

Supplement to:

NAT8 Variants, N-Acetylated Amino Acids, and Progression of CKD

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Supplemental Table 1. All N-acetyl amino acids quantified via untargeted serum metabolomic profiling (N=31) in AASK.

Metabolite	Sub-pathway	PubChem ID	KEGG ID	HMDB ID	Blind duplicate CV	Carbon atom to which acetamido group is attached
N-acetylglutamate	Glutamate Metabolism	70914	C00624	HMDB01138	0.12	C2
N-acetylalanine	Alanine and Aspartate Metabolism	88064	C02847	HMDB00766	0.06	C2
N-acetylleucine	Leucine, Isoleucine and Valine Metabolism	70912	C02710	HMDB11756	0.07	C2
N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	448580	C02712	HMDB11745	0.05	C2
N-acetylvaline	Leucine, Isoleucine and Valine Metabolism	66789		HMDB11757	0.09	C2
N-acetylaspartate (NAA)	Alanine and Aspartate Metabolism	65065	C01042	HMDB00812	0.09	C2
N-acetylglycine	Glycine, Serine and Threonine Metabolism	10972		HMDB00532	0.07	C2
N-acetyltyrosine	Tyrosine Metabolism	68310		HMDB00866	0.13	C2
N-acetylthreonine	Glycine, Serine and Threonine Metabolism	152204			0.08	C2
N-acetylasparagine	Alanine and Aspartate Metabolism	99715		HMDB06028	0.09	C2
N-acetylglutamine	Glutamate Metabolism	182230	C02716	HMDB06029	0.08	C2
N-acetylhistidine	Histidine Metabolism	75619	C02997	HMDB32055	0.12	C2
N-acetylphenylalanine	Phenylalanine Metabolism	74839	C03519	HMDB00512	0.07	C2
N-acetylarginine	Urea cycle; Arginine and Proline Metabolism	67427	C02562	HMDB04620	0.08	C2
N-acetyltryptophan	Tryptophan Metabolism	700653	C03137	HMDB13713	0.08	C2

N-acetylisoleucine	Leucine, Isoleucine and Valine Metabolism	2802421		HMDB61684	0.09	C2
N-acetyl-aspartyl-glutamate (NAAG)	Glutamate Metabolism	5255	C12270	HMDB01067	0.12	C2
N2-acetyllysine	Lysine Metabolism	92907	C12989	HMDB00446	0.18	C2
N6-(epsilon)-acetyllysine	Lysine Metabolism	92832	C02727	HMDB00206	0.07	C6
N-acetyserine	Glycine, Serine and Threonine Metabolism	65249		HMDB02931	0.07	C2
N-acetylputrescine	Polyamine Metabolism	122356	C02714	HMDB02064	0.07	C2
N-delta-acetylornithine	Urea cycle; Arginine and Proline Metabolism	9920500			0.06	C5
N-acetyl-1-methylhistidine	Histidine Metabolism	193270			0.09	C2
N-acetyl-3-methylhistidine	Histidine Metabolism	193270			0.19	C2
N2,N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	10398396			0.11	C2, C5
N-acetylmethionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	193368			0.12	C2
N-acetyltaurine	Methionine, Cysteine, SAM and Taurine Metabolism	159864			0.06	C2
N-acetylcitrulline	Urea cycle; Arginine and Proline Metabolism	656979	C15532	HMDB00856	0.09	C2
N-acetylkynurenine	Tryptophan Metabolism				0.12	C2 (benzene)
(N(1) + N(8))-acetylspermidine	Polyamine Metabolism				0.07	C2, C8
N-acetyl-cadaverine	Lysine Metabolism	189087		HMDB02284	0.16	C2

Supplemental Table 2. Missingness for all covariables of interest.

	Number of missing (proportion)			
	AASK (N=692)	BioMe (N=680)	The ARIC Study (N=1050)	The GCKD Study (N=1624)
N-delta-acetylornithine	0 (0%)	0 (0%)	341 (32%)	146 (9%)
N-acetylcitrulline	0 (0%)	5 (0.7%)	314 (30%)	26 (2%)
N-acetylasparagine	0 (0%)	0 (0%)	291 (28%)	26 (2%)
N-acetylarginine	0 (0%)	0 (0%)	278 (26%)	26 (2%)
N-acetylglutamine	0 (0%)	3 (0.4%)	294 (28%)	27 (2%)
N-acetyl-1-methylhistidine	16 (2%)	10 (1%)	294 (28%)	26 (2%)
N2-acetyllysine	0 (0%)	290 (42%)	497 (47%)	27 (2%)
N-acetylleucine	0 (0%)	0 (0%)	298 (28%)	63 (4%)
N-acetylkynurenine	0 (0%)	0 (0%)	NA	256 (16%)
N-acetylphenylalanine	0 (0%)	0 (0%)	291 (28%)	27 (2%)
N-acetyl-3-methylhistidine	152 (16%)	0 (0%)	365 (35%)	33 (2%)
N-acetyltyrosine	133 (14%)	0 (0%)	324 (31%)	26 (2%)
N-acetylhistidine	4 (0.4%)	5 (0.7%)	329 (31%)	26 (2%)
N2,N5-diacetylornithine	0 (0%)	9 (1%)	311 (30%)	29 (2%)
Age	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Sex	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Race	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Body mass index	0 (0%)	181 (27%)	21 (2%)	0 (0%)
History of smoking	0 (0%)	1 (0.1%)	170 (16%)	0 (0%)
History of heart disease	0 (0%)	1 (0.1%)	12 (1%)	0 (0%)
Glomerular filtration rate	0 (0%)	5 (0.7%)	0 (0%)	0 (0%)
Urine albumin-creatinine ratio or urine albumin-creatinine ratio	4 (0.4%)	430 (71%)	95(9%)	0 (0%)

AASK, African American Study of Kidney Disease and Hypertension. The ARIC Study, the Atherosclerosis Risk in Communities Study. The GCKD Study, the German Chronic Kidney Disease Study. NA: Metabolite not available in the study.

Supplemental Table 3. rs13538 in other populations.

Outcome	Study	Population	N	A1	A2	freqA1	Effect size (SE)	p-value
N/A	GO Exome Sequencing Project	AA	662	A	G	0.473	N/A	N/A
CKD	Pattaro_2016	EA	114472	A	G	0.792	0.046 (0.019)	0.015
eGFRcrea	Pattaro_2016	AA	16428	A	G	0.419	-0.0039 (0.0035)	0.25
eGFRcrea	Pattaro_2016	Non-diabetic, EA	115154	A	G	0.792	-0.0092 (0.0011)	2.8e-16
eGFRcrea	Pattaro_2016	EA	130055	A	G	0.792	-0.0092 (0.0011)	3.1e-17
eGFRcrea	Pattaro_2016	Diabetic, EA	11135	A	G	0.792	-0.0048 (0.0045)	0.28
eGFRcys	Pattaro_2016	EA	33136	A	G	0.792	-0.0092 (0.0023)	6e-05
eGFR	Wuttke_2019	Trans-ethnic	603823	A	G	0.77	-0.0059 (0.0004)	1.2e-47
eGFR	Wuttke_2019	EA	567333	A	G	0.77	-0.0060 (0.0004)	5.5e-48
BUN	Wuttke_2019	Trans-ethnic	268267	A	G	0.76	0.0044 (0.0010)	8.7e-06
BUN	Wuttke_2019	EA	242906	A	G	0.77	0.0047 (0.0010)	6.7e-06
UACR	Teumer_2019	Trans-ethnic	558576	A	G	0.7717	-0.0032 (0.0024)	0.18
UACR	Teumer_2019	AA	6795	A	G	0.4880	0.0169 (0.0178)	0.34
UACR	Teumer_2019	EA	547323	A	G	0.7766	-0.0038 (0.0024)	0.11
UACR	Teumer_2019	Diabetic, trans-ethnic	50818	A	G	0.7592	-0.0071 (0.0075)	0.35
MA	Teumer_2019	Trans-ethnic	344969	A	G	0.7717	-0.0049 (0.0080)	0.55

Bold font: coded alleles.

AA, African ancestry. BUN, blood urea nitrogen. CKD, chronic kidney disease. EA, European ancestry. EGFR, estimated glomerular filtration rate. EGFRcrea, glomerular filtration rate estimated using blood creatinine. EGFRcys, glomerular filtration rate estimated using cystatin. MA, microalbuminuria. N/A, not applicable. UACR, urine albumin-creatinine ratio.

Reference:

https://www.ncbi.nlm.nih.gov/snp/rs13538#frequency_tab

<https://fox.nhlbi.nih.gov/CKDGen>

<https://ckdgen.imbi.uni-freiburg.de>

Supplemental Table 4. Circulating N-acetylated amino acids that are associated with rs13538 in AASK and their correlations with GFR.

Metabolite	Spearman correlation coefficient	p-value
N-delta-acetylornithine	-0.32	7.61E-24
N-acetylcitrulline	-0.40	3.73E-39
N-acetylasparagine	-0.32	1.29E-24
N-acetylarginine	-0.34	8.53E-27
N-acetylglutamine	-0.50	8.93E-61
N-acetyl-1-methylhistidine	-0.56	5.43E-80
N2-acetyllysine	-0.31	2.47E-22
N-acetylleucine	-0.36	2.14E-30
N-acetylkynurenine	-0.25	1.41E-15
N-acetylphenylalanine	-0.29	2.59E-20
N-acetyl-3-methylhistidine	-0.41	5.41E-40
N-acetyltyrosine	-0.21	5.46E-11
N-acetylhistidine	-0.55	4.16E-76
N2,N5-diacetylornithine	-0.60	1.62E-94

Supplemental Table 5. Circulating N-acetylated amino acids that are associated with rs13538 in AASK and their associations with ESKD in AASK, the ARIC Study, and meta-analysis, accounting for death as the competing event.

Study	AASK			ARIC			Meta-analysis*	
Exposure	N(cases/ controls)	sHR	p-value	N(cases/ controls)	sHR	p-value	sHR	p-value
N-delta-acetylornithine	274/688	1.27 (1.08, 1.50)	0.004	117/592	2.31 (1.14, 4.66)	0.02	1.31 (1.12, 1.54)	0.001
N-acetylcitrulline	274/688	1.06 (0.94, 1.19)	0.32	126/610	1.18 (0.72, 1.93)	0.50	1.07 (0.95, 1.20)	0.27
N-acetylasparagine	274/688	1.08 (0.88, 1.34)	0.45	129/630	1.01 (0.35, 2.94)	0.99	1.08 (0.88, 1.32)	0.48
N-acetylgarginine	274/688	1.08 (0.88, 1.33)	0.47	129/630	0.77 (0.33, 1.81)	0.55	1.06 (0.87, 1.30)	0.57
N-acetylglutamine	274/688	1.11 (0.90, 1.36)	0.33	129/628	1.50 (0.47, 4.81)	0.50	1.12 (0.91, 1.37)	0.27
N-acetyl-1- methylhistidine	274/672	1.21 (1.05, 1.38)	0.007	129/627	1.66 (0.82, 3.38)	0.16	1.22 (1.07, 1.40)	0.003
N2-acetyllysine	274/688	1.01 (0.91, 1.12)	0.87	96/457	0.69 (0.36, 1.31)	0.25	1.01 (0.91, 1.12)	0.92
N-acetylleucine	274/688	1.12 (0.84, 1.51)	0.44	127/625	0.93 (0.35, 2.42)	0.88	1.10 (0.83, 1.46)	0.50
N-acetylkynurenine	274/688	1.00 (0.92, 1.10)	0.93	NA	NA	NA	NA	NA
N-acetylphenylalanine	274/688	0.99 (0.81, 1.20)	0.89	129/630	1.30 (0.43, 3.94)	0.64	1.00 (0.82, 1.21)	0.99
N-acetyl-3- methylhistidine	258/552	1.11 (1.02, 1.20)	0.01	121/564	1.42 (0.93, 2.16)	0.10	1.12 (1.03, 1.21)	0.005
N-acetyltyrosine	236/593	1.02 (0.93, 1.13)	0.65	128/598	1.19 (0.48, 2.94)	0.71	1.02 (0.93, 1.13)	0.66
N-acetylhistidine	274/684	1.29 (1.04, 1.60)	0.02	128/593	1.69 (0.87, 3.27)	0.12	1.32 (1.08, 1.63)	0.007
N2,N5-diacetylornithine	274/688	1.24 (1.07, 1.44)	0.004	127/612	3.81 (1.41, 10.3)	0.008	1.27 (1.10, 1.47)	0.001

SHR, sub-hazard ratio, which indicates change in risk per one-fold change in the level of N-acetylated amino acids accounting for death as a competing event.

Co-variables in Cox models: baseline age, sex, measured GFR, urine protein-to-creatinine ratio in AASK or urine albumin-creatinine ratio in the ARIC Study, history of diabetes (ARIC only, AASK participants were non-diabetic at baseline), history of smoking, history of coronary heart disease, and body mass index.

Supplemental Table 6. rs13538 and ESKD.

Study	N (cases/controls)	Coded allele	Coded allele frequency	Effect size	p-value
AASK	274/422	A	0.46	1.19 (0.69, 2.06)	0.53
The ARIC Study	13/887	A	0.47	1.00 (0.49, 2.04)	0.99

AASK, African American Study of Kidney Disease and Hypertension. The ARIC Study, the Atherosclerosis Risk in Communities Study.

Supplemental Figure 1. Overview of study design.

