

SUPPLEMENTARY DATA

Title: **Circulating modified metabolites and a risk of ESRD in patients with Type 1 Diabetes and Chronic Kidney Disease**

Running title: **Modified metabolites and risk of ESRD in diabetes**

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**Supplementary Table 1.** Metabolite associations with eGFR slope in subjects with T1D, proteinuria and CKD3 (main study) in a global profiling of amino acids, purine and pyrimidine metabolites and their derivatives. Metabolites are sorted by biochemical class and presented further in an alphabetical order. Metabolites significantly associated with eGFR slope are marked in bold. r - non parametric correlation coefficient, q value - significance adjusted for multiple testing with positive false discovery rate. PC1\_all - principal component considering all well detected metabolites, PC1\_9 considers only 9 significant modified metabolites identified in the global matrix analysis.

metabolite name	biochemical subclass	correlation with eGFR slope	nominal significance	significance adjusted for multiple testing	Principal component - PC1_all	Principal component - PC1_9
		r	p value	q value	loading	loading
<b>AMINO ACIDS AND DERIVATIVES</b>						
alanine	Alanine and aspartate metabolism	-0.016	8.4E-01	8.4E-01	0.081	
asparagine	Alanine and aspartate metabolism	0.053	5.1E-01	5.1E-01	0.096	
aspartate	Alanine and aspartate metabolism	-0.150	5.9E-02	1.2E-01	0.076	
beta-alanine	Alanine and aspartate metabolism	-0.057	4.8E-01	4.8E-01	0.062	
<b>N-acetylalanine</b>	Alanine and aspartate metabolism	-0.292	2.0E-04	<b>2.0E-03</b>	0.181	0.350
N-acetylaspartate (NAA)	Alanine and aspartate metabolism	-0.086	2.8E-01	2.8E-01	0.101	
N-acetyl-beta-alanine	Alanine and aspartate metabolism	-0.176	2.7E-02	8.0E-02	0.057	
2-aminobutyrate	Butanoate metabolism	0.128	1.1E-01	1.6E-01	-0.010	
creatine	Creatine metabolism	-0.045	5.7E-01	5.7E-01	-0.058	
<b>creatinine</b>	Creatine metabolism	-0.265	7.8E-04	<b>5.2E-03</b>	0.198	
2-hydroxybutyrate (AHB)	Cysteine, methionine, SAM, taurine metabolism	-0.013	8.7E-01	8.7E-01	-0.007	
cysteine	Cysteine, methionine, SAM, taurine metabolism	0.005	9.5E-01	9.5E-01	0.067	
methionine	Cysteine, methionine, SAM, taurine metabolism	0.043	5.9E-01	5.9E-01	0.067	
N-acetylmethionine	Cysteine, methionine, SAM, taurine metabolism	-0.172	3.1E-02	8.2E-02	0.016	
N-formylmethionine	Cysteine, methionine, SAM, taurine metabolism	-0.149	6.2E-02	1.2E-01	0.127	
S-methylcysteine	Cysteine, methionine, SAM, taurine metabolism	0.064	4.2E-01	4.2E-01	0.016	
N-acetylcarnosine	Dipeptide derivative	-0.116	1.5E-01	2.0E-01	0.147	
glutamate	Glutamate metabolism	-0.090	2.6E-01	2.7E-01	0.039	
glutamine	Glutamate metabolism	0.070	3.9E-01	3.9E-01	0.047	
pyroglutamine	Glutamate metabolism	0.098	2.2E-01	2.4E-01	0.122	
5-oxoproline	Glutathione metabolism	-0.043	5.9E-01	5.9E-01	0.000	
beta-hydroxypropyruvate	Glycine, serine and threonine metabolism	-0.017	8.3E-01	8.3E-01	0.047	
betaine	Glycine, serine and threonine metabolism	0.145	7.0E-02	1.2E-01	-0.056	
dimethylglycine	Glycine, serine and threonine metabolism	-0.030	7.1E-01	7.1E-01	0.105	
glycine	Glycine, serine and threonine metabolism	-0.021	8.0E-01	8.0E-01	0.061	
N-acetylglycine	Glycine, serine and threonine metabolism	-0.164	3.9E-02	9.9E-02	0.064	

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<b>N-acetylserine</b>	Glycine, serine and threonine metabolism	-0.291	2.1E-04	<b>2.0E-03</b>	0.177	0.317
<b>N-acetylthreonine</b>	Glycine, serine and threonine metabolism	-0.342	1.1E-05	<b>1.4E-04</b>	0.192	0.359
sarcosine (N-Methylglycine)	Glycine, serine and threonine metabolism	-0.001	9.9E-01	9.9E-01	-0.015	
serine	Glycine, serine and threonine metabolism	-0.069	3.9E-01	3.9E-01	0.057	
threonine	Glycine, serine and threonine metabolism	0.105	1.9E-01	2.2E-01	0.056	
1-methylhistidine	Histidine metabolism	-0.077	3.4E-01	3.4E-01	0.093	
3-methylhistidine	Histidine metabolism	-0.204	1.0E-02	5.2E-02	0.115	
histidine	Histidine metabolism	-0.085	2.9E-01	2.9E-01	0.080	
2-aminoadipate	Lysine metabolism	0.001	9.9E-01	9.9E-01	0.030	
glutarate (pentanedioate)	Lysine metabolism	0.011	9.0E-01	9.0E-01	0.004	
lysine	Lysine metabolism	-0.015	8.5E-01	8.5E-01	0.010	
<b>N6-acetyllysine</b>	Lysine metabolism	-0.261	9.4E-04	<b>5.6E-03</b>	0.124	0.225
pipecolate	Lysine metabolism	-0.031	7.0E-01	7.0E-01	0.024	
3-(4-hydroxyphenyl)lactate	Phenylalanine & tyrosine metabolism	0.141	7.7E-02	1.3E-01	0.047	
3-methoxytyrosine	Phenylalanine & tyrosine metabolism	0.018	8.2E-01	8.2E-01	0.056	
4-hydroxyphenylacetate	Phenylalanine & tyrosine metabolism	-0.157	4.9E-02	1.1E-01	0.131	
5-hydroxymethyl-2-furoic acid	Phenylalanine & tyrosine metabolism	-0.044	5.8E-01	5.8E-01	0.061	
o-cresol sulfate	Phenylalanine & tyrosine metabolism	0.000	1.0E+00	1.0E+00	0.040	
<b>O-sulfo-L-tyrosine</b>	Phenylalanine & tyrosine metabolism	-0.367	2.1E-06	<b>4.6E-05</b>	0.199	0.369
p-cresol sulfate	Phenylalanine & tyrosine metabolism	-0.106	1.9E-01	2.2E-01	0.134	
phenethylamine (isobar with 1-phenylethylamine)	Phenylalanine & tyrosine metabolism	0.103	2.0E-01	2.2E-01	0.035	
<b>phenol sulfate</b>	Phenylalanine & tyrosine metabolism	-0.267	7.0E-04	<b>5.1E-03</b>	0.128	0.240
phenylacetate	Phenylalanine & tyrosine metabolism	0.115	1.5E-01	2.0E-01	0.048	
phenylacetylglutamine	Phenylalanine & tyrosine metabolism	-0.193	1.5E-02	6.3E-02	0.151	
phenylalanine	Phenylalanine & tyrosine metabolism	-0.099	2.2E-01	2.4E-01	0.067	
phenyllactate (PLA)	Phenylalanine & tyrosine metabolism	0.037	6.4E-01	6.4E-01	0.066	
tyrosine	Phenylalanine & tyrosine metabolism	0.066	4.1E-01	4.1E-01	0.033	
3-indoxyl sulfate	Tryptophan metabolism	-0.146	6.7E-02	1.2E-01	0.133	
<b>C-glycosyltryptophan</b>	Tryptophan metabolism	-0.447	4.0E-09	<b>2.6E-07</b>	0.198	0.385
indoleacetate	Tryptophan metabolism	-0.136	8.8E-02	1.4E-01	0.105	
indolelactate	Tryptophan metabolism	-0.174	2.9E-02	8.2E-02	0.145	
indolepropionate	Tryptophan metabolism	0.150	5.9E-02	1.2E-01	-0.009	
kynurenate	Tryptophan metabolism	-0.039	6.3E-01	6.3E-01	0.082	
kynurenine	Tryptophan metabolism	-0.172	3.1E-02	8.2E-02	0.159	
serotonin (5HT)	Tryptophan metabolism	-0.074	3.5E-01	3.5E-01	0.015	
tryptophan	Tryptophan metabolism	0.201	1.1E-02	5.3E-02	-0.032	
tryptophan betaine	Tryptophan metabolism	0.129	1.1E-01	1.6E-01	-0.020	
arginine	Urea cycle; arginine-, proline-, metabolism	-0.024	7.7E-01	7.7E-01	0.055	
citrulline	Urea cycle; arginine-, proline-, metabolism	-0.127	1.1E-01	1.6E-01	0.120	
dimethylarginine (SDMA + ADMA)	Urea cycle; arginine-, proline-, metabolism	-0.189	1.7E-02	6.7E-02	0.115	
homocitrulline	Urea cycle; arginine-, proline-, metabolism	-0.183	2.1E-02	7.3E-02	0.162	
N-methyl proline	Urea cycle; arginine-, proline-, metabolism	-0.112	1.6E-01	2.1E-01	0.060	
ornithine	Urea cycle; arginine-, proline-, metabolism	0.004	9.6E-01	9.6E-01	0.028	

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proline	Urea cycle; arginine-, proline-, metabolism	-0.045	5.8E-01	5.8E-01	0.089	
trans-4-hydroxyproline	Urea cycle; arginine-, proline-, metabolism	-0.099	2.2E-01	2.4E-01	0.079	
urea	Urea cycle; arginine-, proline-, metabolism	-0.129	1.1E-01	1.6E-01	0.169	
2-hydroxy-3-methylvalerate	Valine, leucine and isoleucine metabolism	0.029	7.1E-01	7.1E-01	-0.010	
3-hydroxy-2-ethylpropionate	Valine, leucine and isoleucine metabolism	0.020	8.0E-01	8.0E-01	0.093	
3-hydroxyisobutyrate	Valine, leucine and isoleucine metabolism	0.026	7.5E-01	7.5E-01	0.062	
3-methyl-2-oxobutyrate	Valine, leucine and isoleucine metabolism	0.138	8.4E-02	1.4E-01	0.003	
3-methyl-2-oxovalerate	Valine, leucine and isoleucine metabolism	0.096	2.3E-01	2.4E-01	0.026	
4-methyl-2-oxopentanoate	Valine, leucine and isoleucine metabolism	0.160	4.5E-02	1.1E-01	0.011	
alpha-hydroxyisocaproate	Valine, leucine and isoleucine metabolism	0.028	7.2E-01	7.2E-01	0.006	
alpha-hydroxyisovalerate	Valine, leucine and isoleucine metabolism	0.035	6.7E-01	6.7E-01	-0.036	
beta-hydroxyisovalerate	Valine, leucine and isoleucine metabolism	-0.147	6.5E-02	1.2E-01	0.065	
isoleucine	Valine, leucine and isoleucine metabolism	-0.149	6.1E-02	1.2E-01	0.065	
isovalerylcarnitine	Valine, leucine and isoleucine metabolism	-0.052	5.2E-01	5.2E-01	0.046	
leucine	Valine, leucine and isoleucine metabolism	-0.110	1.7E-01	2.1E-01	0.053	
valine	Valine, leucine and isoleucine metabolism	-0.002	9.8E-01	9.8E-01	0.031	
<b>PURINE AND PYRIMIDINE METABOLITES</b>						
1,3,7-trimethylurate	Purine metabolism	0.103	2.0E-01	2.2E-01	0.034	
1,3-dimethylurate	Purine metabolism	0.090	2.6E-01	2.7E-01	0.028	
1,7-dimethylurate	Purine metabolism	0.134	9.3E-02	1.5E-01	0.019	
1-methylurate	Purine metabolism	0.001	9.9E-01	9.9E-01	0.080	
1-methylxanthine	Purine metabolism	0.177	2.6E-02	8.0E-02	0.003	
3-methylxanthine	Purine metabolism	0.027	7.3E-01	7.3E-01	0.078	
7-methylguanine	Purine metabolism	-0.070	3.8E-01	3.8E-01	0.044	
7-methylxanthine	Purine metabolism	0.084	2.9E-01	2.9E-01	0.048	
allantoin	Purine metabolism	-0.110	1.7E-01	2.1E-01	0.132	
hypoxanthine	Purine metabolism	-0.111	1.6E-01	2.1E-01	0.016	
inosine	Purine metabolism	-0.124	1.2E-01	1.7E-01	0.021	
N1-methyladenosine	Purine metabolism	-0.157	4.9E-02	1.1E-01	0.077	
N2,N2-dimethylguanosine	Purine metabolism	-0.070	3.8E-01	3.8E-01	0.118	
<b>N6-carbamoylthreonyl</b> adenosine	Purine metabolism	-0.373	1.4E-06	<b>4.6E-05</b>	0.169	0.344
urate	Purine metabolism	-0.152	5.7E-02	1.2E-01	0.147	
xanthine	Purine metabolism	-0.008	9.2E-01	9.2E-01	0.064	
3-aminoisobutyrate	Pyrimidine metabolism	-0.024	7.6E-01	7.6E-01	0.100	
5,6-dihydrouracil	Pyrimidine metabolism	-0.151	5.8E-02	1.2E-01	0.045	
5-methyluridine (ribothymidine)	Pyrimidine metabolism	0.116	1.5E-01	2.0E-01	0.044	
dihydroorotate	Pyrimidine metabolism	-0.096	2.3E-01	2.4E-01	0.076	
<b>pseudouridine</b>	Pyrimidine metabolism	-0.349	7.1E-06	<b>1.2E-04</b>	0.201	0.372
uracil	Pyrimidine metabolism	-0.062	4.4E-01	4.4E-01	0.030	
uridine	Pyrimidine metabolism	0.003	9.7E-01	9.7E-01	-0.020	

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**Supplementary Table S2.** Associations of the metabolites with the nephropathy risk in our study that were reported formerly in the independent cohort studies.

name	baseline	eGFR	Hazard ratio HR (95%CI)	Reference no
	eGFR	slope		
	r	r		
C-mannosyltryptophan*	<b>-0.48</b>	<b>-0.45</b>	<b>3.39 (2.22, 5.16)</b>	1,2
O-sulfo-L-tyrosine	<b>-0.46</b>	<b>-0.37</b>	<b>4.13 (2.66, 6.40)</b>	1
Pseudouridine	<b>-0.48</b>	<b>-0.35</b>	<b>3.37 (2.20, 5.16)</b>	1,2
Trans-4-hydroxyproline	-0.09	-0.10	1.13 (0.93, 1.38)	3
Creatine	0.21	-0.05	0.98 (0.78, 1.22)	3
Dimethylarginine <sup>†</sup>	-0.18	-0.19	1.17 (0.95, 1.43)	3
Uracil	0.02	-0.06	1.05 (0.86, 1.30)	3, 5
Citrulline	<b>-0.30</b>	-0.13	<b>1.34 (1.07, 1.68)</b>	4
Kynurenine	<b>-0.26</b>	-0.17	<b>1.53 (1.20, 1.96)</b>	4
Quinolate	<b>-0.29</b>	-0.27	<b>1.62 (1.27, 2.06)</b>	4
Kynurenate	<b>-0.33</b>	-0.04	1.10 (0.90, 1.36)	4
3-hydroxyisobutyrate	-0.09	0.03	0.92 (0.74, 1.13)	5
Beta-hydroxyisovalerate	-0.01	-0.15	1.08 (0.87, 1.33)	5

Hazard ratios are presented per one standard deviation of the metabolite. \*C-mannosyltryptophan is a structurally elucidated compound reported in our study under C-glycosyltryptophan name.

<sup>†</sup> Aggregate of the two forms: asymmetric dimethylarginine/symmetric dimethylarginine.

Legend: r - Spearman non parametric correlation coefficient, HR – hazard ratio, CI – confidence intervals, eGFR – estimated glomerular filtration rate

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**Supplementary Table S3.** Clinical characteristics of the auxiliary study group stratified by outcome. Legend: ESRD – End Stage Renal Disease, HbA1c – Hemoglobin, ACR – albumincreatinine ratio, eGFR – estimated glomerular filtration rate.

	Non progressors (n=39)	ESRD progressors (n=21)	Significance p value
<u>At baseline:</u>			
Male (%)	23 (59%)	12 (57%)	0.89
Age (yr)	35±8	32±5	0.17
Duration of diabetes (yr)	25±8	19±7	0.007
Body mass index (kg/m <sup>2</sup> )	26±5	26±4	0.93
HbA1c (%)	8.8±1.6	9.9±1.3	0.08
HbA1c (mmol/l)	73±18	85±14	0.08
Serum cholesterol (mg/dl)	200±36	228±57	0.02
Systolic blood pressure (mmHg)	133±21	129±20	0.47
Diastolic blood pressure (mmHg)	78±11	82±8	0.15
Antihypertensive/reno-protective			
Treatment (%)	74	52	0.08
ACR (µg/g creatinine)	665 (445,1297)	1243 (850,2000)	0.01
eGFR (ml/min/1.73m <sup>2</sup> )	89±27	81±21	0.22
<u>Follow up</u>			
annual eGFR slope (ml/min/1.73m <sup>2</sup> )	-1.6 (-2.5 - -0.9)	-10.9 (-16.6 - -7.6)	<.0001
ESRD occurrence (%)	0	100	by design

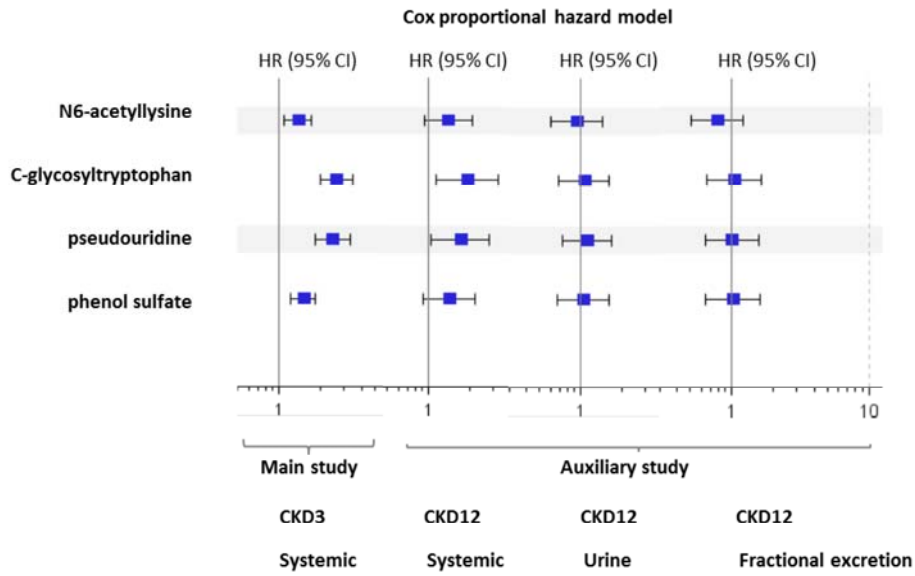
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**Supplementary Figure S1.** Systemic levels and the renal clearance of 4 metabolites measured in main and auxiliary studies according to the risk of nephropathy progression. Crude and adjusted hazard ratios for the progression to ESRD of the metabolites are presented per one standard deviation of the metabolite. Legend: HR – hazard ratio, CI – confidence intervals.





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### Supplementary Results – Protein acetylation

***Quantification of protein-bound acetyl-lysine residues.*** Protein-bound acetyl-lysine residues were quantified by LC/ESI/MS/MS (31) in the 40 samples balanced by caseness in Dr.Pennathur's laboratory (University of Michigan). Briefly, plasma proteins were precipitated with ice-cold 10% trichloroacetic acid and delipidated with water/methanol/water-saturated diethyl ether (1:3:7; vol/vol/vol). Known amounts of isotope-labeled internal standards were added. The precipitated proteins were hydrolyzed at 110°C for overnight in 6M hydrochloric acid, dried and dissolved in 50% methanol. For LC/ESI/MS/MS analysis, an Agilent 6410 triple quadruple MS system equipped with an Agilent 1200 LC system and ESI source was operated in positive ion mode. Acetyl-lysine, lysine, and the isotope-labeled standards were detected in the multiple reaction monitoring (MRM) mode and relative peak areas were obtained.

***Free versus protein-bound N6-acetyllysine residues.*** Free N6-acetyllysine (derived from the global profiling) has been identified among top 9 modified metabolites significantly associated with the eGFR decline. An important source of free acetyllysine could be proteolysis of acetylated proteins. We compared whether free acetyllysine reflected the pattern of circulating protein acetylation at the global level by mass spectrometry. Protein-bound N6-acetyllysine residues did not correlate with free circulating N6-acetyllysine ( $r=0.21$ ) nor with the eGFR slope ( $r=0.05$ ).