGFR (< 60 mL/min) and albuminuria (> 30 mg/24 h), adjusted for age, sex, A1C levels, ACEI/ARB use, protein intake, SBP and DBP. The models were also validated using cross-validation. The performance of the models to be concordant with clinical outcomes was assessed using the area under a receiver operating characteristic (ROC) curve (Harrel's *c statistic*) of estimated probabilities obtained from regression analyses and goodness of fit was assessed using the Hosmer–Lemeshow test. A $p < 0.05$ was considered statistically significant. All statistical analyses were performed using Statistical Package for Social Sciences software (SPSS, version 21.0) and R software (Version 3.4.5).

Results

Clinical and biochemical characteristics of studied subjects

Two-hundred subjects were evaluated as follows: 43 subjects in the control group, 102 in the T2DnonDKD group and 55 in the T2DDKD group (Table 1). Recruitment process and sample size calculation are outlined in Supplementary Material. DKD subjects were significantly older, mostly male and had higher triglyceride, creatinine, A1C and lower HDL-c concentrations compared to other groups ($p < 0.001$). Seventy-four subjects were treated with ACEI/ARB, 19 subjects had overt DN (albuminuria > 300 g/24 h) and 24 subjects had eGFR < 60 mL/min/1.73 m^2.

Metabolite concentrations between groups and correlation with albuminuria and eGFR

We observed significant differences between T2DDKD and other groups in concentrations of blood C0, citrulline, tyrosine and C6, urinary C10:1, U/B proline, C6, C8, C10:1 and C10:2 ($p < 0.001$, Table 2). Next, we explored correlation between albuminuria, eGFR and metabolomic variables, which are shown in Table 3.

Linear clinical-metabolomics models

Using step-wise linear regression, we constructed a clinical model to predict albuminuria using A1C, serum creatinine and dietary protein intake, adjusted for age, sex, ACEI/ARB use, SBP and DBP (Table 4); when we introduced metabolomics, we found significant associations for serum citrulline, C0, C10:2 and urinary C12:1, which increased the explained variability of the model and decreased the AIC, thus improving informative capacity of the model. When using eGFR as the dependent variable, significant clinical associations included A1C, years of T2D exposure and uric acid; the inclusion of metabolomics increased the explained

Table 1 Clinical and biochemical characteristics of studied patients

Parameter	Healthy individuals ($N = 43$)	T2DnonDKD ($N = 102$)	T2DDKD ($N = 55$)	P
Female sex (%)	28 (65.1%)	69 (67.6%)	20 (36.4%)	< 0.001
Age (years)	54.65 ± 9.08*	60.40 ± 8.22	61.71 ± 8.82	< 0.001
BMI (kg/m^2)	25.21 ± 3.51*	28.17 + 3.95	27.11 + 3.87	< 0.001
Waist/hip ratio	0.89 ± 0.15*	0.92 ± 0.07	0.94 ± 0.08	0.015
Systolic BP (mmHg)	105.09 ± 13.79*	125.51 ± 16.79	130.6 ± 19.38	< 0.001
Diastolic BP (mmHg)	70.25 ± 8.87*	74.4 ± 9.98	76.71 ± 11.30	0.004
Fasting glucose (mg/dL)	95.34 ± 9.94*	160.58 ± 57.84	161.03 ± 78.83	< 0.001
A1C (%)	5.58 ± 0.36*	8.5 ± 2.03^{+}	9.35 ± 2.24	< 0.001
Triglycerides (mg/dL)	129.0 (80.0-198.0)*	168.0 (111.3-241.3)	184.0 (126.0-230.0)	0.02
Total cholesterol (mg/dL)	203.28 ± 39.70*	184.32 ± 41.77	193.55 ± 41.21	0.08
HDL-C (mg/dL)	55.53 ± 15.61*	46.14 ± 13.08	46.24 ± 15.41	0.002
LDL-C (mg/dL)	118.35 ± 28.40*	100.23 ± 33.91	105.19 ± 30.45	0.02
Serum creatinine (mg/dl)	0.75 ± 0.18	0.73 ± 0.18	1.17 ± 0.84$^{\&}$	< 0.001
Albuminuria (mg/24 h)	4.02 (0.0-5.90)	7.05 (2.75–13.17)	120.0 (37.8-554.4) $^{\&}$	< 0.001
Uric acid (mg/dL)	5.23 ± 1.28	5.18 ± 1.17	6.21 ± 1.56$^{\&}$	< 0.001
Diabetes duration (yr)	–	16.5 ± 7.31	20.09 ± 8.82	< 0.001
eGFR (ml/min/1.73 m^2)	90.44 (79.5-106.7)	99.8 (76.4-120.3)	73.3 (52.6-104.7)$^{\&}$	< 0.001

Values are means ± SD, unless indicated otherwise

T2D Type 2 diabetes mellitus, *DKD* diabetic kidney disease, *BMI* body mass index, *BP* blood pressure, *A1C* glycosylated hemoglobin, *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *eGFR* estimated glomerular filtration rate

*$p < 0.001$ Healthy vs. T2DnonDKD and T2DDKD, $^{+}$T2DnonDKD vs. T2DDKD, $^{\&}$T2DDKD vs. healthy and T2DnonDKD

Table 2 Levels of amino acids and acylcarnitines in blood, urine and the urine/blood ratio

Sample	Metabolite concentration (μM)	Healthy subjects median ± IQR	T2DnonDKD median ± IQR	T2DDKD median ± IQR
Blood	Citrulline	24.8 (20.2–30.2)*	15.2 (11.4–20.5)[&]	19.5 (16.2–27.0)
	Methionine	5.1 (3.8–6.1)*	3.1 (2.3–4.4)	3.2 (2.6–4.5)
	Phenylalanine	42.9 (38.5–47.2)*	35.8 (31.2–40.3)	36.2 (30.9–41.2)
	Tyrosine	56.3 (51.0–67.2)*	50.0 (40.8–60.7)[&]	46.0 (39.3–53.2)
	Valine	141.8 (122.1–160.1)[#]	119.2 (102.4-139.3)	120.0 (100.0–141.2)
	Ornithine	34.0 (30.0–42.4)*	14.7 (10.0-28.7)	16.8 (12.6–27.6)
	Carnitine	33.6 (28.0–38.7)[#]	34.1 (27.7–41.9)[&]	40.7 (32.1–47.6)
	C4OH:C3D	0.04 (0.03–0.05)*	0.04 (0.03–0.06)	0.05 (0.04–0.06)
	C5DC\C6OH	0.10 (0.09–0.12)[#]	0.13 (0.10–0.16)	0.14 (0.11–0.17)
	C6	0.04 (0.03–0.04)[#]	0.03 (0.027–0.040)[&]	0.04 (0.03–0.05)
	C8	0.08 (0.06–0.10)	0.07 (0.05–0.10)[&]	0.08 (0.06–0.14)
	C8:1	0.12 (0.09–0.14)[#]	0.13 (0.09–0.17)[&]	0.15 (0.10–0.22)
	C10:2	0.01 (0.01–0.02)[#]	0.01 (0.01–0.02)[&]	0.02 (0.01–0.02)
	C14:1	0.05 (0.04–0.07)*	0.04 (0.03–0.05)	0.05 (0.03–0.06)
	C14:2	0.02 (0.02–0.03)*	0.02 (0.01–0.02)	0.02 (0.01–0.02)
	C18:1OH	0.02 (0.02–0.03)[#]	0.02 (0.02–0.03)	0.02 (0.02–0.03)
Urine	Glycine	1855.1 (1042.9-3242.4)[#]	1614.2 (1025.7–3048.0)	1356.4 (716.5-2229.1)
	Proline	28.1 (22.9–37.5)*	51.3 (38.9–81.8)	65.1 (35.7-130.1)
	C10:1	1.13 (0.81–2.09)*	1.00 (0.71–1.49)[&]	0.90 (0.65–1.48)
	C12:1	0.76 (0.64–1.05)[#]	0.73 (0.55-1.00)[&]	0.58 (0.42–0.88)
Urine/blood	Citrulline	0.70 (0.41–1.01)*	1.12 (0.82–1.85)[+]	1.24 (0.78–1.90)
	Methionine	3.67 (2.37–5.28)*	6.23 (4.18–9.48)	5.93 (3.83–9.66)
	Ornithine	0.87 (0.62–1.19)*	2.08 (1.18–3.80)	2.05 (1.26–3.18)
	Proline	0.20 (0.14–0.27)*	0.32 (0.24–0.51)[&]	0.40 (0.26–0.81)
	C6	30.0 (18.6–75.2)*	25.6 (15.5–40.6)	16.7 (9.8–36.5)
	C8	18.1 (10.1–30.9)[#]	17.3 (9.3–23.4)	12.3 (7.5–19.1)
	C10:1	69.0 (40.5-103.9)[#]	64.4 (43.9–88.3)[&]	34.4 (21.0-61.5)
	C10:2	279.3 (167.9-370.6)[#]	196.52 (144.9-345.5)[&]	148.3 (76.4–201.0)
	C12:1	16.8 (11.5–21.1)[#]	18.6 (14.4–25.1)[&]	13.4 (11.1–17.6)
	C14:2	15.2 (12.3–22.0)*	27.4 (17.6–37.3)	20.6 (14.6–31.5)

Post hoc analysis by Fisher LSD. *p* values shown are differences between the group with DKD and the other groups

T2D Type 2 diabetes mellitus, *DKD* diabetic kidney disease, *IQR* interquartile range

*p value < 0.05 for healthy vs. T2DnonDKD and T2DDKD

[+]$p < 0.05$ for healthy vs. T2DnonDKD

[#]$p < 0.05$ for healthy vs. T2DDKD

[&]$p < 0.05$ for T2DnonDKD vs. T2DDKD

variability of the model, with significant associations for serum citrulline, C8:1, C10:2, urinary C10:1 and U/B proline (Table 4), which increased the $r^2$and decreased the AIC of the model.

