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Evaluation of urinary volatile organic compounds as a novel metabolomic biomarker to assess chronic kidney disease progression

Henry H. L. Wu^{1*}, Malcolm Possell², Long The Nguyen¹, Wenbo Peng³, Carol A. Pollock^{1,4} and Sonia Saad¹

Abstract

Background There is a need to develop accurate and reliable non-invasive methods to evaluate chronic kidney disease (CKD) status and assess disease progression. Given it is recognized that dysregulation in metabolic pathways occur from early CKD, there is a basis in utilizing metabolomic biomarkers to monitor CKD progression. Volatile Organic Compounds (VOCs), a form of metabolomic biomarker, are gaseous products of metabolic processes in organisms which are typically released with greater abundance in disease conditions when there is dysregulation in metabolism. How urinary VOCs reflect the abnormal metabolic profile of patients with CKD status is unknown. Our study aimed to explore this.

Methods Individuals aged 18–75 years undergoing kidney biopsy were included. Pre-biopsy urine samples were collected. All biopsy samples had an interstitial fibrosis and tubular atrophy (IFTA) grade scored by standardized assessment. Urine supernatant was extracted from residue and sampled for stir bar sorptive extraction followed by Gas chromatography–mass spectrometry (GC-MS) analysis. Post-processing of GC-MS data separated complex mixtures of VOCs based on their volatility and polarity. Mass-to-charge ratios and fragment patterns were measured for individual VOCs identification and quantification. Linear discriminant analysis (LDA) was performed to assess the ability of urinary VOCs in discriminating between IFTA 0 ('no or minimal IFTA' i.e. <10%, IFTA), IFTA 1 ('mild IFTA' i.e. 10-25% IFTA) and IFTA ≥ 2 ('moderate or severe IFTA' i.e. $\ge 25\%$ IFTA). Linear regression analysis adjusting for age, sex, estimated glomerular filtration rate, diabetes mellitus (DM) status, and albuminuria was conducted to determine significantly regulated urinary VOCs amongst the groups.

Results 64 study participants (22 individuals IFTA 0, 15 individuals IFTA 1, 27 individuals IFTA \geq 2) were included. There were 34 VOCs identified from GC-MS which were statistically associated with correct classification between the IFTA groups, and LDA demonstrated individuals with IFTA 0, IFTA 1 and IFTA \geq 2 could be significantly separated by their urinary VOCs profile (p < 0.001). Multivariate linear regression analysis reported 4 VOCs significantly upregulated in the IFTA 1 compared to the IFTA 0 group, and 2 VOCs significantly upregulated in the IFTA 2 compared to the IFTA 0 group, and 2 VOCs significantly upregulated in the IFTA \geq 2 compared to the IFTA 0 group.

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1 group (p < 0.05). Significantly upregulated urinary VOCs belonged to one of four functional groups - aldehydes, ketones, hydrocarbons, or alcohols.

Conclusions We report novel links between urinary VOCs and tubulointerstitial histopathology. Our findings suggest the application of urinary VOCs as a metabolomic biomarker may have a useful clinical role to non-invasively assess CKD status during disease progression.

Keywords Chronic kidney disease, Volatile organic compounds, Translational diagnostics, Interstitial fibrosis and tubular atrophy, Non-invasive diagnosis

Background

An increase in life expectancy and an increasing prevalence of diabetes mellitus and obesity has amounted to a greater number of individuals affected by chronic kidney disease (CKD), with more than 10% of the global population affected by this condition currently [1]. By 2040, CKD is projected to emerge as the fifth-leading cause of mortality worldwide [2]. Early diagnosis is important to allow for timely intervention which may reduce the excess morbidity and mortality in patients with CKD.

Histopathological evaluation of kidney biopsy tissue remains the gold standard approach which accurately reflects any presence of kidney pathology. Serially performing kidney biopsies to monitor kidney status is not ideal however, as it is invasive and costly. Traditional serum and urine-based tests such as estimated glomerular filtration rate (eGFR) and urinary albumin, whilst considered convenient routinely performed tests to determine kidney function, do have limitations when aiming to accurately assess kidney disease status [3-5]. There remains a need to develop reliable non-invasive methods to evaluate kidney disease status. To this end, the emergence of novel proteomic and metabolomic techniques to determine specific biomarkers which inform on the metabolic and kidney disease status of an individual has taken significant strides [6-8].

Utilization of volatile organic compounds (VOCs) as non-invasive metabolomic biomarkers to evaluate metabolic and kidney status has received growing interest over recent years [9-12]. VOCs are gaseous products of metabolic processes in organisms which are conventionally released with greater abundance in disease conditions when there is dysregulation in metabolism [13]. Due to the kidneys' extraction of soluble wastes from the bloodstream and pre-concentration capabilities, urine has considerable value as a source of VOCs which may reflect the state and function of the kidneys, as well as other organs and pathologies. More than 400 human urinary VOCs - ranging across different organic chemistry functional groups (e.g. alcohols, benzenes, ketones, hydrocarbons, pyrroles, furans, aldehydes, terpenes, sulfur-containing compounds (isocyanates, sulfides), and O- and N-heterocyclic compounds - have been previously identified in normal physiological conditions and in various pathological conditions [14]. Whether the expression levels of VOCs in human urine can play a considerable role in accurately assessing CKD status remains unknown to date. Our study aimed to evaluate whether urinary expression levels of VOCs are significantly associated with the degree of tubulointerstitial fibrosis in the kidney, as reported by kidney biopsy.

Methods

Study participant recruitment and ethical considerations

Adult individuals of either sex aged between 18 and 75 years of age under the care of the Department of Renal Medicine at Royal North Shore Hospital or North Shore Private Hospital, Sydney, Australia referred for kidney biopsy were included in this study. Individuals receiving kidney replacement therapy were excluded from this study. Informed consent was obtained from all study participants. Data collection in this study was carried out in accordance with relevant local guidelines and regulations, and collection of human data was approved by the human ethics committee at Royal North Shore Hospital (Ref: HREC/17/HAWKE/471).

Evaluation of kidney biopsy tissue for interstitial fibrosis and tubular atrophy grading to determine study participant groups

The procurement of kidney biopsy tissue was performed in the Medical Day Procedure Unit at Royal North Shore Hospital. Prior to commencing the procedure, written consent was obtained from study participants to collect the pre-biopsy urine sample for purposes of this study, and to obtain access to the kidney biopsy tissue, which was otherwise performed for clinical indications. Tissue obtained from kidney biopsies were subsequently transferred to the histopathology department and assessed as per standard protocols to determine interstitial fibrosis and tubular atrophy (IFTA) grading. Kidney biopsy samples were processed for light microscopic evaluation via paraffin-embedded sections, supplemented by special and immune histochemical (IHC) stains. Some samples were reserved for immunofluorescence and electron microscopic studies if indicated. Light microscopy assessment included a minimum of two hematoxylin and eosin (H&E), two periodic acid-Schiff (PAS), two Masson's

trichrome (trichrome), and two Jones methenamine silver (silver) stains in complementary fashion. H&E stains provided a general overview of all structures, cytoplasmic and nuclear features, PAS stains highlighted tubular and glomerular basement membranes, trichrome stains accentuated fibrous tissue and fibrin, if present, and silver stains highlighted the glomerular and tubular basement membranes, and also sclerosis. The biopsy assessment was conducted blindly by three accreditated pathologists from the NSW Health Pathology Laboratory, Department of Anatomical Pathology, Northern Sydney Local Health District, Sydney, Australia. Kidney biopsy tissue was assessed as having IFTA 0 ('no or minimal IFTA' i.e. <10%, IFTA), IFTA 1 ('mild IFTA' i.e. 10–25% IFTA) and IFTA ≥ 2 ('moderate or severe IFTA' i.e. >25% IFTA).

Study participants' demographic alongside clinical and biochemical data were acquired from the Royal North Shore Hospital PowerChart Database, summarized using appropriate descriptive statistics and compared between the three groups. For demographic and clinical variables with symmetric normal distributions, the mean and standard deviation were reported. For variables that were skewed or ordinal, the median and interquartile range were used for statistical purposes. Proportions were also presented for categorical variables. Continuous variables between the groups were compared using the Analysis of Variance (ANOVA) test (if normally distributed) or Kruskal-Wallis test (if the distribution was non-parametric). Categorical variables were compared using the Chisquare test or Freeman-Halton extension of the Fisher's exact test accounting for sparsely distributed data.

Collection of urine samples and transferring sample for stir bar sorptive extraction

Using urine bottles with capacity of up to 100 ml, spot urine samples were collected from adult individuals who fulfilled the study criteria. Each collected urine sample was placed on ice immediately after collection for transportation to the Renal Research Laboratory, Kolling Institute of Medical Research and were centrifuged for 20 min at 4° C to isolate urine supernatant from residue. Urine supernatant were stored at -80 °C and defrosted overnight at 4 °C before further sampling. 5 ml of urine was transferred to a 20 ml headspace vial and 3 µl of 15ppm bromobenzene in methanol internal standard (IS) was added along with a conditioned polydimethylsiloxane phase stir bar (Twister, 10 mm x 0.5 mm film thickness; Gerstel, Mülheim an der Ruhr, Germany). The headspace vial was capped and Stir Bar Sorptive Extraction (SBSE) proceeded, with the stir bar spun at 800 rpm for 2 h. The stir bar was then removed, rinsed with double distilled water and patted dry with a lint free tissue before analysis. Two blank samples consisting of 5 ml of double-distilled water, spiked with the same IS were run with each cohort of samples. To determine retention indices, 1 μ l of C8-C20 homologous n-alkanes (containing approximately 40 mg/l of each alkane) was injected onto separate, conditioned SBSE. All reagents were sourced from Sigma-Aldrich (Sydney, Australia).

Gas chromatography-mass spectrometry analysis

Thermal desorption (TD) of the stir bars was done using a Gerstel Thermal Desorption Unit (TDU; Gerstel, Mülheim an der Ruhr, Germany). SBSE stir bars were placed into glass thermal desorption liners that were inserted into the TDU for analysis. Upon insertion into the TDU, the samples were purged with ultra-high purity helium (BOC Ltd, North Ryde, NSW, Australia) at 35 °C for 1 min to eliminate air from the sample and inlet. Samples were then heated by the TDU at 12 °C/s to 250 °C with a helium flow of 50 ml/min. TD products were carried by the helium through to a programmed temperature vaporization (PTV) inlet (CIS-4; Gerstel) installed in an Agilent 7890GC (Agilent Technologies Pty Ltd, Mulgrave, Australia), which was used in solvent mode during the TD. The PTV inlet, containing a glass liner filled with Tenax TA, was held at 30 °C during the TD using liquid CO₂ (BOC Ltd) as the cryogen. After 5 min of TD, the CIS-4 was heated at 12 °C/s to 250 °C and held at that temperature for 3 min while the TD products were injected into the GC without splitting. TD products were separated on a HP-5ms capillary column (30 m x 0.25 mm, 0.25 µm film thickness; Agilent), which was connected to a mass selective detector (Model 5975 C; Agilent). Ultra-high purity helium was used as carrier gas (flow rate through the HP-5ms column was 2.3 ml/min). The initial oven temperature of the GC was 40 °C, held for 2 min, then heated at a rate of 4 °C/min to 250 °C and held for 5 min. The temperature of the Gas chromatography-mass spectrometry (GC-MS) interface was 280 °C, the MS ion source 230 °C and the quadrupole 150 °C. The detector, in electron impact mode (70 eV), scanned the range of 35–300 m/z. Operation of the GC-MS was controlled by Agilent Chemstation (version E.02.01.117) and the TDU by Maestro (version 1.4.36.16; Gerstel).

Quality Control

Pooled urine quality control (QC) samples were generated for each of the three cohorts (IFTA 0; IFTA 1; IFTA \geq 2) by mixing an equal volume of urine of each study sample to make a total of 30 ml of urine. This allowed for 6×5 ml QC samples for each cohort. These were extracted and analyzed as described for the study samples.

Gas chromatography-mass spectrometry post processing of urinary volatile organic compound data

Post-processing of GC-MS data to separate complex mixtures of VOCs based on their volatility and polarity, and measuring mass-to-charge ratios and fragment patterns for individual VOC identification and quantification was performed. Chromatograms were batch processed by metaMS (version 2.1.1) [15], hosted on the Workflow4Metabolomics Galaxy Server [16]. metaMS outputs a data matrix of aligned mass spectra with their corresponding peak area and a mass spectral pattern file. The maximum peak area of aligned mass spectra of the two water blanks run in every batch of samples were subtracted before further analysis. Mass spectra were considered reproducible if they were present in four out of six QC samples, the presence in the QC samples had a coefficient of variation < 30% and the dispersion ratio (a measure of variance in the QC samples to those of the urine samples) was less than 50% [17]. The mass spectra were identified against the NIST14 mass spectral library in NIST MS Search (NIST MS Search v.2.2; NIST, Gaithersburg, MD) using a match factor threshold of 700, and closeness to available retention index value (using nonisothermal Kovats' Retention Indices from the definition of van den Dool and Kratz, for a semi-standard non-polar column) [18].

Statistical analysis of post-processed urinary volatile organic compound data

Expression levels of identified VOCs were compared across the 3 study participant groups. To determine the importance of VOCs and their presence to differentiate IFTA status, linear discriminant analysis (LDA), a supervised learning technique, was used to distinguish the groups. The Mahalanobis distance between each group was calculated to validate the LDA model. Leave-one-out (LOO) cross validation was performed to determine the classification correctness rate of the VOCs across the 3 IFTA groups.

A number of statistical methods were used, including descriptive statistics, one-way ANOVA with post hoc Bonferroni correction, and Kruskal-Wallis test according to the data types and distributions. Associations between the expression levels of identified urinary VOCs and IFTA grading were then evaluated by linear regression analyses. Linear relationships between the dependent and independent variables, multivariate normality (via Q-Q plots of the residuals), and multicollinearity were checked before implementing the regression models. For eligible VOCs, two linear regression models were performed - the univariate model and a multivariate model adjusting for age, sex, estimated glomerular filtration rate (eGFR), diabetes mellitus (DM) status (i.e. no DM or DM), and albuminuria (i.e. no albuminuria, microalbuminuria or macroalbuminuria) of study participants. Covariates were selected a priori. In the multivariate model, a secondary analysis evaluating between the expression levels of identified urinary VOCs and covariates was also completed. Coefficient values, standard error (SE) values and 95% confidence intervals (95%CI) were reported for each model. All statistical tests were 2-sided, and p < 0.05 was considered statistically significant. Statistical analyses were performed using Stata 16 (StataCorp MP, College Station, TX, USA).

Results

Characteristics of study participants

The relevant demographic, clinical and biochemistry characteristics of study participants are presented in Table 1. The three study groups included 22 individuals diagnosed with IFTA 0, 15 individuals diagnosed with IFTA 1, and 27 individuals diagnosed with IFTA ≥ 2 upon

Table 1 Relevan	t characteristics of the study	y participants b	y IFTA status ($n = 64$)
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	All participants	IFTA 0	IFTA 1	IFTA ≥ 2	<i>p</i> -value*
		(<i>n</i> = 22)	(<i>n</i> = 15)	(<i>n</i> = 27)	
Age in years, mean (SD)	46 (16)	38 (13)	50 (14)	51 (17)	0.007
Sex in n (%)					0.771
Female	31 (48%)	12 (39%)	7 (22%)	12 (39%)	
Male	33 (52%)	10 (30%)	8 (24%)	15 (46%)	
eGFR in ml/min/1.73m ² , mean (SD)	65 (26)	90 (0)	71 (18)	40 (15)	<0.001
Diabetes in n (%)					0.113
With diabetes	6 (9%)	0	3 (50%)	3 (50%)	
Without diabetes	58 (91%)	22 (38%)	12 (21%)	24 (41%)	
Albuminuria in n (%)^					
No albuminuria	29 (45%)	22 (100%)	2 (13%)	5 (19%)	<0.001
Microalbuminuria	18 (28%)	0 (0%)	4 (27%)	14 (52%)	
Macroalbuminuria	17 (27%)	0 (0%)	9 (60%)	8 (29%)	

eGFR: Estimated glomerular filtration rate; IFTA: Interstitial fibrosis and tubular atrophy; SD: Standard deviation

*p-values were adjusted by Bonferroni's correction

evaluation of kidney biopsy. There were statistically significant differences in age, level of eGFR and albuminuria among the three groups (both p < 0.05), while sex and the presence of diabetes displayed no statistically significant differences between the three groups. As such, study participants with more severe IFTA were older, had lower eGFR and more severe albuminuria, as expected, compared to the other two groups.

Characteristics of post-processed urinary volatile organic compound data

There were 34 urinary VOCs which were identified following GC-MS post-processing. A summary of the expression levels in relation to each identified urinary VOC across the three IFTA groups is described in Table 2. The expression levels of 29 urinary VOCs have appeared with a 'zero' value in one or two IFTA groups, and 5 urinary VOCs had mean values different from a 'zero' value for all three IFTA groups. These 5 urinary VOCs are Benzeneacetaldehyde, α -methyl-; Benzaldehyde, 4-propyl-; Phenol, 2,5-bis(1,1-dimethylethyl)-; Hexamethylene diacrylate; and 2(3 H)-Furanone, dihydro-5-(2-octenyl)-, (Z)-. Amongst these 5 urinary VOCs, there were statistically significant differences in the Phenol, 2,5-bis(1,1-dimethylethyl)- levels between the three IFTA groups. Compared to study participants with IFTA 0, those with IFTA 1 and IFTA≥2 had statistically significantly higher Phenol, 2,5-bis(1,1-dimethylethyl)- levels. The Phenol,

Table 2 Characteristics of post-processed urinary volatile organic compound data by IFTA status (GC-MS peak area; n = 34)

Compound	IFTA 0	IFTA 1	IFTA ≥ 2	<i>p</i> -value*
	(<i>n</i> = 22)	(<i>n</i> = 15)	(<i>n</i> = 27)	
	Mean (SD)	Mean (SD)	Mean (SD)	
2,3-Butanedione	0	0	255,635 (484396)	<0.001
m/p-xylene	0	31,079(28600)	40,663 (102840)	<0.001
4-Heptanone	869,378 (804062)	487,512 (900704)	0	<0.001
Styrene	0	53,015 (41151)	132,887 (320523)	<0.001
2-Heptanone	0	25,230 (40867)	0	<0.001
2-Heptanone, 4-methyl-	0	0	47,073 (52061)	<0.001
Benzaldehyde	0	0	133,970 (353300)	<0.001
Dimethyl trisulfide	0	0	144,442 (274141)	<0.001
Benzene, 1,2,4-trimethyl-	0	20,059 (15274)	0	<0.001
Eucalyptol	0	10,123 (10887)	0	<0.001
Benzeneacetaldehyde	0	11,595 (8828)	0	<0.001
Benzaldehyde, 4-methyl-	0	19,657 (26858)	37,934 (66294)	<0.001
Benzeneacetaldehyde, α-methyl-	28,315 (49689)	95,472 (108403)	115,515 (267576)	0.378
Nonanal	0	0	200,984 (310859)	<0.001
p-Mentha-1,5-dien-8-ol	0	5725 (12494)	3411 (7159)	0.016
Cyclohexanol, 5-methyl-2-(1-methylethyl)-	0	0	137,948 (361046)	<0.001
Pentanenitrile, 5-(methylthio)-	0	8892 (30567)	5338 (13114)	0.072
Benzaldehyde, 2,5-dimethyl-	0	75,705 (93371)	101,796 (141317)	<0.001
4-(2-Furyl) pyridine	0	0	37,285 (111075)	<0.001
Benzaldehyde, 4-propyl-	63,858 (21117)	110,480 (97065)	126,138 (140007)	0.953
1-Decanol	0	387,312 (424772)	0	<0.001
Benzenamine, 3,5-dichloro-	0	23,736 (37229)	32,300 (78139)	0.003
Propofol	0	0	28,265 (96039)	0.026
Benzene, (isothiocyanatomethyl)-	23,113 (39377)	0	0	0.002
2(3 H)-Furanone, 5-hexyldihydro-	0	123,646 (79831)	0	<0.001
1-Naphthalenecarboxaldehyde	4153 (9168)	0	0	0.018
Phenol, 2,5-bis(1,1-dimethylethyl)-	401,288 (160456)	905,390 (525181)	1,716,810 (188809)	<0.001
Benzoic acid, 4-ethoxy-, ethyl ester	0	3390 (4868)	15,596 (24557)	<0.001
Hexamethylene diacrylate	365,007 (187078)	240,518 (168062)	467,793 (618420)	0.288
2(3 H)-Furanone, dihydro-5-(2-octenyl)-, (Z)-	21,247 (16245)	29,239 (47547)	35,220 (56719)	0.962
Benzyl Benzoate	0	64,092 (152849)	209,976 (719202)	0.010
Caffeine	0	0	492,162 (616067)	<0.001
Lidocaine	0	110,275 (427094)	25,769 (101469)	0.444
Oxybenzone	0	0	9656 (42628)	0.012

IFTA: Interstitial fibrosis and tubular atrophy; SD: Standard deviation

*p-values were obtained via the Kruskal-Wallis Test

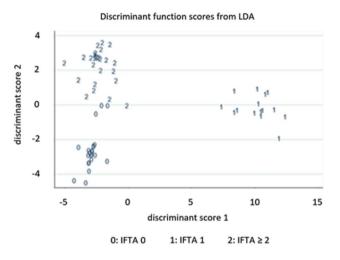


Fig. 1 Linear discriminant analysis demonstrating individuals with IFTA 0, IFTA 1 and IFTA ≥ 2 could be significantly separated by their urinary VOCs profile. IFTA: Interstitial fibrosis and tubular atrophy

 Table 3
 Correct classification rate based on the LOO cross validation method

IFTA group	Number of study participants correctly classified
IFTA 0	86.4%
IFTA 1	86.7%
$IFTA \ge 2$	74.1%
	Ebrosis and tubular atranbul 00 Lagua and out

IFTA: Interstitial fibrosis and tubular atrophy; LOO: Leave-one-out

2,5-bis(1,1-dimethylethyl)- level among people with IFTA \geq 2 was significantly higher than those with IFTA 1 (all *p*<0.05).

Evaluating the separation of the groups of urinary volatile organic compounds by linear discriminant analysis and leave-one-out cross validation

LDA results demonstrated three well-separated groups (i.e. individuals with IFTA 0, individuals with IFTA 1, and individuals with IFTA 2) (Fig. 1). This finding indicates the three IFTA groups are easily separable by their urinary VOC profile. LDA confirmed the pre-identified 34 urinary VOCs were statistically associated with the correct classification of study participants with IFTA 0, study participants with IFTA 1, or study participants with IFTA 2) (p<0.001).

The Mahalanobis distance values were 176, 24, and 162 respectively between study participants with IFTA 0 and IFTA 1; between study participants with IFTA 0 and IFTA \geq 2, and between study participants with IFTA 0 and IFTA \geq 2 (all *p*<0.001). Therefore, the current model displayed a very good discrimination of the three groups, particularly between individuals with IFTA 0 and IFTA 1, and between individuals with IFTA 1 and those with IFTA \geq 2.

According to the LOO cross-validation results (Table 3), 86.4% of study participants (19 of 22 people)

with IFTA 0 were classified correctly by their urinary VOCs profile; 86.7% of study participants (13 of 15 people) with IFTA 1 were classified correctly by their urinary VOCs profile; and 74.1% of study participants with IFTA \geq 2 (20 of 27 people) were classified correctly by their urinary VOCs profile.

Associations between individual urinary volatile organic compounds with IFTA status and covariates

Results from linear regression analysis evaluating associations between IFTA grading amongst the three study participant groups and expression levels of identified urinary VOCs are presented in Table 4. There were 5 VOCs from the univariate model and 4 VOCs from the multivariate model which were significantly upregulated in the IFTA 1 compared to the IFTA 0 group (p < 0.05), of which 2-heptanone; Benzene, 1,2,4-trimethyl; Benzeneacetaldehyde; and 2(3 H)-furanone, 5-hexyldihydro were significantly upregulated VOCs in both the univariate and multivariate analyses. There were 12 VOCs from the univariate model and 2 VOCs from the multivariate model which were significantly upregulated in the IFTA≥2 compared to the IFTA 1 group (p < 0.05), of which 2-heptanone, 4-methyl and Benzaldehyde, 4-methyl were significantly upregulated VOCs in both the univariate and multivariate analyses. There are 2 VOCs (Benzene (isothiocyantomethyl) and Benzaldehyde, 2,5-dimethyl) in the univariate model which were positively associated with IFTA progression across all stages (p < 0.05), while no VOCs in the multivariate model displayed such statistical association.

On evaluating associations between identified urinary VOCs and adjusted covariates within the multivariate linear regression model (Table 4), there were 2 VOCs (4-Hepatanone; and Benzoic acid, 4-ethoxy, ethyl ester) which were downregulated and 2 VOCs (Benzene (iso-thiocyantomethyl); and 1-Napthalenecarboxaldeyde) which were upregulated with the male sex. There were 3 VOCs (Benzaldehyde, 4-propyl; 2-heptanone; and Benzaldehyde, 4-methyl) which were positively associated with decline in eGFR levels. There were 3 VOCs (2,3-butanedione; Benzeneacetaldehyde; and 2(3 H)-Furanone, 5-hexyldihydro) which were positive associated with DM status. Benzeneacetaldehyde was positively associated with albuminuria status.

Discussion

This study is the first that has evaluated the associations between expression levels of urinary VOCs and kidney tubulointerstitial histopathology. It is particularly significant in a CKD context, given IFTA is the hallmark of CKD. Overall, our results identified 34 VOCs which enabled classification between individuals with no tubulointerstitial disease, mild tubulointerstitial disease and

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	Univariate model	del	-		Multivariate model*	odel*		
	Coefficient	SE	95% CI	<i>p</i> -value	Coefficient	SE	95% CI	<i>p</i> -value
Benzeneacetaldehyde, $lpha$ -methyl-								
IFTA grading								
IFTA 0	Reference				Reference			
IFTA 1	67,157	61,800	-56,420, 190,734	0.281	-25,149	95,284	-216,102, 165,804	0.793
$ FTA \ge 2$	87,200	53,009	-18,798, 193,198	0.105	-87,971	117,774	-323,994, 148,053	0.458
Age					3149	1652	-162, 6460	0.062
Sex								
Female					Reference			
Male					-40,090	45,381	-131,036, 50,855	0.381
eGFR					-3046	1799	-6651, 559	0.096
Diabetes presence								
Without diabetes					Reference			
With diabetes					-231,251	88,653	-408,917, -53,586	0.012
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					-30,061	79,520	-189,423, 129,301	0.707
Macroalbuminuria					91,230	81,913	-72,928, 255,388	0.270
Benzaldehyde, 4-propyl-								
IFTA Grading								
IFTA 0	Reference				Reference			
IFTA 1	46,621	34,589	-22,544, 115,787	0.183	52,778	54,059	-55,558, 161,114	0.333 0.777
IFTA ≥ 2	62,279	29,669	2952, 121,606	0.040	-19,056	68,818	-152,963, 114,850	
Age					577	937	-1302, 2455	0.541
Sex								
Female					Reference			
Male					-2476	25,747	-54,073, 49,122	0.924
eGFR					-2743	1021	-4788, -698	0.009
Diabetes presence								
Without diabetes					Reference			
With diabetes					-88,255	50,297	-189,053, 12,542	0.085
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					-77,033	45,115	-167,446, 13,380	0.093
Macroalbuminuria					-43,751	46,473	-136,886, 49,383	0.351
Phenol, 2,5-bis(1,1-dimethylethyl)-								
IFTA Grading								
IFTA 0	Reference				Reference			

Page 7 of 20

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50(102 4.2.502 5.4004(1,36,41) 0.153 5.6010,303(4) 0.153 5.6010,466,41 13552 36.075 59.000,203(3)4 0.001 10305 1.390,132,20030 Prement 13552 36.075 59.000,203(3)4 0.001 1.393 1.390,232,20036 Prement 10335 1.1394 -45.30,650 -1.390,237,26036 Gleen 6667 1.391,12 2.201,252 660,146,541 Intrina 6667 1.391,126 6.661,146,541 -45.30,550 Intrina 6667,146,541 1.393 1.291,279,650 -1.391,279,650 Intrina 6667,146,54 -1.391,379 -1.391,379 -1.391,379 Intrina 6664,146,17 6664,146,17 -1.391,379 -1.391,379 Intrina 8669,169 7.306,199 7.306,199 -1.391,379 Intrina 3669,169 7.306,199 7.306,199 -1.392,305,560,59 Intrina 3669,169 7.306,199 7.306,199 -1.392,305,560,59 Intrina 2.31,240		Coefficient	ł	95% CI	<i>p</i> -value	Coefficient	SE	95% CI	<i>p</i> -value
13522 36,700,200,200,344 0.001 395,54 84/173 1.1927 1.09,273,200,0034 PDENCE 0.031 1.135 1.135,73 1.030,37,240,008 1.030,37,240,008 PDENCE 0.031 1.135,17 1.253,79 0.0357,240,008 0.0357,240,008 PDENCE 0.031 1.135,17 0.0357,240,008 0.001 0.0357,240,008 PDENCE 0.041 0.042 0.043 0.043 0.0357,240,008 PDENCE 0.041 0.043 0.043 0.043 0.043 0.0357,240,008 PDENCE 0.041 0.043	IFTA 1	504,102	422,592	-340,924, 1,349,128	0.238	91,153	686,206	-1,284,034, 1,466,341	0.895
Control Contro <thcontrol< th=""> <thcontrol< th=""> <thco< td=""><td>IFTA ≥ 2</td><td>1,315,522</td><td>362,479</td><td>590,700, 2,040,344</td><td>0.001</td><td>390,564</td><td>848,173</td><td>-1,309,213, 2,090,341</td><td>0.647</td></thco<></thcontrol<></thcontrol<>	IFTA ≥ 2	1,315,522	362,479	590,700, 2,040,344	0.001	390,564	848,173	-1,309,213, 2,090,341	0.647
Freence Element Element <t< td=""><td>Age</td><td></td><td></td><td></td><td></td><td>10,927</td><td>11,897</td><td>-12,914, 34,770</td><td>0.362</td></t<>	Age					10,927	11,897	-12,914, 34,770	0.362
Reference Reference <t< td=""><td>Sex</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	Sex								
475 405 326,82 106,857,340,78 5 19,421 12,941 12,954 45,836,653 dickets 47,82 63,457 45,836,653 45,836,653 dickets 7,82 63,457 45,836,653 45,836,653 nituria 7,92 63,457 45,836,653 45,836,653 nituria 7,92 21,745,11746 29,165 58,6457 45,836,653 nituria 7,461 106,917,166 21,451,371 23,1451,371 23,1451,371 serie 8 8 8 8 66,973,340,99 45,935,356,99 45,935,356,99 45,935,356,99 45,935,356,99 45,935,356,99 45,935,356,99 45,935,356,99 45,935,346,99 45,935,346,99 45,935,346,99 45,935,346,99 45,955,346,99 45,935,346,99 45,935,346,99 45,935,346,99 45,955,346,99 45,955,346,99 45,955,346,99 45,955,346,99 45,955,49 45,955,49 45,955,49 45,955,49 45,955,49 45,955,49 45,955,49 45,955,49 45,955,49 45,955,49 <td>Female</td> <td></td> <td></td> <td></td> <td></td> <td>Reference</td> <td></td> <td></td> <td></td>	Female					Reference			
19421 13921 13924 4532633 650es 654657 653453 653453 1010 667482 63457 159373,66514 1010 674382 63457 15933586655 1010 721625 572681 14335586655 1010 20143 583918 9130731,451273 1010 20143 15023 20143 535534 244e11 105892 7400,43729 0007 150333 193058 555534 255534 9029 7400,43729 0007 150333 192481 143355 5555349 255534 9029 7400,43739 0007 150833 192936 59557,23499 255534 9029 7400,43739 0007 150833 193568 59557,23499 255534 9029 7400,43739 0007 150833 192963 59557,23499 255534 9029 7400,43739 0007 150833 192963 10959763 255564 255564 9029 7400,43739 0007 1950836979 10959753	Male					-405,889	326,822	-1,060,857, 249,078	0.220
Spreame Betweet diaget -614.382 634.57 -1953.779,605214 una -674.382 634.57 -1953.779,605214 una -674.382 534.57 -1953.779,605214 una -674.382 534.57 -1953.779,605214 una -674.382 539.918 -1,953.779,605214 una -261.649 539.53 -913.073,1451,371 una -261.649 200,437.29 0.007 -338.33 -1,439.205,86055 una -3.44e-11 105.92 7,400,437.29 -1,430.237,1451,371 -1,430.235,4699 -1,430.235,4699 una -3.44e-11 105.92 -1,1174,51.1746 -1,130.235,5499 -1,430.235,5499 -1,430.235,5499 -1,430.235,5499 -1,430.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450.235,5499 -1,450	eGFR					-19,421	12,954	-45,382, 6539	0.140
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Deter 674,32 638,47 -1,95,779,662,14 Unio Reference 674,32 538,457 -1,953,779,662,14 Unio Reference 291,625 572,681 -1,459,305,86055 Duminia Reference 291,625 572,681 -1,439,305,86055 Duminia Reference 291,627 589,918 -913,073,1451,371 Duminia Reference 234,611 1000 238,330 154,600 Sator 344,611 1005 238,330 154,600 -913,073,1451,371 Sator 255,634 90,892 21/7,46,211,746 1000 155,723 600,11,16,450 Sator 255,634 90,892 24,004,477,293 0000 155,724,993 656,732,613 Sator Sator Sator 166 2403,473 240,933 243,673 Sator Sator Sator 14,893 51,824,633 133,674 133,674 Sator Sator 133,647 133,647 132,569 133,567 133,567	Without diabetes					Reference			
wtai Reference -391.632 572.681 -1.4393.05.856055 communia -391.632 572.681 -1.4393.05.856055 391.073,1451.371 communia -391.632 572.681 -1.4393.05.856055 391.073,1451.371 communia -344e-11 105.892 2.117.46,211.746 1000 -325.330 155.725 -560611-16,550 conce Reference -344e-11 105.892 74.008,477.259 0.007 -155.833 192.481 -583.4993 conce Reference -344e-11 105.892 74.008,477.259 0.007 -155.833 192.932 593.932 593.932 593.932 593.932 593.932 593.932 51.882,632.53.932 593.932 51.882,632.632.612 74.188 2.42.243,951.149.03 51.882,632.632.612 74.188 2.42.439,51.74.93 51.882,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632.632.612 74.883,632	With diabetes					-674,282	638,457	-1,953,779, 605,214	0.296
minuta Reference umbunia 39,625 572,631 -1,433,05,65055 umbunia 39,625 572,631 -1,433,05,65055 nectione 39,625 559,145 9,913,073,145,1371 serce Reference 39,625 -91,0273,145,1371 serce Reference 3,44e-11 105,892 -21/46,211,46 1000 344e-11 105,892 -21/46,211,746 1000 -383,330 192,481 -536,575,234,090 344e-11 105,892 -21/46,211,746 1000 -383,330 192,481 -536,575,234,090 345 3,44e-11 105,892 -21/46,211,746 1000 -383,330 192,493 555,54 9,029 74,009,437,259 0.007 -136,677 -360,993 50	Albuminuria								
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aminuta anediore serce Reference 3.344e-11 05,892 3.11/46,211/346 1000 3.28530 155/25 640611.16450 3.344e-11 05,892 2.11/46,211/346 1000 3.28530 155/25 640611.16450 3.344e-11 05,892 2.11/346,211/346 1000 3.28530 155/25 64091 3.344e-11 05,892 3.11/346,211/346 1000 3.28530 155/25 64091 4.18 2.700 5.5824,993 4.18 2.700 7.1325 4.18 7.11,1645,1327 4.18 7.11,1645,1327 4.18 7.13264,545,7018 4.18 7.1246,545,7018 4.18 7.1846,545,7018 4.18 7.1846,545,7018 4.18 7.1846,545,7018 4.18 7.1846,545,7018 4.18 7.1446,545,745,745 4.19 7.1446,545,745,745 4.19 7.1446,545,745,745 4.19 7.1446,545,745	Microalbuminuria					-291,625	572,681	-1,439,305, 856,055	0.613
metione Reference Reference S35,53,4 640,611,-16,450	Macroalbuminuria					269,149	589,918	-913,073, 1,451,371	0.650
sere Reference Solution Solution <t< td=""><td>2, 3-Butanedione</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	2, 3-Butanedione								
Reference Reference S3530 15572 640611,16450 -344e-11 105892 -211/46,211,746 1000 -33830 15575 560611,16450 -344e-11 105892 -211/46,211,746 1000 -33850 15575 56057,234909 -344e-11 105892 -211/46,211,746 1000 -33850 15575,34909 255634 90829 7400,437,259 0.007 -16083 192,481 -5808,4993 255634 90829 7400,437,259 0.007 -16089 23039 10999,783 Sprestex Reference Reference -1099,783 -10999,783 -10999,783 Sprestex Reference -10,997 241 144,889 51,882,632,612 Unutai -10,997 -10,997 2402,499 2432,414 -10999,783 Unutai -11,956,57,501,89 -10,992 123,564 -123,563,1140 -123,563,1140 Unutai -11,666 -123,5657,501,89 24359 -133,592 -133593,1140 Unuta	CKD presence								
-344e-11 105,892 -211/346,211/346 1000 -328,530 155/25 -640,611,-16,50 255,634 90,829 74,009,437,259 0.007 -150,833 192,481 -556,57,534,909 255,634 90,829 74,009,437,259 0.007 -150,833 192,481 -556,57,534,909 255,634 90,829 74,009,437,259 0.007 -516,83 102,991,783 -565,793,9699 Spresence Reference -91,657 74,168 -240,293,56,979 -10,999,783 Spresence Reference -91,657 74,168 -10,999,783 Spresence	IFTA 0	Reference				Reference			
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-18 2700 -5828,4933 Reference -91,657 74168 -240,293,56979 Spresence -91,657 74168 -240,293,56979 Glabetes -91,657 74168 -240,293,56979 Glabetes -91,657 74168 -10999,783 Glabetes -91,657 74168 -10999,783 Initria -91,224,71 144,899 51,882,632612 Initria -342,471 144,899 51,882,632612 Initria -107,852 107,852 129,962 -15258,365303 Initria -107,852 107,852 129,962 -15258,365303 Initria -107,852 107,852 129,962 -1525,93,563,033 Initria -107,852 107,852 107,962 -1539,54,1100 Initria -108,866 -11,236,567,-502,189 -0001 -36,364 -1037,594,310,309 Initria -1,236,567,-502,189 -0001 -36,362 -1037,594,310,309 Initria -1,236,567,-502,189 -0001 -478,19 -1037,594,310,309 Initria -1,236,567,-502,189 -0001 -478,19 -1037,594,310,309 Initria -1,236,567,-502,189 -0001 -478,19 -1037,594,310,309 <	IFTA ≥ 2	255,634	90,829	74,009, 437,259	0.007	-150,833	192,481	-536,575, 234,909	0.437
Reference 91,557 74,168 -240,293,56,979 55 Streame -91,557 74,168 -240,293,56,979 5 Streame -91,557 74,168 -10,999,783 5 Streame -91,557 74,168 -10,999,783 5 Streame -91,527 144,889 51,882,632,612 bette -342,47 144,889 51,882,632,612 bette -342,47 144,889 51,882,633,612 minuria -342,47 144,889 51,882,633,633 bette -342,47 107,852 129,962 -152,583,564,310,30 burninuria -381,866 214,082 -309,944,46,217 0079 -355,647,310,309 sence Reference -381,284 -1,235,567,-502,189 -0,001 -478,159 -1,337,544,310,309 sence -381,866 214,082 -309,944,46,217 0079 -355,647,310,309 sence -381,866 214,082 -1,235,567,-502,189 -1,037,594,310,309 sence -383,228 -1,235,567,-502,189 -1,037,594,310,309 sence -333,228 -1,335,544,710,0079 -1,316,667 -1,317,865 sence -1,335,657,-502,189 -1,235,567,-502,189 -1,037,594,310,309 sence -1,	Age					-418	2700	-5828, 4993	0.878
Reference diabetes diabetes betes minuria minu	Sex								
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etes presence Reference out diabetes 342,247 144,889 51,882,632,612 diabetes 342,247 144,889 51,882,632,612 minuria 342,247 144,889 51,882,632,612 burninuria 342,547 107,852 129,962 -152,598,368,303 alburninuria 107,852 107,852 107,852 129,962 -152,598,368,303 alburninuria 245,850 133,874 -22,439,514,140 245,850 133,874 -22,439,514,140 paranore Reference Reference 86 -381,866 107,852 -1,236,567,-502,189 -0079 -363,642 -1,037,594,310,309 2 -869,378 183,628 -1,236,567,-502,189 0.0079 -563,642 -1,037,594,310,309 2 -869,378 183,628 -1,236,567,-502,189 0.0079 -363,642 -1,311,186,354,867 2 -869,378 133,6567,-502,189 0.0079 -363,642 -1,311,186,354,867 2 -869,378 133,6567,-502,189 0.0079 -363,642 -1,311,186,354,867	eGFR					-5108	2939	-10,999, 783	0.088
out diabetes Reference diabetes 342,247 144,899 51,882,632,612 minuria 342,247 144,899 51,882,632,612 burninuria Reference 107,852 129,962 152,598,368,303 alburninuria 107,852 129,962 152,598,368,303 alburninuria 245,850 133,874 -22,439,514,140 oalburninuria 245,850 133,874 -22,439,514,140 pranone 214,082 809,949,46,217 0,079 -363,642 -336,294 0 -381,866 214,082 -380,949,46,217 0,079 -363,642 -1,037,594,310,309 0 -381,866 214,082 -1,236,567,-502,189 <001	Diabetes presence								
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buminuria albuminuria oalbuminuria oolbuminuria ptanone presence 0 1 07852 245,850 133,874 245,850 133,874 245,850 133,874 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 236,949,46,217 007 25 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 245,850 214,08 243,850 21,037,594,310,309 21,037,594,310,594,310,594,594,594,594,594,594,594,594,594,594	Albuminuria								
balbuminuria $107,852$ 129,962 -152,598,363,303 oalbuminuria $245,850$ 133,874 -22,439,514,140 245 and 133,874 -22,439,514,140 245 and 133,874 -22,439,514,140 245 and 133,874 -22,439,514,140 245 and 245 a	No albuminuria					Reference			
oalbuminuria ptanone presence 245,850 133,874 -22,439,514,140 Presence 367,94,310,309 1 -381,866 214,082 -809,949,46,217 0.079 -363,642 336,295 -1,037,594,310,309 1 -381,866 214,082 -809,949,46,217 0.079 -363,642 336,295 -1,037,594,310,309 1 -478,159 415,672 -1,11,186,354,867 2 -363,642 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,1311,186,354,867 2 -369,378 183,628 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,1311,186,354,867 2 -369,378 183,628 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,1311,186,354,867 2 -369,378 183,628 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,1316,354,867 2 -369,378 183,628 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,1316,354,867 2 -369,378 183,628 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,1311,186,354,867 2 -369,378 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,1316,354,867 2 -369,378 -1,236,567,-502,189 <0.001 -478,159 -1,1,382	Microalbuminuria					107,852	129,962	-152,598, 368,303	0.410
ptanone presence Reference Reference 336,295 -1,037,594,310,309 -363,642 336,295 -1,037,594,310,309 -381,866 214,082 -809,949,46,217 0.079 -363,642 336,295 -1,037,594,310,309 -1 -381,866 214,082 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,311,186,354,867 -2 -2 -3 -869,378 183,628 -1,236,567,-502,189 <0.001 -478,159 -1,748,11,882 -1,248,67 -1,248,	Macroalbuminuria					245,850	133,874	-22,439, 514,140	0.072
presence Reference 0 8eference 1 -381,866 214,082 -809,949,46,217 0.079 -363,642 336,295 -1,037,594,310,309 2 -869,378 183,628 -1,236,567,-502,189 0.079 -478,159 198 5830 -11,486,11,882	4-Heptanone								
D Reference Reference 1 -381,866 214,082 -809,949,46,217 0.079 -363,642 336,295 -1,037,594,310,309 ≥ -869,378 183,628 -1,236,567,-502,189 <0.001	CKD presence								
1363,642 -363,642 -1,037,594,310,309 ≥ 2 -869,378 183,628 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,311,186,354,867 198 5830 -11,486,11,882	IFTA 0	Reference				Reference			
≥ 2869,378 183,628 -1,236,567,-502,189 <0.001 -478,159 415,672 -1,311,186,354,867 198 5830 -11,486,11,882	IFTA 1	-381,866	214,082	-809,949, 46,217	0.079	-363,642	336,295	-1,037,594, 310,309	0.284
198 5830 -11,486,11,882	$ FTA \ge 2$	-869,378	183,628	-1,236,567, -502,189	<0.001	-478,159	415,672	-1,311,186, 354,867	0.255
	Age					198	5830	-11,486, 11,882	0.973

Page 8 of 20

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Table

Female Male eGFR Diabetes presence Without diabetes With diabetes With diabetes With diabetes Mbuminuria Albuminuria Microalbuminuria Macroalbuminuria Styrene CKD presence LFTA 0 IFTA 1 IFTA 1 IFTA 2 Mge	Univariate model Coefficient	tel SE			Multivariate model*	odel*		
Female Male eGFR Diabetes presence Without diabetes With diabetes With diabetes With diabetes Microalbuminuria Macroalbuminuria Macroalbuminuria Fyrene CKD presence IFTA 0 IFTA 1 IFTA 1 IFTA 2 IFTA 2 IFTA 2 Sex	Coefficient	SE						
Female Male eGFR Diabetes presence With diabetes With diabetes With diabetes Moroalbuminuria Macroalbuminuria Macroalbuminuria Styrene ETA 0 IFTA 1 IFTA 1 IFTA 2 Age Sex			95% CI	<i>p</i> -value	Coefficient	SE	95% CI	<i>p</i> -value
Male eGFR Diabetes presence Without diabetes With diabetes With diabetes Mbuminuria No albuminuria Macroalbuminuria Macroalbuminuria Styrene CKD presence LFTA 0 IFTA 1 IFTA 1 IFTA 2 IFTA 2 IFTA 2 Sex					Reference			
eGFR Diabetes presence Without diabetes With diabetes Albuminuria No albuminuria Macroalbuminuria Styrene CKD presence IFTA 0 IFTA 1 IFTA 1 IFTA 2 IFTA 2 Sex					-381,066	160,169	-702,052, -60,080	0.021
Diabetes presence Without diabetes With diabetes Albuminuria No albuminuria Microalbuminuria Macroalbuminuria Styrene CKD presence FFA 0 FFA 1 FFA 1 FFA 1 FFA 2 FFA 2 FFA 2 FFA 2 FFA 2 FFA 2 FFA 2 FFA 3 FFA 3					10,035	6348	-2687, 22,757	0.120
Without diabetes With diabetes Albuminuria Nicroalbuminuria Macroalbuminuria Styrene CKD presence IFTA 1 IFTA 2 IFTA 2 Age Sex								
With diabetes Albuminuria No albuminuria Macroalbuminuria Styrene CKD presence IFTA 1 IFTA 1 IFTA 2 Age Sex					Reference			
Albuminuria No albuminuria Microalbuminuria Styrene CKD presence IFTA 0 IFTA 1 IFTA 2 Age Sex					412,139	312,895	-214,916, 1,039,195	0.193
No albuminuria Microalbuminuria Aacroalbuminuria Styrene CKD presence IFTA 0 IFTA 1 IFTA 2 Age Sex								
Microalbuminuria Macroalbuminuria Styrene IFTA 0 IFTA 1 IFTA 2 Age Sex					Reference			
Macroalbuminuria Styrene CKD presence IFTA 0 IFTA 1 IFTA 2 Age Sex					107,627	280,659	-454,827, 670,082	0.703
Styrene CKD presence IFTA 0 IFTA 1 IFTA ≥ 2 Age Sex					144,927	289,107	-434,455, 724,311	0.618
CKD presence IFTA 0 IFTA 1 IFTA ≥ 2 Age Sex								
IFTA 0 IFTA 1 IFTA ≥ 2 Age Sex								
IFTA 1 IFTA ≥ 2 Age Sex	Reference				Reference			
lFTA ≥ 2 Age Sex	53,014	70,379	-87,717, 193,746	0.454	-82,217	110,326	-303,317, 138,883	0.459
Age Sex	132,886	60,367	12,173, 253,599	0.032	-23,865	136,367	-297,152,249,421	0.862
Sex					2931	1912	-901, 6765	0.131
Female					Reference			
Male					-66,780	52,545	-172,085, 38,523	0.209
eGFR					-1895	2082	-6069, 2278	0.367
Diabetes presence								
Without diabetes					Reference			
With diabetes					-107,838	102,649	-313,553, 97,876	0.298
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					-7446	92,074	-191,968, 177,075	0.936
Macroalbuminuria					155,481	94,845	-34,593, 345,557	0.107
2-Heptanone								
IFTA Grading								
IFTA 0	Reference				Reference			
IFTA 1	25,229	6555	12,120, 38,338	<0.001	34,885	10,630	13,582, 56,188	0.002
$ FTA \ge 2$	-7.11e-12	5623	-11,244, 11,244	1.000	24,240	13,139	-2091, 50,571	0.070
Age					-101	184	-470, 268	0.585
Sex								
Female					Reference			
Male					-3589	5062	-13,735, 6556	0.481
eGFR					428	200	26, 830	0.037
Diabetes presence								

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E 95% Cl p-value 11,381 -22,757,22,557 1,000 9762 27,552,66,593 <0.001 9764 27,552,66,593 <0.001 59,929 -119,836,119,836 1000 59,929 -119,836,119,836 1000 51,404 41,652,247,231 0.007		Univariate model	del			Multivariate model*	odel*		
Allower Allower <t< th=""><th></th><th>Coefficient</th><th></th><th>95% CI</th><th><i>p</i>-value</th><th>Coefficient</th><th></th><th>95% CI</th><th><i>p</i>-value</th></t<>		Coefficient		95% CI	<i>p</i> -value	Coefficient		95% CI	<i>p</i> -value
etea 1674 980 -960.3665 muna	Without diabetes					Reference			
Main Hereice Distribution 3423 891 21,023,4335 Distribution 3012 939 21,023,4335 Distribution Meterice 3012 3023 2033,4301 Distribution Meterice 3012 3012 21,023,4335 Distribution Meterice 3012 3233,533 21,033 9343 Distribution 3012 3233,533 2333,533 3203 3203 333 Street 3012 3233,533 3203 3203 333 333 Street 47,033 3203 3203 333 <	With diabetes					14,874	9890	-4946, 34,695	0.138
muna Belience 373 861 3733 <	Albuminuria								
Immunia	No albuminuria					Reference			
Duminuia -501 913 -7125,14701 Duminuia - <th< td=""><td>Microalbuminuria</td><td></td><td></td><td></td><td></td><td>-3423</td><td>8871</td><td>-21,202, 14,355</td><td>0.701</td></th<>	Microalbuminuria					-3423	8871	-21,202, 14,355	0.701
none t-methyt- ading Reference 11,391 2,255,235,333 4001 11,897 2360 10,801 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 0355 0356 <td>Macroalbuminuria</td> <td></td> <td></td> <td></td> <td></td> <td>-3612</td> <td>9138</td> <td>-21,925, 14,701</td> <td>0.694</td>	Macroalbuminuria					-3612	9138	-21,925, 14,701	0.694
Allon Reference Selence Selence <t< td=""><td>2-Heptanone, 4-methyl-</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	2-Heptanone, 4-methyl-								
Reference Reference <threference< th=""> <threference< th=""> <thr< td=""><td>IFTA Grading</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></thr<></threference<></threference<>	IFTA Grading								
2.15-11 1.381 -2.257,2257 1000 1.497 18,91 -556,4555 47072 9762 27552,66.93 <001	IFTA 0	Reference				Reference			
4707 702 7555.66393 5000 57247 21856 11081 532 530 57431 330 594,310 591 57541 330 594,310 591 57541 349 557,341 591 661 141 349 557,341 591 661 141 349 557,341 591 861 141 349 557,341 501 861 17,305 141 349 557,341 501 861 141 349 557,341 141 501 861 14307 9361 1705 9369 51 344 51,4031 1537 25359 1537 93591 51 51,403 15335 1000 15432 21531,40337 51 51 510 15397 25597,5167 51 51 510 15397 25597,5167 51 51 510 15395 5597,5167 51 51 510 15395 5597,5167 51 51 510 15336 1007 1521 51 51 51336 100 1574 1277,505 <td>IFTA 1</td> <td>2.15e-11</td> <td>11,381</td> <td>-22,757, 22,757</td> <td>1.000</td> <td>11,497</td> <td>18,491</td> <td>-25,561, 48,555</td> <td>0.537</td>	IFTA 1	2.15e-11	11,381	-22,757, 22,757	1.000	11,497	18,491	-25,561, 48,555	0.537
32 32 97,310 5 5 5 5 5 5 692 807 -2552,12757 6 692 807 -2552,12757 11 349 -2552,12757 -49269,19660 bete 6492 807 -2552,421357 bete -14799 17,205 -49269,19660 minuta -14799 17,205 -49269,19660 bunnuna -14799 17,205 -49269,19660 bunnuna -14799 17,205 -49269,19660 bunnuna -14799 17,205 -3157,40337 bunnuna -1479 1440 -14799 -1435337 bunnuna -14799 17,205 -147337 bunnuna -1441 11,305,119836 -14353 bunnuna -1441 11,305 -14353 bunnuna -1442 11,91356 -14353 adiop -344-11 9929 -119356,13337 adiop -14442 11,91356,119836 -110530 adiop -14442 11,91356,119836 -110531 adiop -14442 11,91356,119836 -110531 adiop -14442 11,91356,119836 -110531 <td>IFTA ≥ 2</td> <td>47,072</td> <td>9762</td> <td>27,552, 66,593</td> <td><0.001</td> <td>57,787</td> <td>22,856</td> <td>11,981, 103,593</td> <td>0.014</td>	IFTA ≥ 2	47,072	9762	27,552, 66,593	<0.001	57,787	22,856	11,981, 103,593	0.014
Reference Reference Reference 2.254,12757 spreance -492 88/7 -2254,12757 spreance Reference -492 557,841 bers Reference -492 557,841 bers Reference -492 557,841 minuia Reference -14,789 17,205 -49,260,19,690 minuia Reference -14,789 17,205 -49,260,19,690 minuia Reference -14,789 17,205 -49,260,19,690 minuia Reference -14,442 51,404 -13,2959,158,650 -14,7033 Misula Reference -14,442 51,404 41,622,247,231 000 -14,44 93,61 -14,703 -14,7033 Misula -14,442 51,404 41,622,247,231 000 -14,74 93,61 -14,7302 -14,9033 Misula -14,442 51,404 41,622,247,231 000 -14,74 93,61 -14,7302 -14,7502 Spreace -14,442	Age					-332	320	-974, 310	0.305
Belence Belence 4802 8807 -22542,12757 4802 557,841 557,841 spresence 8807 -22542,12757 dabetes 8807 -22542,12757 betes 8807 -22542,12757 betes 8807 -22542,12757 betes 14,128 12,059 -49269,19560 minuia 1-1,1283 17,205 -49269,19560 minuia 9410 15,897 -38549,25167 betes -119336,119336 1000 15,897 -38549,25167 betes -344e-11 5929 -119336,119336 1000 15,897 -38549,25167 betes -344e-11 5929 -119336,119336 10216 122,814 -143797,34331 betes -344e-11 59299 -119336,119336 102216 -3359,158650 betes -344e-11 59299 102016 122,814 -1437,903,318650 betes -344e-11 59299 192417 102,316 -140,	Sex								
•492 8807 -25-42,1257 •te presence 141 349 -557,841 •te detecs -14789 17205 -49269,19590 rid detecs -14789 17205 -49269,19590 rid detecs -14789 17205 -49269,19590 rid munia Reference -14789 -557,841 munia Reference -14789 17205 -49269,19560 munia Reference -4400 15,897 -38549,25167 Munia Reference -4441 -3929,18650 -38549,25167 Munia Reference -44442 -41652,247231 0007 102216 122361 -1497,5026 2 144442 51,404 41652,247231 0007 102216 122364 -1393,503,54650 2 144442 51,404 41652,247231 0007 102216 12374 -1497,5026 Reference 102416 122281 12326 -1393,503,54650 -1405,633690 2 144442 51,404 41652,247231 0007 12216 -1497,5026 Reference 102416 12246 12236 -1497,5026 -1405,63369 Reference 10444 1552,47231 0007	Female					Reference			
141 349 -57,7841 texpresence -4,799 17,205 -49,269,19,690 texpresence -14,799 17,205 -49,269,19,690 unimunia Reference -14,793 -49,00 15,412 -21,517,40,337 unimunia Reference -14,793 -66,00 15,897 -33,54,55167 Wrtsuhlde Reference -66,00 15,897 -33,54,55167 Wrtsuhlde -34,4-11 59,292 -119,836,119,836 1000 -34,441 -13,907,343,441 2 14,442 51,404 41,652,247,231 0007 102,216 122,144 -13,907,343,941 2 14,442 51,404 41,652,247,231 0007 102,216 -1877,5026 8	Male					-4892	8807	-22,542, 12,757	0.581
te presene ut diabetes Reference -14,789 17,205 -49,269,19,690 uninuia uninuia uninuia uninuia uninuia Reference -49,269,19,690 -49,269,19,690 uninuia uninuia uninuia Reference -41,789 -17,205 -49,269,19,690 uninuia uninuia Reference -14,789 -17,302 -49,269,19,690 uninuia Reference -13,992 11,9336,119,836 -11,9336,119,836 -13,992 -13,992,91,58,650 uninuia Ade-11 59,929 -11,19336,119,836 1000 -14,474 -33,590,138,650 uninuia -34,442 51,404 41,652,247,231 0.007 157,4 -139,907,343,431 uninuia -44,42 51,404 41,652,247,231 0.007 10,216 -139,07,343,431 e -44,42 51,404 41,652,247,231 0.007 10,216 -130,505,343,341 e -44,42 51,404 10,521 122,814 -130,676,48399 e -44,42 51,404 10,521 10,516 -137,597,9338 e	eGFR					141	349	-557, 841	0.686
It diabetes and the following	Diabetes presence								
liabetes inuria inur	Without diabetes					Reference			
ninutia Reference Reference	With diabetes					-14,789	17,205	-49,269, 19,690	0.394
uninuia uninuia albuninuia albuninuia tytisufide tytisufide tytisufide tytisufide tytisufide tytisufide tytisufide tytisufide tytisufide tytisufide tytisufide tytisufide totion tytisufide treference totion tytisufide tytisufide treference totion tytisufide treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference treference	Albuminuria								
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albuminufa ihy trisuffde isolation iby trisuffde isolation iby trisuffde isolation Reference 2 2404 41,652,247,231 0.000 -40,474 99,361 -239,59,158,650 -40,474 99,361 -239,59,158,650 -40,474 99,361 -239,59,158,650 -40,474 99,361 -239,59,158,650 -41,4442 51,404 41,652,247,231 0.000 -40,474 99,361 -239,59,158,650 1574 1722 -1877,5026 -1877,5026 -1877,5026 -1877,5026 -1877,5026 -10,6890 -40,74 99,361 -239,59,158,650 -106,890 92,447 -292,159,78,378 -106,890 92,447 -292,150,78,378 -106,890 92,447 -292,150,78,378 -106,890 -204 -204 -204 -204 -204 -204 -204 -20	Microalbuminuria					9410	15,432	-21,517, 40,337	0.545
thytrisufide Reference Reference iading Reference 335591158560 3.44e-11 59292 -119835,119835 1000 -40744 99.361 -239599,158650 2 3.44e-11 59292 -119835,119835 1000 -40744 99.361 -143907,348,341 2 144,442 51,404 41,652,247,231 0.007 102,216 122,814 -143907,348,341 e 144,442 51,404 41,652,247,231 0.007 102,216 122,814 -143907,348,341 e 144,442 51,404 41,652,247,231 0.007 102,216 122,814 -143507,348,341 e 144,442 51,404 41,652,247,231 0.007 102,216 122,814 -143,507,3026 e 144,442 51,404 41,652,247,231 0.007 102,216 1722 -1877,5026 e 144,442 144,442 144,442 146,646 -140,696 -140,696 -140,696 -140,696 -180,696 -140,696 -140,696 -140,696 -140,696 -1416,697 -140,696 -1416,697	Macroalbuminuria					-6690	15,897	-38,549, 25,167	0.675
iading Reference 3.44e-11 5.9229 -119,836,119,836 1.000 -40,474 99,361 -239,599,158,650 3.44e-11 5.9229 -119,836,119,836 1.000 -40,474 99,361 -239,599,158,650 1.44,442 51,404 41,652,247,231 0.007 102,216 122,814 -143,907,348,341 1574 1722 -1877,5026 1574 1722 -1877,5026 1574 1732 -140,676,48,999 47,323 -140,676,48,999 47,323 -140,676,48,999 47,323 -140,676,48,999 47,323 -140,676,48,999 16,680 1875 -1877,5026 16,680 2,447 2,247,231 0.007 1875 -190,78,378 16,680 2,447 2,247,5378 16,680 2,447 2,247,5378 16,680 2,447 2,247,5378 16,680 2,447 2,247,5378 16,680 2,447 2,247,5378 16,680 2,447 2,247,5378 16,680 2,447 2,547,5378 16,680 2,447 2,477 2,547,5378 16,680 2,447 2,547,5378 16,680 2,447 2,547,5378 16,680 2,447 2,547,5378 16,680 2,447 2,547,5378 16,680 2,447 2,547,5378 16,680 2,5447 2,547,547 16,780 2,547,547 17,780 2,547,547 17,780 2,547,547 17,780 2,547,547 17,780 2,547,547 17,780 2,547,547 17,780 2,547,547 17,780 2,547,547 17,780 2,547 17,780 2,547 17,780 2,547 17,780 2,547 17,780 2,547 17,780 2,547 17,780 2,547 17,780 2,547 17,780 2,547 17,780 2,547	Dimethyl trisulfide								
Reference Reference 3.44e-11 59,929 -119,836,119,836 1000 -40,474 99,361 -239,599,158,650 3.44e-11 59,929 -119,836,119,836 1002 -40,474 99,361 -239,599,158,650 2 144,442 51,404 41,652,247,231 0.007 102,216 122,814 -143,907,348,341 e 1574 1722 1722 1877,5026 e -45,838 47,323 -140,676,48,999 ut diabetes -46,00 1875 -4159,3358 to diabetes -46,00 1875 -4159,3358 iabetes -106,890 92,447 -292,159,78338 ut diabetes -106,890 92,447 -292,159,78338 uturia -106,890 92,447 -292,159,783378	IFTA Grading								
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e 1574 1722 -1877,5026 Reference -45,838 47,323 -140,676,48,999 -400 1875 -4159,3358 tes presence Reference -106,890 92,447 -292,159,78,378 nibetes ninuria Reference -106,890 92,447 -292,159,78,378 minuria Reference -106,890 92,447 -292,159,78,378	IFTA = 2	144,442	51,404	41,652, 247,231	0.007	102,216	122,814	-143,907, 348,341	0.409
e 45,838 47,323 -140,676,48,999 -400 1875 -4159,3358 tet presence It diabetes fiabetes ninuria Minuria Reference Reference -106,890 92,447 -292,159,78,378 Reference Reference	Age					1574	1722	-1877, 5026	0.365
e Reference -45,838 7,323 -140,676,48,999 -400 1875 -4159,3358 -4159,3358 -400 1875 -4159,3358 reference Reference -106,890 92,447 -292,159,78,378 inbutia Minutia Minutia	Sex								
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-400 1875 -4159, 3358 tes presence Reference -106,890 ut diabetes -106,890 92,447 -292,159,78,378 ninuria Reference -106,890 92,447 -292,159,78,378	Male					-45,838	47,323	-140,676, 48,999	0.337
ce Reference -106,890 92,447 -292,159,78,378 Reference	eGFR					-400	1875	-4159, 3358	0.832
Reference -106,890 92,447 -292,159,78,378 Reference	Diabetes presence								
-106,890 92,447 -292,159,78,378 Reference	Without diabetes					Reference			
	With diabetes					-106,890	92,447	-292,159, 78,378	0.253
	Albuminuria								
	No albuminuria					Reference			

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Tablo	

lable 4 (continued)								
	Univariate model	del			Multivariate model*	iodel*		
	Coefficient	SE	95% CI	<i>p</i> -value	Coefficient	SE	95% CI	<i>p</i> -value
Microalbuminuria					-3593	82,923	-169,775, 162,589	0.966
Macroalbuminuria					66,709	85,419	-104,474, 237,893	0.438
Benzene, 1,2,4-trimethyl-								
IFTA Grading								
IFTA 0	Reference				Reference			
IFTA 1	20,058	2450	15,159, 24,958	<0.001	20,856	4005	12,829, 28,882	<0.001
IFTA ≥ 2	-5.93e-12	2101	-4202, 4202	1.000	705	4950	-9215, 10,626	0.887
Age					76	69	-62, 215	0.274
Sex								
Female					Reference			
Male					1042	1907	-2780, 4865	0.587
eGFR					Ø	75	-142, 160	0.908
Diabetes presence								
Without diabetes					Reference			
With diabetes					-8334	3726	-15,802, -866	0.029
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					-1243	3342	-7942, 5455	0.711
Macroalbuminuria					608	3443	-6292, 7508	0.860
Benzeneacetaldehyde								
IFTA Grading								
IFTA 0	Reference				Reference			
IFTA 1	11,594	1416	8736, 14,426	<0.001	9902	2162	5569, 14,236	<0.001
IFTA ≥ 2	0	1214	-2428, 2428	1.000	2314	2672	-3041, 7670	0.390
Age					-14	37	-89, 60	0.696
Sex								
Female					Reference			
Male					1328	1029	-735, 3392	0.203
eGFR					93	40	11, 175	0.026
Diabetes presence								
Without diabetes					Reference			
With diabetes					4047	2011	15, 8079	0.049
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					1594	1804	-2022, 5210	0.381
Macroalbuminuria					3813	1858	87, 7538	0.045
Benzaldehyde, 4-methyl-								
IFTA Grading								

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Coefficient E 95% CI Reference - - Reference 19556 15,119 -10,576,49,889 19556 12,001,63,866 - - Adabetes 12,009 - - Betes 12,006 - - - Adiabetes - - - - - Adiabetes - <t< th=""><th>95% Cl</th><th></th><th></th><th></th><th></th></t<>	95% Cl				
Reference 1,0,5,6 1,5,1,19 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49889 -10,576,49886 -10,576,49886 -10,526 -11,52,886,135,886 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,529 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 -10,528 <t< th=""><th></th><th>Coefficient</th><th>SE</th><th>95% CI</th><th><i>p</i>-value</th></t<>		Coefficient	SE	95% CI	<i>p</i> -value
19656 15,119 -10,576,4989 37,934 12,906 12,001,63366 daberes daberes 12,001,63366 daberes atria 12,001,63366 daberes burnufa 12,001,63366 atria ninuta 12,001,63366 burnufa 0 6,955 atria ninuta 84,26,315,866 burnufa 0 6,955 atria 13,586,135,866 13,5866 atria 13,586 13,5866 atria ninuta 84,26,317,540 burnufa 0 6,955 84,26,317,540 atria ninuta 84,26,317,540 13,5866 betes ninuta 84,26,317,540 13,5866 betes 10 6,955 84,26,317,540 atria 10,055 84,26,317,540 betes 10,055 84,26,317,540 betes 10,056 34,32,317,540 betes 10,056 13,556 betes 10,056 13,556 betes 10,056 14,557 betes 10,056 14,357 code 23,23 23,23		Reference			
37,934 12,968 12,001,63,866 and albeets diabetes diabetes diabetes muria munuia munuia buminutia		30,518	23,315	-16,207, 77,245	0.196
s presence diabetes betes minuria minuria uninuria ding Reference 0 67,956 -135,886,135,886 0 67,956 -135,886,135,886 0 67,956 -135,886,135,886 0 5,956 -135,886,135,886 0 67,956 -135,886,135,886 adiabetes betes betes betes minuria uninuria betes		79,607	28,819	21,852, 137,362	0.008
s presence diabetes betes munia minufa minufa minufa buminuta diag Beference diabetes betes munia minufa minufa Mi		-372	404	-1182, 437	0.361
s presence diabetes betes muria muria muria muria muria muria muria buminuta di ading s presence diabetes di ading s presence di ading Reference di ading Reference di ading Reference di ading Reference di ading Reference ading Adi					
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s presence diabetes betes munia uminuria uminuria uminuria uminuria ding Reference diabetes diabetes diabetes thetes thria minuria auminu albyde, 25-dimethyt- s Reference diabetes betes betes thria aminuria diabetes betes thria aminuria diabetes		1152	440	269, 2034	0.011
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nuria minuta buminutia buminutia ding eding eding eding estes betes betes betes betes betes butia buminuti		14,104	21,693	-29,370, 57,579	0.518
minuia buminuia buminuia ding Reference ading Reference 0 67,956 -135,886,135,886 0 67,956 -135,886,135,886 0 67,956 -135,886,135,886 0 67,956 -135,886,135,886 0 67,956 -135,886,135,886 0 67,956 -135,886,135,886 0 0 67,956 -135,886,135,986 0 0 67,956 -135,886,135,986 0 0 67,956 -135,886,135,986 0 0 7 7,940 0 0 0 7 7,940 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0					
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Il ading Reference Reference 0 67,956 -135,886,135,886 0 0 67,956 -135,886,135,886 200,983 58,289 84,426,317,540 200,983 58,289 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 84,426,317,540 73,743 84,426,317,540 84,436,317,540 84,436,317,556,317,556,317,556,317,566,317		5991	20,044	-34,178, 46,160	0.766
ading Beference 0 67,956 -135,886, 135,886 200,983 58,289 84,426, 317,540 200,983 58,289 84,426, 317,540 30,983 58,289 84,426, 317,540 30,983 58,289 84,426, 317,540 84,426, 317,540 30,983 58,135,886 34,426, 317,540 34,426, 317,540 34,556, 317,540 34,556, 34,566,					
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200,983 58,289 84,426,317,540 s: presence diabetes betes betes nuria minuria minuria buminuria		-59,269	110,067	-279,848, 161,310	0.592
s presence diabetes betes betes moria minuria minuria buminuria buminuria buminuria behyde. 2,5-dimethyl- deng Beference 75,704 34,332 7052, 144,357		89,490	136,046	-183,153, 362,133	0.513
s presence diabetes betes muria minutia minutia buminutia buminutia tehyde, 2,5-dimethyt- ding Reference 75,704 34,332 7052, 144,357		1218	1908	-2606, 5042	0.526
s presence diabetes betes muria minuria minuria buminuria behyde, 2,5-dimethyl- dehyde, 2,5-dimethyl- 36,704 34,332 7052, 144,357					
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tes presence at diabetes iabetes inurria uminuria buminuria buminuria buminuria buminuria buminuria buminuria Buminuria		-104,244	52,422	-209,301, 811	0.052
tes presence at diabetes iabetes inuria uminuria lbuminuria		-884	2077	-5048, 3279	0.672
rt diabetes iabetes inuria uminuria Ibuminuria albuminuria dehyde, 2,5-dimethyl- rading Reference 75,704 34,332 7052, 144,357					
iabetes inuria uminuria buminuria albuminuria albuminuria ddehyde, 2,5-dimethyl- rading Reference 75,704 34,332 7052, 144,357		Reference			
inuria uminuria Ibuminuria albuminuria Idehyde, 2,5-dimethyl- rading Reference 75,704 34,332 7052, 144,357		-94,279	102,408	-299,510, 110,950	0.361
uminuria Ibuminuria Ibuminuria Idehyde, 2,5-dimethyl- rading Reference 75,704 34,332 7052, 144,357					
Ibuminuria albuminuria Idehyde, 2,5-dimethyl- rading Reference 75,704 34,332 7052, 144,357		Reference			
albuminuria Idehyde, 2,5-dimethyl- Reference 75,704 34,332 7052, 144,357		116,500	91,857	-67,586, 300,587	0.210
ldehyde, 2,5-dimethyl- rading Reference 75,704 34,332 7052, 144,357		40,073	94,622	-149,553, 229,701	0.674
Reference 75,704 34,332 7052, 144,357					
75,704 34,332 7052, 144,357		Reference			
		91,458	57,975	-24,726, 207,643	0.120
IFTA ≥ 2 101,795 29,448 42,909,160,682		109,524	71,659	-34,083, 253,132	0.132
Age		-839	1005	-2853, 1174	0.407

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Conficient C PSAct PSAct PSAct Streence Entence Entence Entence Entence Streence Entence Entence 25/13 25/13 25/13 25/13 Streence Entence Entence Entence 25/13 25/13 25/13 25/13 Streence Entence 25/13		Univariate mo				Multivariate m			
Reference Reference <t< th=""><th></th><th>Coefficient</th><th>SE</th><th>95% CI</th><th><i>p</i>-value</th><th>Coefficient</th><th>SE</th><th>95% CI</th><th><i>p</i>-value</th></t<>		Coefficient	SE	95% CI	<i>p</i> -value	Coefficient	SE	95% CI	<i>p</i> -value
Reference Reference <t< td=""><td>Sex</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	Sex								
Streets 24,43 275,5,632 2310,206 Streets 646 2310,206 2310,206 detect 646 646 646 2310,206 detect 646 646 646 646 detect 646 646 646 647,60 detect 646 647,60 773,603 773,603 unitia 646 647,60 773,603 773,603 unitia 100 100 646 647,60 binnitia 100 100 646 647,61 113,55 100 700 639,00 719,003 binnitia 100 1037 0043 623 binnitia 100 1037 0043 630,1139 113,56 100 1037 0043 639,1139 113,56 100 1037 0043 113,957 113,56 100 1037 0043 113,957 113,56 100 1037 1037 113,957 113,57 100 1037 1037 113,957 113,56 100 1037 1045 113,957 114,156 1000 1037 1045 113,957	Female					Reference			
Fibererece -17 084 -230,206 distrete	Male					-28,403	27,612	-83,739, 26,932	0.308
species beside	eGFR					-117	1094	-2310, 2076	0.915
didence detection animal munitation munitati	Diabetes presence								
teete tinua minua minua minua minua minua minua minua minua strinta minua minua strinta minua strinta minua minua strinta minua minua strinta minua minua strinta minua mi	Without diabetes					Reference			
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munuta Elefence Former Former <thform< th=""> <thform< th=""> Former<td>Albuminuria</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></thform<></thform<>	Albuminuria								
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building -19789 49840 -119,670,80022 anine, 35-stethore- Reference -105,70,8002 -0706,71461 anine, 35-stethore- 23375 18095 -12,448,39921 0,03 20200 -4706,71461 23375 18095 -12,448,39921 0,03 20200 -4706,71461 23375 18095 -12,448,39921 0,03 20200 -4706,71461 23320 15,511 122,63337 0,02 22,29 -40569,17139 2445 -11,115 -22,219 -4059,34076 -40569,17139 2446 -11,115 -22,219 -3653,4076 -40569,34076 Aninual -11,115 14,038 -3653,46769 -4056,34076 Aninual -11,115 14,038 -3653,46769 -4055,34076 Aninual -11,115 11,015 -3120 -3653,46769 Aninual -11,115 -11,115 -3112 -3653,46769 Aninual -11,115 -11,115 -31650 -3653,46769 Aninual -11,115 -11,115 -31650 -3653,46769 Aninual -11,115 -11,215 -314,215 -3653,46769 Aninual -11,115 -11,215 -3653,47649 <td>Microalbuminuria</td> <td></td> <td></td> <td></td> <td></td> <td>7671</td> <td>48,383</td> <td>-89,291, 104,635</td> <td>0.875</td>	Microalbuminuria					7671	48,383	-89,291, 104,635	0.875
amoa 3.5 dictoro- ading Reference 23/36 8.005 -1248.5921 0.155 3337 0.077 3020 -49/06/1/461 23/306 15521 1252.63337 0.042 2.8275 37,366 -49/06/1/461 23/306 -13521 1252.63337 0.042 2.8275 37,366 -49/06/1/461 -68/667 -68/25 -68/	Macroalbuminuria					-19,789	49,840	-119,670, 80,092	0.693
efference Reference Solution	Benzenamine, 3,5-dichloro-								
Reference Elerence	IFTA Grading								
23736 8095 -1-2445,5921 0.195 10,877 30,300 -4706,71461 32,300 15,21 15,22 15,52 15,52 1265,63,337 0.042 8,3159,66607 8,3159,66607 32,300 15,52 15,52 15,52 1265,63,337 0.042 8,275 5,3159,66607 Reference	IFTA 0	Reference				Reference			
32300 15,21 1262,63337 0.042 8275 37,366 63,159,6607 Spreame -2 24 -113, 967 -113, 967 -113, 967 Spreame -2 -11,715 14,398 -40,569,17139 Spreame -11,715 14,398 -40,569,17139 Glabeles -11,715 14,398 -7050 1893,3933 Spreame -11,715 14,398 -78559,34076 Bete -2,2291 281127 2789 -6353,64589 Duminal -11,415 -2,2291 28127 -78559,34076 Duminal -11,416 -2,2291 2619 -6552,64589 Duminal -11,418 -2,2291 2619 -6552,64589 Duminal -11,418 2009 2509 2509 -6573,64589 Duminal -11,418 2509 2509 -6532,64589 Duminal -11,402 2509 2509 -6532,64589 Duminal -11,402 2509 2509 -6532,64589 Duminal -11,402 2509 2512,64589 Duminal -11,402 25161 -45712,01612 Duminal -231,112 7736 -3538,-7643 Duminal	IFTA 1	23,736	18,095	-12,448, 59,921	0.195	10,877	30,230	-49,706, 71,461	0.720
	IFTA ≥ 2	32,300	15,521	1262, 63,337	0.042	-8275	37,366	-83,159, 66,607	0.826
Reference diabetes diabetes betes that annual butia nuria nuria nuria nuria nuria nuria nuria sumuria butia nuri	Age					-82	524	-1133, 967	0.875
Reference intri 5 14398 -40569,17139 is presence clubetes betes betes minuta mi	Sex								
II,715 14,398 -40.569,17,139 Spreace Spreace Helence Helence Helence Helence Helence Intuia I	Female					Reference			
spresence -750 570 -1893, 393 diabetes diabetes Reference -78659, 34076 unitia -22291 28127 -78659, 34076 nutia -22291 28127 -78659, 34076 nutia -22291 28127 -78659, 34076 nutia -22312 28127 -78659, 34076 nutia -22312 28127 -78659, 34076 nutia -23312 2309 25229 -36532, 64589 burniutia -23312 736 -38582, -7643 2004 23162 e.(sothicyanstomethy)- -3331, -9844 0.004 -23152 12176 -4775, 1249 ading Reference -33112 6635 -36381, -9844 0.001 -19550 -4775, 10612 233112 7736 -38382, -7643 0.004 -23152 12176 -4775, 10612 -233,112 6635 -36381, -9844 0.001 -19550 -4775, 10612 -233,112 6635 -36381, -9844 0.001 -19550 -4775, 10612 -233,112 6635 -36381, -9844 0.001 -19550 -4775, 10612 -233,112 7736 -377543 -377543 -4775, 10612 <td>Male</td> <td></td> <td></td> <td></td> <td></td> <td>-11,715</td> <td>14,398</td> <td>-40,569, 17,139</td> <td>0.419</td>	Male					-11,715	14,398	-40,569, 17,139	0.419
s presence diabetes betes muria minutia minutia minutia buminutia	eGFR					-750	570	-1893, 393	0.194
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minuria buminuria buminuria e, (isothiocyanatomethy)- ading Reference -23,112 7736 -33,582, -7643 0.004 25,988 -49,773, 54,391 ading Reference -23,112 7736 -33,582, -7643 0.004 23,152 12,176 -47,555, 1249 -23,112 6635 -36,381, -9844 0.001 19,550 15,050 -49,712, 10,612 -23,112 6635 -36,381, -9844 0.001 19,550 15,050 -49,712, 10,612 -33,152 6635 -36,381, -9844 0.001 19,550 15,050 -49,713, 10,612 -33,152 6635 -36,381, -9844 0.001 19,550 15,050 -49,713, 10,612 -33,152 6635 -36,381, -9844 0.001 19,550 15,050 -49,713, 10,612 -33,152 6635 -36,381, -9844 0.001 19,550 15,050 -49,715, 10,612 -33,154 -30,50 -49,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -40,530 -40,515 -	Albuminuria								
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e, (isothiocyanatomethy)- ading Reference -23,152 12,176 -47,555, 1249 -23,112 7736 -38,582, -7643 0.004 -23,152 12,176 -47,555, 1249 -23,112 6635 -36,381, -9844 0.001 -19,550 15,050 -49,712, 10,612 -23,112 6635 -36,381, -9844 0.001 -19,550 15,050 -49,712, 10,612 -33,112 6635 -36,381, -9844 0.001 19,550 15,050 -49,712, 10,612 -33,112 6635 -36,381, -9844 0.001 19,550 15,050 -49,712, 10,612 -33,112 6635 -36,381, -9844 0.001 19,550 15,050 -40,712, 10,612 -33,715 6635 -36,381, -9844 0.001 19,550 15,050 -40,730,90 -377,543	Macroalbuminuria					2309	25,988	-49,773, 54,391	0:930
Reference Reference -23,112 7736 -38,582, -7643 0.004 -23,152 12,176 -47,555, 1249 -23,112 6635 -36,381, -9844 0.001 -19,550 15,050 -49,712, 10,612 -23,112 6635 -36,381, -9844 0.001 -19,550 15,050 -49,712, 10,612 -23,112 6635 -36,381, -9844 0.001 -19,550 15,050 -49,712, 10,612 -23,112 6635 -36,381, -9844 0.001 -19,550 15,050 -49,712, 10,612 -53 211 -19,550 15,050 15,050 7045, 369 -80 18,667 5799 7045, 30,290 82 229 -377, 543	Benzene, (isothiocyanatomethyl)- IETA Condition								
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-23,112 7736 -38,582, -7643 0.004 -23,152 12,176 -47,555, 1249 -23,112 6635 -36,381, -9844 0.001 -19,550 15,050 -49,712, 10,612 -53 211 -476, 369 Reference 18,667 5799 7045, 30,290 82 229 -377, 543	IFTA 0	Reference				Reference			
-23,112 6635 -36,381,-9844 0.001 -19,550 15,050 -49,712,10,612 -53 211 -476, 369 Reference 18,667 5799 7045, 30,290 82 229 -377,543	IFTA 1	-23,112	7736	-38,582, -7643	0.004	-23,152	12,176	-47,555, 1249	0.062
-53 211 -476,369 Reference 18,667 5799 7045,30,290 82 229 -377,543	$ FTA \ge 2$	-23,112	6635	-36,381, -9844	0.001	-19,550	15,050	-49,712, 10,612	0.199
Reference 18,667 5799 7045,30,290 82 229 -377,543	Age					-53	211	-476, 369	0.802
Reference 18,667 5799 7045,30,290 82 229 -377,543	Sex								
18,667 5799 7045,30,290 82 229 -377,543	Female					Reference			
82 229 -377, 543	Male					18,667	5799	7045, 30,290	0.002
	eGFR					82	229	-377, 543	0.719

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	Univariate model	del			Multivariate model*	odel*		
	Coefficient	SE	95% CI	<i>p</i> -value	Coefficient	SE	95% CI	<i>p</i> -value
Diabetes presence								
Without diabetes					Reference			
With diabetes					-2036	11,329	-24,741, 20,667	0.858
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					-2488	10,162	-22,854, 17,876	0.807
Macroalbuminuria					3062	10,468	-17,915, 24,041	0.771
2(3 H)-Furanone, 5-hexyldihydro-								
IFTA Grading								
IFTA 0	Reference				Reference			
IFTA 1	123,646	12,806	98,038, 149,253	<0.001	131,455	18,911	93,556, 169,354	<0.001
IFTA ≥ 2	2.85e-11	10,984	-21,964, 21,964	1.000	13,709	23,375	-33,135, 60,554	0.560
Age					521	327	-135, 1178	0.117
Sex								
Female					Reference			
Male					10,037	2005	-8013, 28,087	0.270
eGFR					219	357	-496, 934	0.542
Diabetes presence								
Without diabetes					Reference			
With diabetes					46,048	17,595	10,785, 81,310	0.011
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					-15,483	15,782	-47,112, 16,146	0.331
Macroalbuminuria					-26,354	16,257	-58,935, 6226	0.111
1-Naphthalenecarboxaldehyde								
IF IA Grading								
IFIA U	Keterence	000			keterence 2000			000
	-4100	1 00 1	100-,001/-	CZN.U	0065-	0542	-9/04, 1903	0.109
IFTA ≥ 2	-4153	1544	-7242, -1064	0.009	-3257	3629	-10,530, 4015	0.373
Age					-26	50	-128, 75	0.600
Sex								
Female					Reference			
Male					3333	1398	530, 6135	0.021
eGFR					13	55	-97, 124	0.807
Diabetes presence								
Without diabetes					Reference			
With diabetes					-54	2731	-5529, 5420	0.984
Albuminuria								

Page 14 of 20

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	Univariate model	del			Multivariate model*	odel*		
	Coefficient	SE	95% CI	<i>p</i> -value	Coefficient	SE	95% CI	<i>p</i> -value
No albuminuria					Reference			
Microalbuminuria					-591	2450	-5502, 4318	0.810
Macroalbuminuria					384	2524	-4673, 5443	0.879
Benzoic acid, 4-ethoxy-, ethyl ester								
IFTA Grading								
IFTA 0	Reference				Reference			
IFTA 1	3389	5424	-7458, 14,237	0.534	4073	8811	-13,585, 21,733	0.646
$IFTA \ge 2$	15,596	4653	6291, 24,900	0.001	21,213	10,891	-614, 43,040	0.057
Age					78	152	-227, 384	0.611
Sex								
Female					Reference			
Male					-8659	4196	-17,069, -248	0.044
eGFR					166	166	-167, 499	0.323
Diabetes presence								
Without diabetes					Reference			
With diabetes					-830	8198	-17,261, 15,600	0.920
Albuminuria								
No albuminuria					Reference			
Microalbuminuria					3681	7354	-11,056, 18,418	0.619
Macroalbuminuria					2277	7575	-12,903, 17,459	0.765
Caffeine								
IFTA Grading								
IFTA 0	Reference				Reference			
IFTA 1	1.37e-10	134,677	-269,303, 269,303	1.000	-156,089	218,784	-594,541, 282,363	0.479
$IFTA \ge 2$	492,162	115,519	261,166, 723,157	<0.001	213,178	270,424	-328,763, 755,120	0.434
Age					3821	3793	-3779, 11,423	0.318
N Sex								
Female					Reference			
Male					-158,626	104,201	-367,450, 50,197	0.134
eGFR					-4065	4130	-12,342, 4211	0.329
Diabetes presence								
Without diabetes					Reference			
With diabetes					-320,246	203,560	-728,190, 87,697	0.121
Albuminuria								
No albuminuria					Reference			

	Univariate model	e				odel		
	Coefficient	SE	95% CI	<i>p</i> -value	Coefficient	SE	SE 95% CI	<i>p</i> -value
Microalbuminuria					59,197	182,588	-306,718, 425,113	0.747
Macroalbuminuria					157,724	188,084	-219,204, 534,654	0.405

Fable 4 (continued)

Adjusted for age, sex, the level of eGFR, diabetes mellitus status, and albuminuria status

moderate/severe tubulointerstitial disease. Our multivariate regression analysis model evaluating the association between expression levels of urinary VOCs and CKD adjusted for age, sex, eGFR, diabetic and albuminuria status, given these covariates were determined to be significantly associated with VOCs expression and CKD progression from previous studies [19-21]. In the multivariate analysis, we identified 4 VOCs significantly upregulated in the mild IFTA compared to the no IFTA group and 2 VOCs significantly upregulated in the moderate/severe IFTA compared to the mild IFTA group.

Metabolic dysregulation that occurs with CKD progression is primarily characterized by oxidative stress and inflammation [22]. Increased production of reactive oxygen species (ROS) results in oxidative damage to lipids, proteins and DNA through their reactive properties [23]. Emerging evidence suggests ROS also function as important secondary messengers in cellular signalling pathways [24, 25]. For one, cytoplasmic ROS induces the activity of AMP-activated protein kinase, which has a crucial role in glucose and lipid metabolism, cell survival, growth, and inflammation, all of which are affected in CKD [24, 26]. Oxidative stress can also activate the transcription factor NF-κB, which induces the expression of cytokines and chemokines to regulate inflammatory responses in the kidneys [27]. The inflammatory cascade in CKD is characterized by the generation and/or accumulation of proinflammatory cytokines (e.g. tumour necrosis factor-a and interleukin-1) from intrinsic and/or extrinsic kidney damage not limited to uraemia, dyslipidaemia, malnutrition, infection and gut microbiota, resulting in increased blood flow, upregulation of chemical mediators and leukocyte infiltration [28]. Prior investigations established physiological links between VOCs and oxidative stress, lipid and amino acid metabolism, and inflammation [29, 30]. Hence, there is a basis in CKD for utilizing metabolomic markers such as VOCs to capture the extent of oxidative stress and inflammation, and translationally inform on the degree of CKD progression.

The majority of the 34 identified urinary VOCs in our study, and all of the significantly upregulated urinary VOCs belonged to one of four key organic chemistry functional groups - aldehydes, ketones, hydrocarbons, and alcohols. Urinary aldehydes can be exogenous or endogenous in origin. They can be produced during lipid peroxidation via the beta-cleavage reaction of lipid alkoxyl radicals [31]. It is well-known that there are lipid metabolic disturbances in patients with kidney disease [32]. Therefore, abnormal urinary aldehyde levels in these conditions may be explained by the lipid peroxidation damage that occurs. Ketones typically originate from exogenous sources and from the decarboxylation of oxo-acids [33, 34]. In healthy humans, ketones are mainly formed in hepatocytes from acetoacetate during the decarboxylation of excess acetyl-CoA [34]. Human breath, blood and urine all contain ketones in the form of acetone [34]. Heptanone in urine is supposedly the product of beta-oxidation of 2-ethylhexanoic acid, a metabolic product of the plasticizer di-(2-ethylhexyl)-phthalate [10]. Impairment of kidney function may reduce the filtration of ketones, leading to decreased concentration of ketones detected in the urine of kidney disease patients [35]. There is emerging evidence nevertheless, which observed increased urinary ketone (2-pentanone) levels in kidney disease aetiologies such as idiopathic membranous nephropathy (IMN) [36]. Further study is needed to delineate the intricacies that are linked between kidney pathology and ketone physiology. Hydrocarbons are thought to be the by-product of cholesterol biosynthesis [37, 38]. Change in levels of urinary VOCs stemming from the hydrocarbon group (i.e. benzaldehydes and carbonyl groups) in kidney disease may indicate disorders in tryptophan metabolism and alterations in pyruvate, glycine, serine, and threonine metabolisms, respectively [39]. Alcohols originate from aliphatic alcohol in human tissue fluids, and various processes formed from acetaldehyde metabolism or exogenous intake [40]. Its role in oxidative stress and inflammation pathways in kidney disease is well-established [41].

Although there were no previous studies which evaluated associations between expression of urinary VOCs and CKD as defined by tubulointerstitial pathology, urinary VOCs have been previously studied for their potential as biomarkers in multiple glomerular diseases such as mesangial proliferative glomerulonephritis, Immunoglobulin A nephropathy, IMN and minimal change disease [36, 42-44]. In the preliminary studies that were conducted, a different panel of significantly upregulated (or downregulated) VOCs with progressing disease severity were identified, in comparison to the identified VOCs from our study [36, 42–44]. Wang et al. [42] evaluated urine samples in 15 mesangial proliferative glomerulonephritis (MPGN) patients, 21 Immunoglobulin A nephropathy (IgAN) patients and 15 healthy controls. Five VOCs (Carbamic acid, monoammonium salt; Carbon disulfide; Silanediol, dimethyl-; 2 H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5phenyl-1-(trimethylsilyl)- and Butylated Hydroxytoluene) had significantly elevated expression levels in the MPGN group compared with the control group, whilst 3 VOCs (Carbamic acid, monoammonium salt; Carbon disulfide and 2 H-1,4-Benzodiazepin-2-one,7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-) were found at increased expression levels in the IgAN group compared to normal controls. In addition, 5 VOCs (Tartronic acid; Carbamic acid; Sulfide, allyl methyl; Hydrogen azide and N-[(pentafluorophenyl)methy-Benzeneethanamine, lene]-.beta,4-bis[(trimethylsilyl)oxy]-) were significantly increased in IgAN patients compared with MPGN patients, suggesting these urinary VOCs may be specific biomarkers which differentiate between the two conditions. 4-heptanone, 2-pentanone and pyrrole were identified at decreased urinary levels in IgAN and MPGN patients compared to the control groups. Wang et al. [43] also aimed to detect urinary VOCs which could distinguish between patients with idiopathic membranous nephropathy (IMN) and normal controls. The investigators assessed the urine collected from 63 IMN patients and 15 normal controls, in which 6 VOCs (Carbamic acid, monoammonium salt; 2-pentanone; 2,4-dimethylpentanal; Hydrogen azide; Thiourea and 4-heptanone) displayed significantly higher expression levels in IMN patients compared to normal controls. The same investigator group [36] also collected urine samples from 38 minimal change disease (MCD) patients and 15 healthy controls. They identified 6 VOCs (Trans-2,2-dimethyl-4-decene; Pyrrole; Carbamic acid, monoammonium salt; 1-butyne, 3,3-dimethyl-; Diisopropylamine and 4-heptanone) that are present at reduced urinary expression levels in MCD patients. Further work is needed to validate the use of these urinary VOCs as biomarkers to predict MCD status and disease progression. A more recently conducted study by Ligor et al. [44], which separated and identified urinary VOCs via gas chromatography timeof-flight mass spectrometry, aimed to determine urinary VOC profiles between 27 patients diagnosed with glomerular diseases and 20 healthy controls. Amongst those diagnosed with glomerular disease, there were 4 VOCs (Methyl hexadecanoate; 9-hexadecen-1-ol; 6,10-dimethyl-5,9-undecadien-2-one and 2-pentanone) found to be at elevated urinary expression levels.

Otherwise, links between exhaled air VOCs from human breath with CKD were recently investigated. Romani et al. [45] examined the utility of selected ion flow tube-mass spectrometry (SIFT-MS) to measure breath VOCs in CKD patients and healthy subjects, and evaluated the possible correlation between breath VOC expression levels with the presence of CKD and CKD progression as determined by the Kidney Disease Improving Global Outcomes guideline diagnostic criteria [46]. The investigators enrolled 68 Stage I-IV CKD patients (all were receiving conservative therapy) and 54 healthy subjects. Analysis of the VOCs from exhaled air of the enrolled subjects was performed by SIFT-MS. They observed increased breath VOCs expression levels for numerous VOCs in CKD compared to healthy subjects and with progressing CKD severity, albeit these were different VOCs from the ones identified in our study. The most relevant results by receiver operating characteristic curves were observed for trimethylamine (TMA), acetone, ammonia, and dimethyl sulfide. Romani et al. [45] noted that an individual's breath TMA concentration

superior to 26 parts per billion by volume characterizes a 6.11 times greater risk of having CKD, compared to those with lower levels of breath TMA concentration. Moreover, they detected an increased concentration of acetone and ammonia in CKD patients compared to healthy subjects. SIFT-MS is considered a superior mass spectrometry option for measuring nitrogen- and sulfur-containing VOCs, which are more challenging to measure when using other mass spectrometry modalities. Future studies evaluating urinary VOCs within a CKD context using SIFT-MS is anticipated.

Whilst our study findings provide novel evidence into the associations between urinary VOCs and CKD, there remain important gaps in our knowledge base which require evaluation. For one, the exact mechanisms for the generation of most urinary VOCs is unclear at a molecular level, and they could be perturbed in many physiological and pathological states outside of tubulointerstitial disease alone, Although we adjusted for several potential confounding factors in our analyses, there may be other factors challenging to control, not limited to dietary habits, physical stress and environmental exposure to toxins, which could affect the accuracy of urinary VOCs profiling [47]. Hence, further studies with larger clinical cohorts are required to validate our data, adjusting for other potential covariates that may be relevant to kidney disease. Another issue relates to the vast quantity of urinary VOCs that were found to be potentially useful biomarkers of CKD across different IFTA stages, also considering there may be other clinically significant urinary VOCs that remain unidentified currently. Further evidence to specify and narrow towards the key urinary VOCs that could be confidently applied in clinical practice to predict CKD progression is required. While most urinary VOCs and other metabolomic studies reported to date used GC-MS as the analytical method, complementary analysis could be performed by reversed-phase liquid chromatography-mass spectrometry (RP-LC-MS), hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-LC-MS), and capillary electrophoresis-mass spectrometry (CE-MS) methods as well [48]. This would broaden the range of potential disease markers that could be investigated. Alternative types of mass spectrometry analysis approaches could also be considered to improve sensitivity of metabolite detection but this must be balanced against their increasing price, operating costs and complicated operation in a clinical setting [49]. Hence, improving biosensing software platforms to detect clinically useful urinary VOCs is an attractive proposition where ongoing technological developments are foreseeable. For one, the feasibility of metal oxide biosensor platforms to determine urinary VOCs with significant predictive capability for detecting genitourinary cancers (i.e. renal cell carcinoma,

transitional cell carcinoma and prostate cancer) has been recently demonstrated to good levels of accuracy. Future studies could perhaps consider extending its use for this purpose in CKD [50]. Furthermore, a mass spectrometry-based electronic nose (MS-EN) approach possesses tremendous potential but has been seldomly applied for urinary VOCs and so far, has not been explored within in CKD yet though it has been trialled within the context of kidney cancer [51, 52]. This is also a potential avenue of further research to be considered.

Conclusions

Our study demonstrated that the urinary expression levels of various aldehydes, ketones, hydrocarbons and alcohols are significantly associated with tubulointerstitial histopathology, which suggests urinary VOCs may indeed have a clinically useful role in CKD as a metabolomic biomarker. Additional studies are required to validate our findings in a larger cohort and examine the potential of utilizing urinary VOCs to reliably assess CKD progression in clinical practice.

Abbreviatio

Abbreviat	ions
ANOVA	Analysis of Variance
CKD	Chronic Kidney Disease
DM	Diabetes Mellitus
eGFR	Estimated Glomerular Filtration Rate
GC	Gas Chromatography
GC-MS	Gas Chromatography–Mass Spectrometry
IFTA	Interstitial Fibrosis and Tubular Atrophy
IMN	Idiopathic Membranous Nephropathy
IS	Internal Standard
LDA	Linear Discriminant Analysis
LOO	Leave-one-out
MS-EN	Mass Spectrometry-based Electronic Nose
PTV	Programmed Temperature Vaporization
QC	Quality Control
ROS	Reactive Oxygen Species
SBSE	Stir Bar Sorptive Extraction
SIFT-MS	Selected Ion Flow Tube-Mass Spectrometry
VOCs	Volatile Organic Compounds
TD	Thermal Desorption
TDU	Thermal Desorption Unit
TMA	Trimethylamine
95%CI	95% Confidence Intervals

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Author contributions

HHLW conceptualized the study, collected the urine samples, obtained the patient demographic and clinical data, prepared the original manuscript version and revised the manuscript; MP processed the urine samples including Stir Bar Sorptive Extraction and Gas Chromatography-Mass Spectrometry to obtain the raw data for further analysis, and was involved in revising the manuscript; LTN was involved in urine sample collection and obtained the patient demographic and clinical data; WP was involved in formal statistical analysis of the post-processed data obtained from Gas Chromatography-Mass Spectrometry and prepared the data presented in the results section of this manuscript; CAP conceptualized the study and revised the manuscript; SS conceptualized the study, provided the resources for the

study, revised the manuscript, and was in charge of the project administration and supervision. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Data collection in this study was carried out in accordance with relevant local guidelines and regulations, and collection of human data was approved by the human ethics committee at Royal North Shore Hospital (Ref: HREC/17/ HAWKE/471). Informed consent was obtained from all study participants.

Consent for publication

No individual patient data has been disclosed in this manuscript. Individual consent obtained from all study participants in this study included consent for publication of study results.

Competing interests

HHLW is a member of the editorial board in BMC Nephrology. The other authors have no competing interests to declare in relation to the contents of this manuscript.

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