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ORIGINAL ARTICLE

Can kidney parenchyma metabolites serve as prognostic biomarkers for long-term kidney function after nephrectomy for renal cell carcinoma? A preliminary study

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ABSTRACT

Objective. Nephrectomy, the standard of care for localized renal cell carcinoma (RCC), may lead to kidney function loss. Our goal was to identify prognostic biomarkers of postoperative renal function using metabolomics.

Methods. Metabolomics data from benign kidney parenchyma were collected prospectively from 138 patients with RCC who underwent nephrectomy at a single institution. The primary endpoint was the difference between the postoperative and preoperative estimated glomerular filtration (eGFR) rate divided by the elapsed time (eGFR slope). eGFR slope was calculated ~2 years post-nephrectomy (GFR1), and at last follow-up (GFR2). A multivariate regularized regression model identified clinical characteristics and abundance of metabolites in baseline benign kidney parenchyma that were significantly associated with eGFR slope. Findings were validated by associating gene expression data with eGFR slope in an independent cohort ($n = 58$).

Results. Data were compiled on 78 patients (median age 62.6 years, 65.4% males). The mean follow-up was 25 ± 3.4 months for GFR1 and 69.5 ± 23.5 months for GFR2 and 17 (22%) and 32 (41%) patients showed eGFR recovery, respectively.

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Nephrectomy type, blood lipids, gender and 23 metabolites from benign parenchyma were significantly associated with eGFR slope. Some metabolites associated with eGFR slope overlapped with previously reported chronic kidney disease-related processes. Subgroup analysis identified unique 'metabolite signatures' by older age, nephrectomy type and preoperative eGFR.

Conclusions. Nephrectomy type, gender, blood lipids and benign parenchyma metabolites at nephrectomy were associated with long-term kidney function. On further study, these metabolites may be useful as potential biomarkers and to identify novel therapeutic targets for malignancy-associated renal disease.

Keywords: chronic kidney disease, fatty acid oxidation, kidney function, metabolomics, nephrectomy, renal cell carcinoma

INTRODUCTION

Surgical resection is considered the standard of care for localized renal cell carcinoma, either in the form of partial or radical nephrectomy (RN) [1]. However, nephrectomy involves the reduction of kidney mass and often leads to the loss of kidney function. This may increase cardiovascular risk, and may result in the need for renal replacement therapy, in reduced quality of life, and in premature death [1]. Moreover, patients undergoing nephrectomy tend to be older, and many suffer from significant medical comorbidities including diabetes mellitus (DM), hypertension (HTN), hyperlipidemia and chronic kidney disease (CKD), which may have an adverse impact on kidney function independently or synergistically with surgery, although several studies have shown that kidney function may recover at longer post-operative follow-up [1–3].

Metabolomics refers to the systematic analysis of all metabolites (molecules involved in metabolism, such as sugars, amino acids, lipids, etc.) in biological specimens. The kidney serves as a pivotal organ in the production, conversion and clearance of metabolites, as well as in the hormonal regulation of systemic metabolism, thus accounting for the intensive study of metabolomics in renal disease [4]. Indeed, several studies have shown associations between relevant metabolites and CKD [5], acute kidney injury [6], diabetic nephropathy [7] and kidney cancer [8], among other kidney pathologies.

Histologic analysis of benign kidney parenchyma, which is found adjacent to tumor, at the time of nephrectomy can provide prognostic information for long-term kidney function [9]. Therefore, we hypothesized that a metabolomics molecular analysis of tumor-adjacent benign kidney parenchyma, adding functional data to pathologic description, might unveil metabolites that may be associated with long-term renal function and hence serve as tissue-based prognostic biomarkers for kidney function following nephrectomy. We accordingly designed a hypothesis-generating study with the goal of defining kidney tissue metabolomics that could be used to enhance post-nephrectomy kidney function prediction in addition to the patient's clinical characteristics and comorbidities.

MATERIALS AND METHODS

This research was approved by Memorial Sloan Kettering (MSK) Cancer Center Institutional Review Board and all patients from which data were collected provided written informed consent. These data were previously described elsewhere [8]. Briefly, for kidney metabolomics, samples of benign kidney parenchyma, as identified by the MSK pathologist, were prospectively collected from 138 specimens of partial or radical nephrectomies performed at MSK Cancer Center (New York). The samples were fresh frozen in liquid nitrogen immediately after their surgical removal and transferred to a −80°C storage at the MSK Translational Kidney Research Program. At a later time point, these samples were sent to Metabolon Inc. (Durham, NC, USA) for abundance quantification, by means of a nontargeted metabolomics gas and liquid chromatography coupled to mass spectrometry approach [8], of 877 metabolites.

Abundances of metabolites were median normalized, and those which fell below the limit of detection per each sample were removed. Subsequently, any metabolite which below the limit-of-detection removal eliminated >75% of its samples was further eliminated, eventually leaving a total of 736 of the 877 metabolites.

The patients were followed-up in the MSK Surgical Clinic as per standard of care. Medical records were retrospectively reviewed, and patient and disease-associated characteristics, such as comorbidities, tumor pathological and clinical stages and nuclear grade (collectively referred to as covariates) were recorded (Table 1). Surgical specimens of the nephrectomies were reviewed by two expert genitourinary pathologists.

Kidney function was calculated as the estimated glomerular filtration rate (eGFR) by means of the CKD Epidemiology Collaboration formula. The intermediate follow-up time point was defined as the follow-up time point closest to 24 months and at least 15 months following surgery (hereby termed GFR1). The long-term follow-up time point was defined as the latest follow-up time point and additionally requiring it to be at least one additional year after the intermediate follow-up time point (hereby termed GFR2).

For patients that underwent a second surgical intervention during follow-up that caused additional renal insult with immediate renal function deterioration, eGFR at GFR2 was evaluated as the last available follow-up prior to that intervention. No patients received a renal transplant or dialysis during the study period.

For each patient, its corresponding eGFR slope at GFR1 was calculated as its eGFR at GFR1 minus its preoperative eGFR and divided by the elapsed time (i.e. GFR1 minus the timep oint at operation). eGFR slope at GFR2 was similarly calculated as the difference between eGFR at GFR2 and preoperative eGFR divided by the difference between its GFR2 and operation time point. Thus, eGFR slopes are a proxy measurement of kidney function outcome, and a positive slope represents an improved eGFR.

A subgroup analysis was performed using the dichotomous variables of age (>70 years versus ≤70 years), nephrectomy type (radical versus partial) and preoperative eGFR (eGFR $\geq$ 60 mL/min/1.73 m^2 versus eGFR $<$ 60 mL/min/1.73 m^2).

Our aim was to identify benign tissue metabolite abundances and/or patient and disease covariates which are significantly associated with variations of intermediate (GFR1) and long-term eGFR (GFR2) findings and, therefore, could serve as potential prognostic biomarkers. Since the number of

Table 1. Clinical characteristics of the entire cohort

Characteristics	*n* (%)
Age (mean ± SD), years	62.6 ± 11.3
Gender	
Male	51 (65.4)
Female	27 (34.6)
Race	
Caucasian	68 (87.2)
Afro-American	5 (6.4)
Other	5 (6.4)
Preop GFR (mean±SD), mL/min per 1.73 m^2	67 ± 15
HTN	53 (67.9)
Diabetes	12 (15.4)
Coronary artery disease	11 (14.1)
Hyperlipidemia	37 (47.4)
Smoking	38 (48.7)
Pack years (mean ± SD)	9.1 (±13.5)
BMI (mean ± SD), (kg/m2)	31.6 ± 5.9
Tumor (T) stage	
pT1a	17 (21.8)
pT1b	9 (11.5)
pT2a	4 (5.1)
pT3a	14 (17.9)
pT3b	33 (42.3)
pT4	1 (1.3)
Fuhrman nuclear grade	
G2	35 (44.9)
G3	38 (48.7)
G4	5 (6.4)
Lymph nodes (N) stage	
N0	41 (52.6)
N+	37 (47.4)
Distant metastasis (M) stage	
M0	76 (97.4)
M1	2 (2.6)
AJCC stage	
1	26 (33.3)
2	4 (5.1)
3	47 (60.3)
4	1 (1.3)
Nephrectomy type	
Radical	37 (47.4)
Partial	41 (52.6)
EBL (mean ± SD), cc	408 ± 359

Preop GFR: preoperative estimated GFR; BMI: body mass index; AJCC: American Joint Committee of Cancer (nodes and metastasis stage were included in the AJCC stage analysis); EBL: estimated blood loss.

independent variables in our data is much higher than the number of samples, a standard linear model would be inadequate to account for the collinearities in this high dimensionality setting. Therefore, we used a multi-response Gaussian family Least Absolute Shrinkage and Selection Operator (LASSO) regularized regression model in order to detect strong associations between the eGFR slopes at both time points and the clinical covariates (Table 1) and metabolite abundances. We treated the eGFR slopes as the response matrix and the covariates and metabolite abundances as factors, including all 736 metabolites and all covariates described in Table 1 (see below). Prior to fitting the LASSO model, we sought to remove all outliers, both in the response as well as in the metabolite abundances and covariates, due to their high potential to drive spurious associations. To this end, the distribution of the responses, the abundances of each metabolite and of the covariates, were each searched for significant outliers using the Grubbs' test for outliers [10]. We then fitted the LASSO model (employing the R statistical language package glmnet using the mgaussian response family), applying a leave-one-out cross-validation strategy and optimizing the regularization parameter lambda (Supplementary data, Figure S1). From this optimal lambda value, we obtained effect estimates of the factors selected by the LASSO model. The glmnet algorithm uses a cyclical coordinate descent, which successively optimizes the objective function over each parameter with others fixed, and cycles repeatedly until convergence. As a result, this package identifies a list of only relevant factors. Unlike a standard linear model, LASSO does not obtain a P-value as a measure of statistical significance of an effect size. Hence, as such, we report the multiple-hypothesis corrected P-value [11] obtained by using a univariable linear model associating eGFR slope with each individual metabolite and covariate selected by the LASSO model.

A separate cohort of subjects with gene expression data from tumor-adjacent benign kidney parenchyma and eGFR slopes was used for confirmation of the metabolomics results. These samples were collected during surgical radical/partial nephrectomy (PN) at the Albert Einstein College of Medicine/Montefiore Medical Center between 2007 and 2011, as described by Gluck *et al.* [12]. Clinical data were available for these subjects at the time of sample collection as well as before and after nephrectomy. To use these data for confirmation of the metabolomics data analysis, we used the Biochemical Genetic andGenomic (BiGG) database [13] to identify genes involved in the reactions of metabolites we found to be significantly associated with eGFR slope (Supplementary data, Table S1). We additionally required that expression levels of these genes have a significant association with an adjusted eGFR slope in their cohort (Figure 2 and Supplementary data, Figure S2. In other words, intersecting metabolites whose abundances were found to be significantly associated with eGFR slope in one cohort with genes involved in the pathways that generate these metabolites, and which expression levels were found to be significantly associated with eGFR slope in another cohort.

Estimation of eGFR slopes for the validation cohort was determined by linear regression across all available eGFR measurements. As described by Gluck *et al.* [12], subjects were excluded if their unadjusted eGFR slopes were <-40 mLmin/1.73 m2/year or >40 mLmin/1.73 m^2/year. We used a best linear unbiased predictor to determine the adjusted eGFR slope and the variance of the slope. Using the adjusted eGFR slope as the response, we applied LASSO to all available clinical and histological variables that were associated with eGFR slope on univariate analysis ($P < 0.05$). It emerged that DM, age and baseline eGFR were the variables that best explained adjusted eGFR slopes. These three variables were then used in a weighted linear regression model for adjusted eGFR slopes which was weighted by inverse variance of the slope. The gene expression level was then individually added to this baseline model to identify genes whose expression level improved the model fit [akaike information criterion (AIC) <176] and were significantly associated with outcome of adjusted eGFR slopes ($P < 0.05$).

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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RESULTS

Out of the 138 patients with available metabolomics data, 6 patients were excluded due to intra-operative complications and 51 patients did not meet the inclusion criteria for both intermediate and long-term eGFR follow-up data (GFR1 and GFR2). Three additional patients were defined as outliers (see Materials and Methods section). Our final cohort included 78 patients characterized as described in Table 1. The mean intermediate follow-up (GFR1) was 25 ± 3 months, and the mean long-term follow-up (GFR2) was 69.5 ± 23.5 months. The mean preoperative eGFR was 67 ± 15 mL/min/1.73 m^2, and the mean eGFR slopes for GFR1 and GFR2 were −0.17 ± 0.28 and −0.04 ± 0.22, respectively. Seventeen (22%) patients had improved eGFR at GFR1, and 32 (41%) had improved eGFR at GFR2 (i.e. positive eGFR slope). Significant associations between eGFR slopes and nephrectomy type, hyperlipidemia, and gender were identified by the LASSO model for both GFR1 and GFR2 (Figure 1).

Overall, 23 metabolites were found to have a significant association with eGFR slopes, and 18 of them are identified (Supplementary data, Table S2). Three identified and two unidentified metabolites that were significantly associated with both eGFR slopes (GFR1 and GFR2) were identified by the analysis of the entire cohort (Table 2 and Supplementary data, Table S2) .

In the subgroup analysis, we investigated the relevance of a preoperative eGFR (≥60 mL/min/1.73 m^2 cutoff, $n = 51$, versus <60 mL/min/1.73 m^2, $n = 27$), age (>70 years, $n = 21$ versus ≤70 years, $n = 57$) and nephrectomy type (radical, $n = 37$ versus partial, $n = 41$). Nephrectomy type, blood lipids and gender were significantly associated with eGFR slope regardless of preoperative kidney function. The same three clinical covariates were significantly associated with eGFR slope in patients who were ≤70 years. No clinical covariates or metabolites were significantly associated with eGFR slope in patients undergoing PN, while blood lipids and gender had significant associations with eGFR slope in patients undergoing RN (Supplementary data, Table S3). Metabolites associated with eGFR slopes in the subgroup analyses are shown in Tables 3, 4 and Supplementary data, Table S3.

To attempt to validate our metabolomics data, we evaluated a second cohort of 58 patients from another institution from which we had obtained genomic data Supplementary data, Figure S2a The mean follow-up for this confirmation cohort was 2.4 ± 1.5 years. The mean age of the subjects at the time of nephrectomy was 65.5 ± 11.1 years. Fifty percent of the subjects had confirmed DM and 78% of subjects had confirmed HTN. The mean baseline estimated eGFR was 65.8 ± 26.5 mL/min/1.73 m^2. Of the original cohort, nine of the metabolites associated with eGFR slope had genes that were identified as being involved in their chemical reactions according to the BiGG database [13] (Supplementary data, Table S1). In our confirmation cohort, two of these genes were significantly associated with adjusted eGFR slope ($P < 0.05$) and improved model fit (AIC < 176). Both genes related to the same metabolite, 1-arachidonoylglycerophosphoethanolamine. Addition of *lecithin-cholesterol acyltransferase* (LCAT) gene expression to the model lowered the AIC of the model to 166, and it was negatively associated with adjusted eGFR slope ($\beta = -0.767$, $P = 0.0011$) (Figure 2). Addition of *phospholipase A2 group III* gene expression to the model lowered the AIC of the model to 173, and it was negatively associated with adjusted eGFR slope (beta = −0.525, $P = 0.03$) (Supplementary data, Figure S2).

DISCUSSION

It is well recognized that renal function is often affected after either partial or RN, yet other than using the criteria of CKD at diagnosis, it is not known which patients may or may not recover renal function post-operatively. Recent reports from our institution had shown that kidney function may indeed recover following nephrectomy [2, 3]. Histological analysis of non-neoplastic renal parenchyma at time of nephrectomy and severity of glomerulosclerosis was found to be associated with renal function deterioration and increased cardiovascular risk, suggesting that this analysis could be used as a personalized post-nephrectomy follow-up tool [9]. In this study, we evaluated the association of clinical covariates and abundances of metabolites in benign kidney parenchyma with eGFR slope in order to

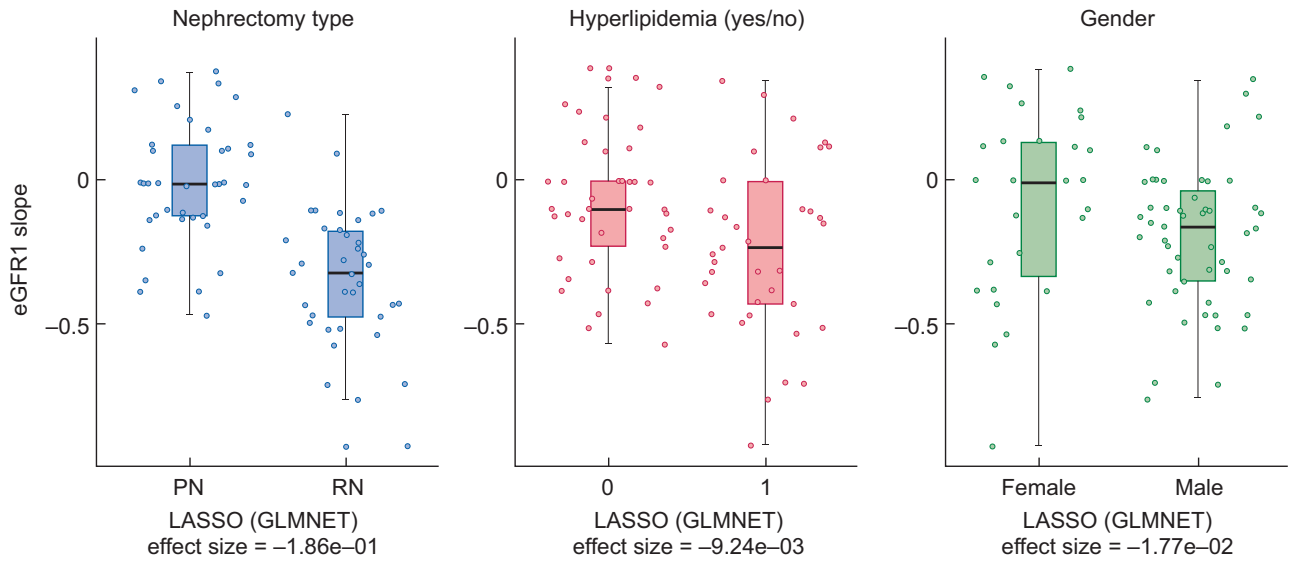

FIGURE 1: Comorbidities identified by the LASSO model to have an association with eGFR slopes (q-value < 0.1 for all). eGFR1 slope = eGFR slope at an intermediate time point (~2 years following nephrectomy); 0 = no hyperlipidemia; 1 = hyperlipidemia; effect size = effect size of the comorbidity on the eGFR1 slope as estimated by the LASSO model.

Table 2. Abundances of all metabolites in benign kidney parenchyma significantly associated with eGFR slopes and their effect size (GFR1 data presented)

Biochemical name	Super pathway	Sub-pathway	Effect size	q-value
1-Arachidonoylglycerophosphoethanolamine	Lipid	Lysolipid	0.01589	0.01682
Leucylserine	Peptide	Dipeptide	−0.00319	0.08498
Stachydrine	Xenobiotics	Food component/plant	−0.03130	0.00014
X-11315	NA	NA	−0.08447	0.00059
X-15691	NA	NA	−0.04079	0.01251

Negative and positive effect size, respectively, indicate that kidney function (eGFR slope) is negatively and positively associated with the abundance of that metabolite (i.e. a possible biomarkers of deterioration and recovery, respectively). X-11315 = unnamed metabolite number 11315 (LC/MS positive, Retention Time Index = 1210, Mass = 130.2, data adopted from Metabolon Inc.); X-15691 = unnamed metabolite number 15691; (LC/MS negative, Retention Time Index = 5170, Mass = 387.2, data adopted from Metabolon Inc.); NA, not available.

Table 3. Subgroup analysis by age for abundances of metabolites in benign kidney parenchyma significantly associated with eGFR slopes and their effect size

Biochemical name	Super pathway	Sub-pathway	>70 years		<70 years	
			Effect size	q-value	Effect size	q-value
Pentadecanoate (15:0)	Lipid	Long chain fatty acid	0.22627	0.00306	–	–
Glycerophosphorylcholine	Lipid	Glycerolipid metabolism	0.02065	0.08561	–	–
2-Hydroxypalmitate	Lipid	Fatty acid monohydroxy	−0.13646	0.00306	–	–
Glycerol	Lipid	Glycerolipid metabolism	−0.01032	0.00349	–	–
Uracil	Nucleotide	Pyrimidine metabolism uracil containing	−0.00929	0.01412	–	–
p-cresol sulfate	Amino acid	Phenylalanine and tyrosine metabolism	−0.02526	0.00306	–	–
Pyroglutamine	Amino acid	Glutamate metabolism	−0.00340	0.02842	–	–
N-acetyl-aspartyl-glutamate	Amino acid	Glutamate metabolism	–		0.00059	0.07296
Nicotinamide ribonucleotide	Cofactors and vitamins	Nicotinate and nicotinamide metabolism	–		0.00101	0.01575
Phenylalanylmethionine	Peptide	Dipeptide	–		−0.00630	0.00193

>70 = subgroup analysis for patients >70 years old; <70 = subgroup analysis for patients 70 years old or younger. Negative and positive effect size indicate that kidney function (eGFR slope) is negatively and positively associated with the abundance of that metabolite, respectively (i.e. possible biomarkers of deterioration and recovery, respectively). GFR1 data are presented. Four unnamed metabolites are not shown.

further identify potential future biomarkers of renal recovery as well as possible therapeutic targets. This analysis revealed significant associations between eGFR slope and nephrectomy type, gender and hyperlipidemia. Nephrectomy type and female gender had already been reported as being associated with eGFR slope [2, 3, 14]. Gonadal steroids and other endocrine pathways have been suggested as potential explanations for the gender-based difference in kidney function recovery following nephrectomy [15].

Despite the extensive knowledge regarding lipid abnormalities in patients with end-stage renal disease, clinical evidence supporting an association between cholesterol levels and the development of renal dysfunction is relatively limited in humans, although it has been reported and is well-supported by animal models [16–18]. In earlier studies looking at the role of fatty acid oxidation (FAO) in CKD pathogenesis, a lower expression of key enzymes and regulators of FAO, as well as an increased intracellular lipid deposition were demonstrated in tubulointerstitial fibrosis [19]. Our current analysis associated kidney function recovery with an FAO metabolite, 1-arachidonoylglycerophosphoethanolamine, which is the product of cholesterol c + phosphatidylethanolamine metabolized to cholesterol ester+ 1-arachidonoylglycerophosphoethanolamine, facilitated by the activity of the LCAT enzyme [13] (Figure 2). 1-Arachidonoylglycerophosphoethanolamine is also a product of phosphatidylethanolamine hydrolysis mediated by phospholipase A2 1-arachidonoylglycerophosphoethanolamine [13]. Both of these findings were further validated by an independent cohort, significantly associating these enzymes' genes expression levels with eGFR slope (Figure 2 and Supplementary data, Figure S2).

Interestingly, the LCAT enzyme had been described as part of the lipid metabolism, specifically, high-density lipoprotein [20]. Lack of the LCAT enzyme is characterized by the association of dyslipidemia, corneal opacities, anemia and progressive nephropathy and kidney transplantation was described in a patient carrying this homozygosity [21]. Our current validation cohort associated higher LCAT expression levels with negative eGFR slope (Figure 2). Despite this apparently counter-intuitive finding (i.e. higher rather than lower), biological reactions often show comparable mechanisms (e.g.HMG) CoA reductase under statin treatment), and may suggest compensation for a malfunctioning enzyme. Our finding that the expression of phospholipase A2, another enzyme related to a reaction creating this metabolite, correlated with eGFR slope further supports the importance of these reactions in post-nephrectomy kidney function recovery.

Given that renal function tends to decrease with aging, there is some controversy over the benefits of curative surgical treatment for renal masses versus conservative treatments and renal function preservation as populations grow older [22], thus increasing the importance of risk stratification of surgical

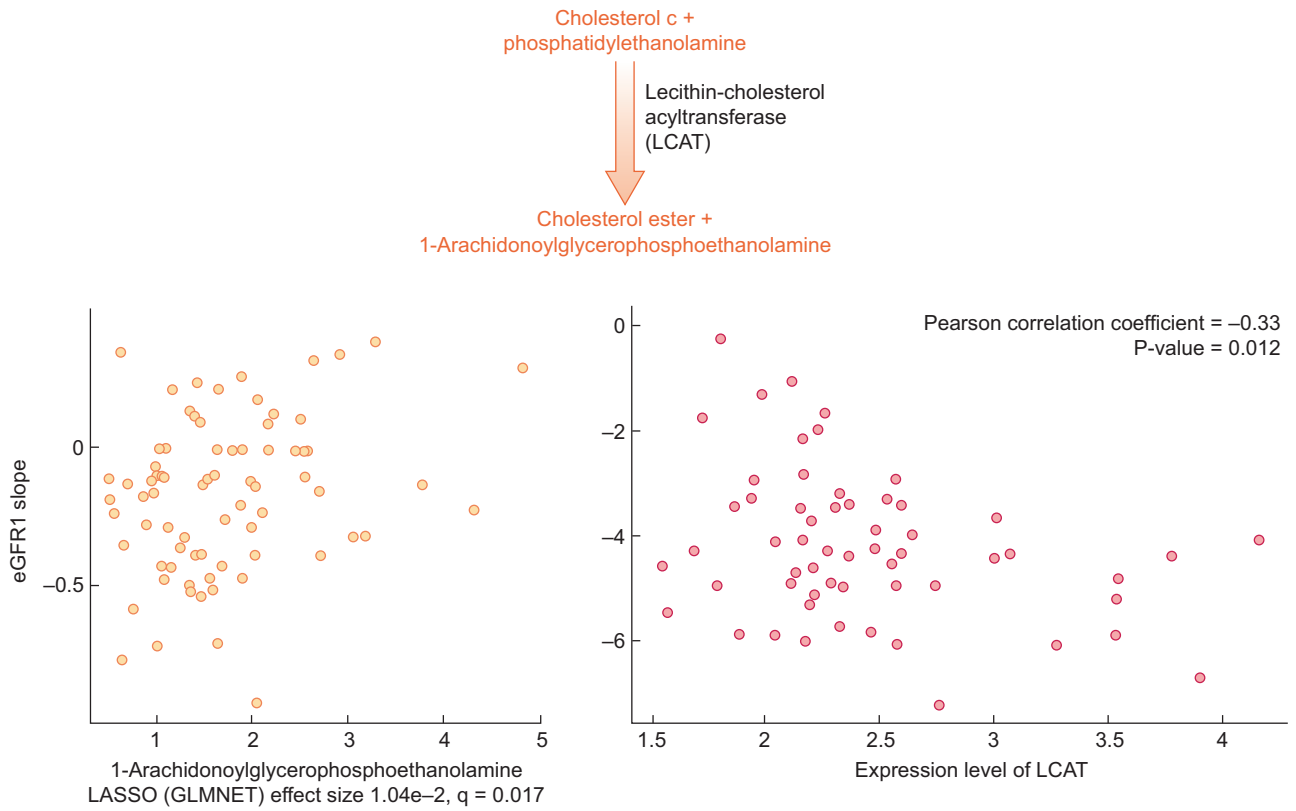

FIGURE 2: Cholesterol c + phosphatidylethanolamine metabolized to cholesterol ester + 1-arachidonoylglycerophosphoethanolamine by the activity of LCAT enzyme. Lower left panel shows the association of the 1-arachidonoylglycerophosphoethanolamine metabolite with eGFR slope as found in the metabolite dataset. Lower right panel shows the LCAT enzyme gene expression facilitating this reaction to associate with eGFR slope in the validation cohort.

candidates among elderly patients. Our cohort included 21 (26.9%) patients >70 years of age, and 10 metabolites were associated with eGFR slope among them (Table 3). Two of these 10 metabolites were positively associated with eGFR slope: one of them is glycerophosphocholine, a renal medullary organic osmolyte that protects renal medullary cells from the high interstitial concentrations of NaCl and urea [23]. Glycerol and *p*-cresol sulfate were negatively associated metabolites. The effect of glycerol on renal function has been applied in animal models for renal failure induction [24], while *p*-cresol sulfate has been reported as a uremic toxin associated with CKD stage, CKD progression and mortality in CKD patients, as well as with the phenomenon of compensatory renal growth [25, 26].

Interestingly, several of the metabolites found to be associated with eGFR slope in older patients have also been associated with old age in different pathologies. For example, metabolites related to gut bacterial metabolism (e.g. *p*-cresol sulfate), which are altered in response to peroxisome proliferator-activated receptor-alpha activation and take part in FAO (e.g. 2-hydroxypalmitate), have been associated with physical function in functionally limited older adults [27].

Although many urologists support PN over RN based on perceived benefits, such as improvement in all-cause mortality and eGFR preservation, studies on such benefits have shown conflicting results [28]. Furthermore, the benefits of PN have been argued to be limited to certain patient subgroups [29]. Our analysis isolated some metabolites which may provide prognostic information and, as such, aid in risk stratification of these patients. Our analysis of the RN subgroup found gender and blood lipids to correlate with eGFR slope. Metabolites associated with eGFR slope in that subgroup fully coincided with the results for the entire cohort (Supplementary data, Table S3), as noted above. Not surprisingly, neither clinical covariates nor metabolomics correlated with eGFR slope in patients undergoing PN, which can be explained by contralateral kidney compensation and the smaller changes in eGFR slope following nephron-sparing surgery.

Abundances of several metabolites that have been previously associated with CKD were also identified in the present work as predictors of a negative eGFR slope, e.g. stachydrine (Table 2) [30]. Zabor *et al.* correlated various covariates with kidney function recovery following nephrectomy in patients with a preoperative eGFR of <60 mL/min/1.73 m^2 [2]. Moreover, a confirmatory study validated preoperative eGFR as a predictor of kidney function recovery [3]. This subgroup was also represented in this study, and three metabolites (*p*-cresol sulfate, pyroglutamine and phenylacetylglutamine, Table 4) showed a negative association with eGFR slope in patients with a preoperative eGFR < 60 mL/min/1.73 m^2. As noted earlier, *p*-cresol sulfate has been associated with both CKD and compensatory renal growth [25, 26, 31], while phenylacetylglutamine, another gut-derived uremic toxin, has been associated with cardiovascular disease and mortality in CKD patients [32]. The abundances of five metabolites were associated with eGFR slope in patients with a preoperative eGFR ≥ 60 mL/min/1.73 m^2, among which is octanoylcarnitine, that had been reported in plasma and urine of CKD patients [33]. Despite this resemblance to already reported CKD metabolite profiles, the identification of the signature of new metabolites in the setting of post-nephrectomy eGFR slope described in this study may support

Table 4. Subgroup analysis by preoperative eGFR for abundances of metabolites in benign kidney parenchyma significantly associated with eGFR slope and their effect size

Biochemical name	Super pathway	Sub-pathway	Pre ≥ 60		Pre < 60	
			Effect size	q-value	Effect size	q-value
3-Dephosphocoenzyme A	Cofactors and vitamins	Pantothenate and CoA metabolism	0.00334	0.0775	–	–
N-methylglutamate	Amino acid	Glutamate metabolism	−0.00383	0.00073	–	–
Octanoylcarnitine	Lipid	Carnitine metabolism	−0.02766	0.01026	–	–
Stachydrine[a]	Xenobiotics	Food component/plant	−0.01857	0.00011	–	–
Xylose	Carbohydrate	Nucleotide sugars pentose metabolism	–	–	0.00336	0.00619
p-cresol sulfate	Amino acid	Phenylalanine and tyrosine metabolism			−0.02109	0.00149
Pyroglutamine	Amino acid	Glutamate metabolism			−0.09306	0.00322
Phenylacetylglutamine	Amino acid	Phenylalanine and tyrosine metabolism			−0.00738	0.00619

Pre ≥ 60 = subgroup analysis for patients with preoperative eGFRs of 60 mL/min/1.73 m^2 or higher; Pre < 60 = subgroup analysis for patients with preoperative eGFRs < 60 mL/min/1.73 m^2. Negative and positive effect size, respectively, indicate that kidney function (eGFR slopes) is negatively and positively associated with the abundances of that metabolite (i.e. possible biomarkers of deterioration and recovery, respectively). GFR1 data are presented. Two unnamed metabolites are not shown.
[a]Metabolite significant for both 'All' and preoperative eGFR subgroup analysis cohorts.

an alternative pathophysiology for CKD versus post-nephrectomy renal function, as has been suggested before [34]. Incorporation of metabolomics as renal function progonstic tools can be used to guide a patient-tailored post-nephrectomy follow-up protocol. Renal mass biopsy, recommended by some, can possibly elaborate such application to the pre-surgical setting [35].

Limitations of this study include the possible effect of cancer on its metabolic surroundings. While such effect has been previously suggested, conflicting evidence exists regarding actual alteration of benign parenchyma metabolomics adjacent to cancer [36]. Furthermore, even if some alterations do exist, the fact that we correlated tissue metabolomics with final clinical outcomes (i.e. kidney function), the relevance of such might not be of the essence to the suggested hypothesis. Also, the small size of our cohort relative to the number of comorbidities and the abundances of the tested metabolites renders the study underpowered to detect small but nevertheless significant effects. In addition, due to the limited size of our data, we did not test for associations between eGFR slopes and any type of interactions between the comorbidities and metabolite abundances. From a clinical perspective, possible sources of bias include the retrospective nature of this study. Only 86 out of 138 samples had eGFR data available at both time points. Long-term kidney function is a major factor in preoperative decision-making, and while GFR1 may represent long-term surgical insult following compensatory renal growth, adding GFR2 represents the longest-term function available. The fact that our confirmation analysis found only one metabolite to stand out in both cohorts might question the importance of the other metabolites we found. Only 50% of the identified metabolites (9 out of the 18) had corresponding genes identified in the BiGG database, and could, therefore, be assessed in a confirmation analysis. This may explain the lack of other metabolites to be validated by this external cohort genes analysis. Furthermore, the small size of both cohorts and, accordingly, the dramatically higher number of independent variables compared to the number of samples in both cohorts may mask metabolic processes with smaller clinical effects. We, therefore, believe that the fact that we identified two genes to correspond with a single metabolite emphasizes its strong effect rather than the lack of importance for the rest of the metabolites identified by the primary analysis.

To the best of our knowledge, this study is the first to report an association between abundances of metabolites in benign kidney parenchyma at the time of nephrectomy and postoperative kidney function. This finding serves as proof of concept for chemical processes taking place at that time to be associated with long-term kidney function. We believe that identifying the significance of several metabolites and further specifying and confirming the importance of a single chemical process by the correlation between 1-arachidonoylglycerophosphoethanolamine and LCAT enzyme should support future endeavors in the search for kidney tissue prognostic biomarkers and potential therapeutic targets.

CONCLUSION

Nephrectomy type, gender and blood lipids, as well as benign parenchyma metabolites at the time of nephrectomy are associated with long-term kidney function. A subgroup analysis further identified unique metabolite patterns according to older age, type of nephrectomy and preoperative eGFR. These data propose that metabolites can serve as potential future biomarkers and that related metabolic reactions can serve as possible therapeutic targets.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

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CONFLICT OF INTEREST STATEMENT

Dr E.A.J. reports other financial activities outside the submitted work as Chief Medical Officer and Stockholder at Goldilocks Therapeutics, Inc. All other authors declare no COI.

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