Logistic clinical-metabolomics models

When evaluating specific outcomes (Table 5), decreased eGFR was significantly associated with BMI and uric acid levels. The inclusion of metabolomics (C10:2) increased the explained variability and AUC of the model. Similarly, albuminuria > 30 mg/day was associated to years of T2D exposure, A1C, uric acid, creatinine and protein intake; the inclusion of serum C0, C10:2 and urinary C12:1 increased the r^2 and AUC of the models. Finally, we developed a clinical-metabolomics model for DKD, which included BMI, A1C, uric acid, age, C0, C10:2, C8:1 and urinary C12:1, which had a higher r^2 and AUC compared to the clinical model.

Table 3 Partial correlations between microalbuminuria and eGFR with biochemical and metabolomic variables adjusted by blood pressure, A1C, gender, age, and body mass index

		Albuminuria		Glomerular filtration rate	
Blood	Variable	*r*	*p* Value	*r*	*p* Value
	Citrulline	0.293	< 0.001	− 0.282	< 0.001
	C0	0.272	< 0.001	− 0.234	< 0.001
	C5DC/C6O	0.045	0.53	− 0.26	< 0.001
	C6	0.233	< 0.001	− 0.232	< 0.001
	C8	0.15	0.037	− 0.161	0.025
	C8:1	0.15	0.037	− 0.438	< 0.001
	C10:2	0.312	< 0.001	− 0.474	< 0.001
	C14:1	0.124	0.085	− 0.173	0.016
	C14:2	0.111	0.124	− 0.181	0.012
	C18:1OH	0.199	0.005	− 0.133	0.065
Urine	Glycine	− 0.122	0.09	0.208	0.004
	Proline	0.152	0.035	− 0.266	< 0.001
	C10:1	− 0.225	0.002	0.312	< 0.001
	C12:1	− 0.242	0.001	0.245	0.001
Urine/blood	Proline	0.154	0.032	− 0.257	< 0.001
	C6	− 0.142	0.048	0.214	0.003
	C8	− 0.165	0.022	0.22	0.002
	C10	− 0.195	0.007	0.255	< 0.001
	C10:1	− 0.29	< 0.001	0.439	< 0.001
	C10:2	− 0.288	< 0.001	0.392	< 0.001
	C12:1	− 0.312	< 0.001	0.383	< 0.001
	C14:2	− 0.204	0.004	− 0.207	0.004

Clinical-metabolomics model validation

We then evaluated both the linear and binary logistic regression models using training ($N = 118$) and validation datasets ($N = 82$) to correct findings for over-optimism and validate the findings. We observed an increase in r^2 and a decrease in AIC with the inclusion of metabolomics without evidence of multicollinearity for all models, which was replicated in both datasets (Table 6).

Discussion

Our study highlights the development of clinical-metabolomics models related to kidney dysfunction in DKD. Here, we replicate previously reported abnormalities in metabolomics linked to DKD using a validated method of dried biological samples (DBS) blotted in filter paper. This method may facilitate sample handling and could be applied in large-scale efforts to identify new metabolomics-based biomarkers of DKD. Targeted metabolomics has recently been introduced in the study of diverse models of disease and its use in DKD has previously been reported in some animal models and more recently in human subjects. Differences in plasma and/or urine metabolomics between T2DDKD and controls suggest that this condition is associated with abnormalities in glycolysis and lipid and amino acid pathways [20–25]. At this time, identification of specific biomarkers of DKD using metabolomics is a topic that remains largely unexplored [26].

First, we evaluated clinical-metabolomics profiles that explain the variability in identifying kidney dysfunction in our cohort. We observed that the inclusion of metabolomic and clinical variables improved the explained variability of linear models for albuminuria and eGFR and yielded predictive improvements. In addition, performances of estimated probabilities from clinical models are improved with the inclusion of metabolomics to detect decreased glomerular function, albuminuria and DKD. These observations are consistent with the expected course of kidney dysfunction in DKD, since models included A1C, BMI and years of T2D exposure, as well as protein intake for albuminuria and markers of kidney dysfunction including serum creatinine and uric acid, which is a byproduct of purine metabolism and is elevated in the setting of cellular hypoxia, oxidative stress and inflammation, processes which have been linked to kidney dysfunction, particularly albuminuria, in DKD [27, 28]. Overall, our results demonstrate that inclusion of metabolomics improves the detection threshold of glomerular dysfunction over traditional clinical variables and confirms the significance of studying metabolomics to evaluate kidney dysfunction patients with DKD [29–32].

As our observations confirmed, altered metabolic pathways in amino acid biosynthesis might be relevant in DKD [33]. Citrulline concentrations are decreased in subjects with T2D regardless of the presence of DKD. However, we observed higher serum concentrations in T2DDKD compared to T2DnonDKD. Elevated levels of citrulline and other urea cycle metabolites have been shown to be related to kidney disease progression in T2D [22, 25, 26]. A possible alteration of citrulline to arginine conversion has been proposed as an explanation, given that these metabolites normally compete with endothelial nitric oxide synthase to increase nitric oxide production, stabilizing endothelial function [34]. In the case of eGFR, increased urinary and U/B proline were also identified in T2DDKD subjects, which indicates increased proline production. High proline levels have been related to insulin deprivation and products of proline metabolism have been linked to glomerular dysfunction in advanced chronic kidney disease [34, 35]. Finally, we observed altered blood concentrations of phenylalanine in patients with DKD, which is similar to previous reports of low tyrosine levels in patients with type 2 diabetes and advanced CKD [36]. Plasma elevations of acylcarnitines in patients with albuminuria has previously been described [37, 38], in our work we only found differences in U/B medium

Table 4 Multiple linear regression analysis showing independent variables associated with albuminuria and glomerular filtration rate

	Model	Parameters	Parameters	β	Standarized β	t	p value	95% CI
Albuminuria	Clinical	$R^2 = 0.318$ $F = 10.772$ $P < 0.001$ AIC = 224.26	A1C	0.191	0.208	3.016	0.003	0.066–0.317
			T2D duration	0.041	0.201	2.542	0.012	0.009–0.073
			Protein intake	−0.776	−0.217	−3.257	0.001	−1.246 to −0.306
	Clinical + metabolomics	$R^2 = 0.460$ $F = 13.376$ $P < 0.001$ AIC = 183.57	A1C	0.256	0.279	4.408	< 0.001	0.142–0.371
			T2D duration	0.031	0.152	2.062	0.041	0.001–0.060
			Protein intake	−0.616	−0.172	−2.855	0.005	−1.041 to −0.190
			Citrulline	2.508	0.217	3.126	0.025	0.213–3.087
			C0	2.441	0.137	2.058	0.002	1.290–5.438
			C10:2	2.256	0.175	2.703	0.025	0.001–0.020
			C12:1 U	−2.187	−0.213	−3.365	< 0.001	−3.982 to −1.577
eGFR	Clinical	$R^2 = 0.395$ $F = 13.146$ $P < 0.001$ AIC = −392.14	A1C	0.034	0.180	2.750	0.007	0.010–0.059
			T2D duration	−0.008	−0.196	−2.622	0.010	−0.014 to −0.002
			Uric acid	−0.116	−0.372	−5.902	< 0.001	−0.155 to −0.077
	Clinical + metabolomics	$R^2 = 0.650$ $F = 24.056$ $P < 0.001$ AIC = −490.0	A1C	0.041	0.218	4.085	< 0.001	0.021–0.061
			Uric acid	−0.065	−0.209	−4.101	< 0.001	−0.097 to −0.034
			Citrulline	−0.333	−0.136	−2.688	0.008	−0.577 to -0.088
			C8:1	−0.537	−0.233	−4.314	< 0.001	−0.782 to −0.291
			C10:2	−0.003	−0.205	−4.054	< 0.001	−0.005 to −0.002
			C10:1 U	0.462	0.300	6.083	< 0.001	0.312–0.611
			Proline U/B	−0.329	−0.259	−5.400	< 0.001	−0.449 to −0.209

Models adjusted by age, sex, BMI, SBP, DBP, A1C, T2D duration and ACEI/ARB use

T2D Type 2 diabetes, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* dyastolic blood pressure, *A1C* glycosylated hemoglobin, *eGFR* estimated glomerular filtration rate

and large chain acylcarnitines between T2DDKD and the other groups. Previous reports have suggested that accumulation of various acylcarnitines in plasma demonstrates impaired metabolite clearance due to CKD and other authors have proposed that an increase of urinary acylcarnitines is associated with early kidney damage, reflecting alterations in the β-oxidation pathway, which has also demonstrated alterations in murine models of diabetic nephropathy [39–44]. The observed progressive increase of serum carnitine concentrations in our study ranging from controls, T2DnonDKD and T2DDKD subjects has been formerly documented as related to impairment of acylcarnitine excretion and decreased carnitine clearance, indicating mitochondrial damage, which could lead to activation of oxidative stress pathways [43, 45]. Available evidence supports the benefit of carnitine supplementation in hemodialyzed T2D subjects, but the use of carnitine in DKD deserves more profound studies [40]. The contribution of acylcarnitines to our clinical-metabolomics models to identify diseased individuals with albuminuria or decreased glomerular function, indicates the elevated importance of acylcarnitines as markers of glomerular disease in DKD and its implications in identifying kidney dysfunction in subjects with T2D. Future studies should evaluate the role of metabolomics to evaluate treatment response and prediction of changes in kidney function related to acylcarnitine supplementation in individuals with DKD, as well as the role metabolomics to evaluate the impact of T2D medication in ameliorating kidney dysfunction [46]; proving such approach could be useful as a further clinical application for metabolomics research.

As shown, we were able to replicate findings from previous metabolomics approaches using DBS blotted in filter paper, which demonstrates that this technique is both viable and useful for targeted metabolomics in the study of DKD. Studies of the metabolome require collection and storage of biological samples, which is complex and costly [23]. DBS collection in filter paper is a relevant method to study complex biological samples, particularly in studies that face challenges of large sample size, longitudinal assessment or frequent sampling in which DBS collection in filter paper would reduce storage costs and facilitate sample collection and handling [47]. In general terms, any analyte that can be measured from whole blood, serum or plasma can be measured from DBS on filter paper, with the additional advantage of stabilizing and reducing the degradations of numerous analytes due to buffering by the dried blood-matrix [48, 49]. Our results indicate that the use of this approach could be helpful in studying altered metabolic pathways linked to

Table 5 Logistic regression analyses using decreased GFR, albuminuria and DKD as dependent variables

	Model	Parameters	Parameter	β	OR	95%CI	p Value
Glomerular filtration rate < 60 mL/min	Clinical	$R^2 = 0.344$ $P < 0.001$ $\chi^2 = 6.42$, $p = 0.600$ c-statistic = 0.853 (95%CI 0.776–0.931)	BMI	−0.170	0.844	0.719–0.990	0.037
			Uric acid	0.711	2.036	1.370–3.025	< 0.001
	Clinical + metabolomics	$R^2 = 0.547$ $P < 0.001$ $\chi^2 = 10.31$, $p = 0.244$ c-statistic = 0.924 (95%CI 0.863–0.984)	BMI	−0.197	0.821	0.682–0.989	0.038
			Uric Acid	0.580	1.786	1.149–2.776	0.010
			C10:2	0.063	1.065	1.033–1.098	< 0.001
Albuminuria > 30 mg/day	Clinical	$R^2 = 0.489$ $P < 0.001$ $\chi^2 = 5.69$, $p = 0.682$ c-statistic = 0.891 (95%CI 0.845–0.938)	T2D duration	0.058	1.060	1.006–1.117	0.029
			A1C	0.412	1.510	1.212–1.882	< 0.001
			Uric acid	0.472	1.603	1.138–2.259	0.007
			Creatinine	0.918	2.504	1.054–5.946	0.038
			Protein intake	−1.102	0.332	0.138–0.802	0.014
	Clinical + metabolomics	$R^2 = 0.545$ $P < 0.001$ $\chi^2 = 7.51$, $p = 0.483$ c-statistic = 0.908 (95%CI 0.865–0.951)	T2D duration	0.054	1.051	0.997–1.107	0.062
			A1C	0.464	1.590	1.262–2.004	< 0.001
			Uric acid	0.394	1.482	1.031–2.132	0.034
			Creatinine	0.322	1.380	0.584–3.262	0.463
			Protein intake	−0.861	0.423	0.166–1.077	0.071
			C0	0.053	1.054	0.999–1.112	0.056
			C10:2	0.021	0.099	0.017–0.582	0.010
			C12:1 U	−2.309	1.021	1.000-1.043	0.049
Diabetic kidney disease	Clinical	$R^2 = 0.485$ $P < 0.001$ $\chi^2 = 11.41$, $p = 0.180$ c-statistic = 0.879 (95%CI 0.830–0.929)	BMI	−0.167	0.846	0.743-.963	0.012
			A1C	0.353	1.424	1.153–1.758	0.001
			Uric acid	0.595	1.813	1.299–2.530	< 0.001
			T2D duration	0.071	1.074	1.017–1.134	0.011
	Clinical + metabolomics	$R^2 = 0.589$ $P < 0.001$ $\chi^2 = 4.082$, $p = 0.850$ c-statistic = 0.913 (95% CI 0.874–0.953)	BMI	−0.192	0.825	0.715–0.951	0.008
			A1C	0.520	1.682	1.307–2.165	< 0.001
			Uric acid	0.506	1.659	1.138–2.421	0.009
			Age	0.078	1.081	1.005–1.162	0.037
			C0	0.055	1.056	1.000-1.115	0.050
			C10:2	0.033	1.034	1.012–1.056	0.002
			C8:1	−0.157	0.854	0.747–0.977	0.021
			C12:1 U	−1.751	0.174	0.033–0.919	0.039

Models adjusted for by age, sex, BMI, SBP, DBP, A1C, T2D duration and ACEI/ARB use

BMI body mass index, *SBP* systolic blood pressure, *DBP* dyastolic blood pressure, *A1C* glycosylated hemoglobin, *eGFR* estimated glomerular filtration rate, *OR* Odds ratio, *95% CI* 95% confidence interval, *AUC* area under the curve

DKD and these results could be extrapolated to other disease models.

Our study had some strengths and limitations. First, we were able to replicate previous findings in metabolomics of DKD using a low-cost approach in both training and replication datasets, which could be implemented in other studies to reduce costs associated to sample processing and storage. Second, we could collect both serum and urine samples to estimate differences in metabolite concentrations in a cohort of patients including healthy individuals, which allowed us to construct clinical-metabolomics models to identify kidney dysfunction in DKD using a targeted metabolomics approach. Furthermore, duration of T2D in our cohort had a wide range of disease exposure, with a minimum of 10 years; this reassures that kidney dysfunction is attributable to T2D. Amongst the limitations of our study is the cross-sectional design, which precluded us from estimating the role of metabolites in identifying progression of in kidney dysfunction and the relatively small number of cases with albuminuria > 300 mg/24 h, which did not allow for comparison of overt diabetic nephropathy cases. Furthermore, since variables were controlled in statistical analysis, there exists a possibility of residual confounding.

Table 6 Model parameters for linear logistic regression clinical and clinical-metabolomics models using a training ($N=118$) and validation ($N=82$) datasets

Linear model	Model	Sample	Adjusted r^2	AIC	p value
Albuminuria	Clinical	Training	0.197	133.06	0.004
		Validation	0.278	118.61	< 0.001
	Clinical + metabolomics	Training	0.420	110.61	< 0.001
		Validation	0.411	99.66	< 0.001
eGFR	Clinical	Training	0.445	− 168.32	< 0.001
		Validation	0.354	− 232.52	< 0.001
	Clinical + metabolomics	Training	0.720	− 218.88	< 0.001
		Validation	0.615	− 286.33	< 0.001
		Validation	0.524	4.81	0.903 (0.835–0.970)
Logistic model	Model	Sample	Adj. r^2	χ^2	c-statistic (95%CI)
Albuminuria (> 30 mg/g)	Clinical	Training	0.465	3.34	0.883 (0.819–0.947)
		Validation	0.524	4.81	0.903 (0.835–0.970)
	Clinical + metabolomics	Training	0.584	9.14	0.903 (0.848–0.959)
		Validation	0.578	4.32	0.901 (0.830–0.971)
eGFR (< 60 mL/min)	Clinical	Training	0.323	3.35	0.845 (0.745–0.945)
		Validation	0.487	15.12	0.918 (0.840–0.997)
	Clinical + metabolomics	Training	0.530	3.30	0.918 (0.840–0.997)
		Validation	0.717	16.14	0.931 (0.835-1.000)
DKD	Clinical	Training	0.469	3.880	0.874 (0.808–0.940)
		Validation	0.540	2.948	0.889 (0.815–0.962)
	Clinical + metabolomics	Training	0.673	4.193	0.930 (0.884–0.975)
		Validation	0.596	6.432	0.886 (0.884–0.975)

eGFR estimated glomerular filtration rate, *DKD* diabetic kidney disease, *AIC* Akaike's information criteria, *AUC* area under the curve (Harrel's *c*-statistic)

In conclusion, our study demonstrates the applications of targeted metabolomics in the study of metabolic alterations in DKD using a low-cost approach. The use of metabolomics evaluated in DBS in filter paper as a complementary method for DKD identification offers a practical alternative that could also shed light on the pathophysiology of DKD. Implementation of predictive models grouping clinical variables to identify glomerular dysfunction and albuminuria are improved with the use of recognized altered metabolites. The role of these metabolites as biomarkers of DKD remains to be studied and confirmed in independent longitudinal follow-up and replication cohorts. Targeted metabolomics in the study of DKD, performed simultaneously in blood and urine samples, is feasible and accessible in DBS collected in filter paper, which is a simple recollection device that allows the possibility of massive sampling, storage and analysis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Written informed consent was obtained before the examination from each patient, as well as the approval from our institutional ethics committee.

References

1. American Diabetes Association (2001) Clinical practice recommendations 2001: diabetic nephropathy (position statement). Diabetes Care 24(suppl):S69–S72
2. Giorda CB, Carnà P, Salomone M, et al (2018) Ten-year comparative analysis of incidence, prognosis, and associated factors for dialysis and renal transplantation in type 1 and type 2 diabetes versus non-diabetes. Acta Diabetol 55(7):733–740
3. Penno G, Solini A, Bonora E, Renal Insufficiency Events C (RIACE) Study Group, et al (2018) Defining the contribution of chronic kidney disease to all-cause mortality in patients with type 2 diabetes: the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study. Acta Diabetol 55(6):603–612
4. Mora-Fernández C, Domínguez-Pimentel V, de Fuentes MM, Górriz JL, et al (2014) Diabetic kidney disease: from physiology to therapeutics. J Physiol 592(18):3997–4012
5. Zhang J, Wang Y, Gurung P, et al (2018) The relationship between the thickness of glomerular basement membrane and renal outcomes in patients with diabetic nephropathy. Acta Diabetol 55(7):669–679
6. Susztak K, Böttinger EP (2006) Diabetic nephropathy: a frontier for personalized medicine. J Am Soc Nephrol 17(2):361–367
7. Urbschat A, Obermüller N, Haferkamp A (2011) Biomarkers of kidney injury. Biomarkers 16(Suppl 1):S22–S30
8. Rhee EP (2015) Metabolomics and renal disease. Curr Opin Nephrol Hypertens 24(4):371–379
9. Suhre K, Meisinger C, Döring A, Altmaier E, Belcredi P, Gieger C et al (2010) Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. PLoS One 5(11):e13953
10. Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL, Adams SH (2010) Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African–American women. PLoS One 5(12):e15234
11. Floegel A, Stefan N, Yu Z, Mühlenbruch K, Drogan D, Joost HG et al (2013) Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes 62(2):639–648
12. Niewczas MA, Sirich TL, Mathew AV, Skupien J, Mohney RP, Warram JH et al (2014) Uremic solutes and risk of end-stage renal disease in type 2 diabetes: metabolomic study. Kidney Int 85(5):1214–1224
13. Li M, Wang X, Aa J, et al (2013) GC/TOFMS analysis of metabolites in serum and urine reveals metabolic perturbation of TCA cycle in db/db mice involved in diabetic nephropathy. Am J Physiol Renal Physiol 304(11):F1317–F1324
14. Solini A, Manca ML, Penno G, Pugliese G, Cobb JE, Ferrannini E (2016) Prediction of declining renal function and albuminuria in patients with type 2 diabetes by metabolomics. J Clin Endocrinol Metab 101(2):696–704
15. Huang T, Cao Y, Zeng J (2016) Tandem mass spectrometry-based newborn screening strategy could be used to facilitate rapid and sensitive lung cancer diagnosis. Onco Targets Ther 9:2479–2487. https://doi.org/10.2147/OTT.S99099
16. Wang TJ, Larson MG, Vasan RS, et al (2011) Metabolite profiles and the risk of developing diabetes. Nat Med 17(4):448–453
17. Chace DH, Kalas TA, Naylor EW (2003) Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clin Chem 49(11):1797–1817
18. Arreola-Guerra JM, Rincón-Pedrero R, Cruz-Rivera C, Belmont-Pérez T, Correa-Rotter R, Niño-Cruz JA (2014) Performance of MDRD-IDMS and CKD-EPI equations in Mexican individuals with normal renal function. Nefrologia 34(5):591–598
19. Teruel Briones JL, Gomis Couto A, Sabater J, et al (2011) Validation of the chronic kidney disease epidemiology collaboration (CKD-EPI) equation in advanced chronic renal failure. Nefrologia 31(6):677–682
20. Aittokalio T, Schwikowski B (2006) Graph-based methods for analyzing networks in cell biology. Brief Bioinform 7(3):243–255
21. Han LD, Xia JF, Liang QL, Wang Y, Wang YM, Hu P (2011) Plasma esterified and non-esterified fatty acids metabolic profiling using gas chromatography-mass spectrometry and its application in the study of diabetic mellitus and diabetic nephropathy. Anal Chim Acta 689(1):85–91
22. Hirayama A, Nakashima E, Sugimoto M, et al (2012) Metabolic profiling reveals new serum biomarkers for differentiating diabetic nephropathy. Anal Bioanal Chem 404(10):3101–3109
23. Mäkinen VP, Kangas AJ, Soininen P, Würtz P, Groop PH, Ala-Korpela M (2013) Metabolic phenotyping of diabetic nephropathy. Clin Pharmacol Ther 94(5):566–569
24. Lanza IR, Zhang S, Ward LE, Karakelides H, Raftery D, Nair KS (2010) Quantitative metabolomics by H-NMR and LC–MS/MS confirms altered metabolic pathways in diabetes. PLoS One 5(5):e10538
25. Pena MJ, Lambers Heerspink HJ, Hellemons ME, Friedrich T, Dallmann G, Lajer M (2014) Urine and plasma metabolites predict the development of diabetic nephropathy in individuals with type 2 diabetes mellitus. Diabet Med 31(9):1138–1147
26. Stec DF, Wang S, Stothers C, et al (2015) Alterations of urinary metabolite profile in model diabetic nephropathy. Biochem Biophys Res Commun 456(2):610–614
27. Zhang J, Wang Y, Zhang R, et al (2018) Implication of decreased serum complement 3 in patients with diabetic nephropathy. Acta Diabetol 55(1):31–39
28. Feng G, Gao JL, Zhang P, et al (2017) Decreased serum extracellular superoxide dismutase activity is associated with albuminuria in Chinese patients with type 2 diabetes mellitus. Acta Diabetol 54(11):1047–1055
29. Qi S, Ouyang X, Wang L, Peng W, Wen J, Dai Y (2012) A pilot metabolic profiling study in serum of patients with chronic kidney disease based on (1) H-NMR-spectroscopy. Clin Transl Sci 5(5):379–385
30. Sun J, Shannon M, Ando Y, et al (2012) Serum metabolomic profiles from patients with acute kidney injury: a pilot study. J Chromatogr B Analyt Technol Biomed Life Sci 893:107–113
31. Goek ON, Döring A, Gieger C, et al (2012) Serum metabolite concentrations and decreased GFR in the general population. Am J Kidney Dis 60(2):197–206
32. Campion CG, Sanchez-Ferras O, Batchu SN (2017) Potential role of serum and urinary biomarkers in diagnosis and prognosis of diabetic nephropathy. Can J Kidney Health Dis 4:2054358117705371
33. You H, Gao T, Cooper TK, Morris SM Jr, Awad AS (2013) Arginase inhibition mediates renal tissue protection in diabetic nephropathy by a nitric oxide synthase 3-dependent mechanism. Kidney Int 84(6):1189–1197
34. Persson P, Fasching A, Teerlink T, Hansell P, Palm F (2014) L-Citrulline, but not L-arginine, prevents diabetes mellitus-induced glomerular hyperfiltration and proteinuria in rat. Hypertension 64(2):323–329
35. Shah VO, Townsend RR, Feldman HI, Pappan KL, Kensicki E, Vander Jagt DL (2013) Plasma metabolomics profiles in different stages of CKD. Clin J Am Soc Nephrol 8(3):363–370
36. Duranton F, Lundin U, Gayrard N, Mischak H, Aparicio M, Mourad G et al (2014) Plasma and urinary amino acid metabolomics profiling in patients with different levels of kidney function. Clin J Am Soc Nephrol 9(1):37–45
37. Ahmad S (2001) L-carnitine in dialysis patients. Semin Dial 14(3):209–217

38. Wanner C, Förstner-Wanner S, Rössle C, Fürst P, Schollmeyer P, Hörl WH (1987) Carnitine metabolism in patients with chronic renal failure: effect of L-carnitine supplementation. Kidney Int Suppl 22:S132–S135
39. Nkuipou-Kenfack E, Duranton F, Gayrard N, Argilés À, Lundin U, Weinberger KM, Dakna M et al (2014) Assessment of metabolomics and proteomic biomarkers in detection and prognosis of progression of renal function in chronic kidney disease. PLoS One 9(5):e96955
40. Fouque D, Holt S, Guebre-Egziabher F, Nakamura K, Vianey-Saban C, Hadj-Aïssa A et al (2006) Relationship between serum carnitine, acylcarnitines, and renal function in patients with chronic renal disease. J Ren Nutr 16(2):125–131
41. Atzler D, Schwedhelm E, Zeller T (2014) Integrated genomics and metabolomics in nephrology. Nephrol Dial Transplant 29(8):1467–1474
42. van der Kloet FM, Tempels FW, Ismail N, van der Heijden R, Kasper PT, Rojas-Cherto M et al (2012) Discovery of early-stage biomarkers for diabetic kidney disease using ms-based metabolomics (FinnDiane study). Metabolomics 8(1):109–119
43. Rebouche CJ (2004 Nov) Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. Ann NY Acad Sci 1033:30–41
44. Rossi C, Marzano V, Consalvo A, et al (2018) Proteomic and metabolomic characterization of streptozotocin-induced diabetic nephropathy in TIMP3-deficient mice. Acta Diabetol 55(2):121–129
45. Hocher B, Adamski J (2017) Metabolomics for clinical use and research in chronic kidney disease. Nat Rev Nephrol 13(5):269–284
46. Chang YH, Hwu DW, Chang DM, An LW, Hsieh CH, Lee YJ (2017) Renal function preservation with pioglitazone or with basal insulin as an add-on therapy for patients with type 2 diabetes mellitus. Acta Diabetol 54(6):561–568
47. Mei JV, Alexander JR, Adam BW, Hannon WH (2001) Use of filter paper for the collection and analysis of human whole blood specimens. J Nutr 131(5):1631S–1631S6S
48. McDade TW, Williams S, Snodgrass JJ (2007) What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography 44(4):899–925
49. Brindle E, O´Connor KA, Garret DA (2014) Applications of dried blood spots in general human health studies. In: Dried blood spots applications and techniques, 1st edn. Wiley, Hoboken, pp 114–129

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