Supplemental Information

A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors

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Table S1. Baseline characteristics of the patients in the Discovery Cohort. Related to Fig. 1.

Characteristics	Discovery Cohort (n=1,162)
Age (years)	64.0 ± 10.9
Sex male (%)	63.7
T2DM (%)	22.1
History of hypertension (%)	72.1
History of hyperlipidemia (%)	85.8
Former/current smoking (%)	65.4
History of CAD (%)	75.6
HDL cholesterol (mg/dL)	34.3 (28.5-41.2)
LDL cholesterol (mg/dL)	96.0 (80.0-116.0)
C-reactive protein (mg/L)	2.3 (1.0-5.4)
eGFR (ml/min/1.73 m^2)	81.1 (68.0-93.7)
Baseline medications (%)	
Aspirin (%)	76.8
ACE inhibitors (%)	49.9
Beta blocker (%)	65.3
Statin (%)	61.4

The Discovery Cohort consists of sequential stable subjects who underwent elective diagnostic coronary angiography (cardiac catheterization or coronary computed tomography) for evaluation of coronary artery disease (CAD). MACE (Major Adverse Cardiac Event) was defined as death, nonfatal myocardial infarction, or nonfatal cerebrovascular accident (stroke) following enrollment. Continuous data are presented as mean ± standard deviation or median (interquartile range). Categorical variables are presented as %; T2DM = type 2 diabetes mellitus; eGFR = estimated glomerular filtration rate; ACE = angiotensin-converting-enzyme.

Table S2. Top ranked plasma metabolites from the untargeted metabolomics in the Discovery Cohort (n=1,162). Related to Fig. 1.

	Hazard ratio MACE 3 years Q_4 vs Q_1		T2DM vs non-T2DM	Spearman correlation					
				Fasting glucose		HGBA1c		Insulin/glucose	
Compound	**HR**	**5-95% (CI)**	**p**	**rho**	**p**	**rho**	**p**	**rho**	**p**
Knowns									
PE (38:4)	2.63	1.50-4.61	0.0003	0.038	0.1974	0.150	3.5e-7	0.051	0.0831
TML	2.53	1.44-4.45	0.0034	0.114	0.0001	0.069	0.0201	0.167	1.0e-8
TMAO	2.42	1.44-4.08	1.7e-5	0.165	2.0e-8	0.119	5.6e-5	0.058	0.0724
PC (34:5)	2.32	1.35-3.99	0.0003	0.041	0.1592	0.154	1.7e-7	0.053	0.0721
PE (34:2)	2.12	1.27-3.57	2.7e-8	0.113	0.0001	0.149	4.3e-7	0.119	4.9e-5
Unknowns (*m/z*)									
265.1188	2.69	1.61-4.52	0.0002	0.036	0.2261	0.093	0.0018	-0.031	0.2897
126.0905	2.60	1.53-4.43	7.2e-10	0.096	0.0011	0.111	0.0002	0.006	0.8517
185.1278	2.59	1.54-4.36	2.0e-10	0.108	0.0002	0.117	0.0001	0.007	0.8200
906.771	2.44	1.36-4.38	0.0289	0.008	0.7758	0.074	0.0130	0.047	0.1072
740.5187	2.37	1.38-4.07	6.6e-8	0.090	0.0022	0.149	4.5e-7	0.048	0.1034

Table S2 represents the top five metabolites that were structurally identified at the time of study (Knowns) and the top five analytes with unknown structure (Unknowns), Unknowns were characterized by high resolution mass spectrometry of the analyte and retention time (not shown), that had significant Hazard Ratio (HR) for major averse cardiac events (MACE: MI, stroke or death) for fourth quartile (Q4) vs first quartile (Q1), showed levels that were significantly elevated in diabetics vs non-diabetics, and were poorly associated with multiple indices of glucose control (fasting glucose, HGBA1c and insulin/glucose ratio). CI, 95% confidence interval; HR, Hazard ratio; Q=quartile; T2DM, Type 2 diabetes mellitus; *m/z*, mas to charge ratio.

Table S3. Comparison of experimental high resolution collision induced dissociation mass spectral data from the plasma analyte with *m/z* 265.1188 and phenylacetylglutamine (PAGln). Related to Fig. 1.

Analyte	Elemental composition	m/z (mass to charge ratio)		Difference (Δppm)
		Theoretical (Experimental PAGln standard)	Measured	
Molecular ion	**$C_{13}H_{17}N_2O_4$**	**265.1188 (265.1185)**	**265.1179**	**-3.3 (-2.3)**
Fragment 1	$C_5H_{11}N_2O_3$	147.0770 (147.0760)	147.0758	8.1 (-1.4)
Fragment 2	$C_8H_{10}N_1O_1$	136.0762 (136.0752)	136.0755	5.1 (2.2)
Fragment 3	$C_5H_8N_1O_3$	130.0504 (130.0496)	130.0497	5.3 (0.8)
Fragment 4	C_7H_7	91.0548 (91.0545)	91.0547	1.0 (2.2)
Fragment 5	$C_4H_6N_1O_1$	84.0449 (84.0449)	84.0450	-1.2 (-1.2)

High resolution mass spectrometry data were collected for the unknown compound with *m/z* 265.1188 (in the current experiment *m/z* 265.1179) and compared with the theoretical values and experimental values obtained from the pure phenylacetylglutamine (PAGln) standard (values shown in parenthesis). Difference between values is expressed in parts per million (ppm).

Table S4. Baseline characteristics of patients in the Validation Cohort. Related to Fig. 1.

Characteristics	Validation Cohort (n=4,000)
Age (years)	62.9 ± 10.9
Sex male (%)	64.4
Diabetes mellitus (%)	31.5
History of hypertension (%)	71.7
History of hyperlipidemia (%)	84.2
Former/current smoking (%)	65.1
History of CAD (%)	72.4
HDL cholesterol (mg/dL)	34.1 (28.3-41.2)
LDL cholesterol (mg/dL)	96.0 (78.0-117.0)
C-reactive protein (mg/L)	2.4 (1.0-5.9)
eGFR (ml/min/1.73 m^2)	82.1 (68.8-94.8)
Baseline medications (%)	
Aspirin (%)	73.8
ACE inhibitors (%)	50.1
Beta blocker (%)	63.1
Statin (%)	60.2

The Validation Cohort consists of sequential stable subjects who underwent elective diagnostic coronary angiography (cardiac catheterization or coronary computed tomography) for evaluation of coronary artery disease (CAD). MACE (Major Adverse Cardia Events) was defined as death, nonfatal myocardial infarction, or nonfatal cerebrovascular accident (stroke) following enrollment. Continuous data are presented as mean ± standard deviation or median (interquartile range). Categorical variables are presented as %; T2DM = type 2 diabetes mellitus; eGFR = estimated glomerular filtration rate; ACE = angiotensin-converting-enzyme.

Table S5. Distribution of plasma PAGln levels in the Validation Cohort. Related to Fig. 1.

	Phenylacetylglutamine (µM)						
PAGln range (%)	**Whole cohort (n=4,000)**	**History of CVD in the whole cohort**		**History of CVD in non-T2DM**		**History of CVD in T2DM**	
		No (n=811)	**Yes (n=3,189)**	**No (n=650)**	**Yes (n=2,089)**	**No (n=161)**	**Yes (n=1,100)**
Min	0.081	0.081	0.108	0.081	0.109	0.311	0.108
5	0.808	0.745	0.833	0.767	0.797	0.622	1.005
10	1.112	0.951	1.154	0.945	1.084	0.975	1.340
25	1.825	1.572	1.912	1.540	1.806	1.775	2.137
50	3.007	2.548	3.190	2.508	2.923	2.752	3.685
75	4.893	3.945	5.094	3.897	4.656	4.400	5.962
90	7.444	6.134	7.784	5.799	6.946	6.525	9.425
95	9.937	7.862	10.435	8.036	9.047	7.382	13.100
97.5	13.691	10.220	14.519	9.948	12.161	10.35	20.282
99	23.716	14.374	25.268	13.392	16.573	12.98	70.298
Max	267.1	152.1	267.1	152.1	267.1	18.17	217.1

Results in the Table show plasma PAGln concentrations at the indicated percentile cut off (in µM) within the whole cohort in the indicated subgroups. CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus.