Supplementary Data

Title: Gut Microbial Product Predicts Cardiovascular Risk in Chronic Kidney Disease Patients

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Running title: Valerate and cardiovascular outcomes

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Supplementary Table 1: Comparing baseline characteristics of patients with and without CAD in the training and validation sets

	Training Set			Validation Set		
	With CAD	Without CAD	P value	With CAD	Without CAD	P value
Anthropometric parameters						
Number of subjects	40	67		41	66	
Age (years)	68 ± 9	52 ± 16	<0.001	67 ± 12	58 ± 17	0.002
Male Sex (%)	23 (57.5)	36 (53.7)	0.84	22 (53.7)	29 (43.9)	0.43
White Race (%)	28 (70)	46 (68.6)	1.00	28 (68.3)	48 (72.7)	0.66
SBP (mmHg)	142 ± 25	135 ± 23	0.13	141 ± 23	132 ± 20	0.002
DBP (mmHg)	75 ± 12	76 ± 12	0.82	75 ± 11	75 ± 12	0.91
Height (m)	1.72 ± 0.10	1.71 ± 0.11	0.84	1.70 ± 0.11	1.67 ± 0.16	0.39
Weight (kg)	92 ± 22	92 ± 21	0.97	93 ± 23	85 ± 20	0.07
BMI (Kg/m^2)	30.9 ± 6.2	31.4 ± 7.2	0.75	31.8 ± 5.9	30.2 ± 7.6	0.26
Smoking (%)	3 (7.5)	9 (13.4)	0.53	6 (14.6)	3 (4.5)	0.08
Etiology						
Hypertension (%)	11 (27.5)	10 (14.9)	0.03	10 (24.4)	13 (19.7)	0.01
Diabetes (%)	9 (22.5)	11 (16.4)		11 (26.8)	16 (24.2)	
Glomerular (%)	8 (20.0)	33 (49.3)		5 (12.2)	27 (40.9)	
Tubulointerstitial (%)	1 (2.5)	3 (4.5)		1 (2.4)	2 (3.0)	
Others, n (%)	11(27.5)	10 (14.9)		14 (34.1)	8 (12.1)	
Comorbidities						
Hypertension (%)	37 (92.5)	51 (76.1)	0.04	36 (87.8)	52 (78.8)	0.30
Diabetes (%)	17 (42.5)	21 (31.3)	0.30	27 (65.9)	24 (36.4)	0.005
Stroke (%)	10 (25)	7 (10.4)	0.06	7 (17.1)	6 (9.1)	0.24
Heart failure (%)	10 (25)	4 (6.0)	0.007	16 (39.0)	1 (1.5)	<0.001
PVD (%)	12 (30)	3 (4.5)	<0.001	9 (21.9)	5 (7.6)	0.04
Arrhythmia (%)	8 (20)	7 (10.4)	0.25	10 (24.4)	8 (12.1)	0.12
Medications						
Statins (%)	29 (72.5)	24 (35.8)	<0.001	24 (58.5)	37 (56.1)	0.84
Fibrates (%)	5 (12.5)	5 (7.5)	0.50	3 (7.3)	5 (7.6)	1.000
Niacin (%)	1 (2.5)	2 (3.0)	1.00	2 (4.9)	2 (3.0)	0.64
ACEI/ARB (%)	28 (70.0)	43 (64.2)	0.54	32 (78.0)	47 (71.2)	0.43
PPI (%)	17 (42.5)	19 (28.4)	0.13	10 (24.4)	12 (18.2)	0.44
ESA (%)	7 (17.5)	4 (6.0)	0.06	3 (7.3)	5. (7.6)	0.96
Probiotics/Antibiotics (%)	1 (2.5)	1 (1.5)	1.0	1 (2.4)	2 (3.0)	1.0
Biochemical parameters						
Albumin (g/dL)	4.0 ± 0.5	4.1 ± 0.4	0.22	4.0 ± 0.4	4.1 ± 0.4	0.18
Hemoglobin (g/dL)	12.4 1.5	12.0 1.8	0.23	12.1 1.8	11.7 1.6	0.20
CRP (g/dL)†	0.3 0.3	1.4 3.3	0.44	1.5 1.9	0.7 0.7	0.43
Cholesterol (mg/dL)	158 ± 59	180 ± 53	0.05	163 ± 49	168 ± 48	0.54
LDL (mg/dL)	79 ± 42	92 ± 44	0.14	83 ± 33	85 ± 38	0.77
HDL (mg/dL)	37 ± 17	42 ± 19	0.18	36 ± 16	38 ± 17	0.43
Triglycerides (mg/dl)	140 ± 136	163 ± 111	0.33	173 ± 87	170 ± 105	0.87
UPCR, median [IQR]	1.2 [0.4 – 1.9]	1.2 [0.2 – 2.6]	0.15	1.2 [0.3 – 1.7]	0.9 [0.2 – 1.8]	0.78
eGFR, mL/min, median [IQR]	35 ± 17	42 ± 25	0.09	41 ± 20	43 ± 22	0.68
eGFR categories						
>60 ml/min, n (%)	3 (7.5)	16 (23.9)	0.15	5 (12.2)	12 (18.2)	0.22
30-59 ml/min, n (%)	20 (50)	29 (43.3)		22 (53.6)	28 (42.4)	
15-29 ml/min, n (%)	14 (35)	14 (20.9)		12 (29.3)	21 (31.8)	
<15 ml/min, n (%)	3 (7.5)	8 (11.9)		2 (4.9)	5 (7.6)	

BMI, body mass index; CAD, coronary artery disease; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein; PVD, peripheral artery disease; ACEI, Angiotensin converting enzyme inhibitor; ARB, Angiotensin Receptor Blocker, PPI, proton pump inhibitor; ESA, erythropoietin stimulating agent; SBP, systolic blood pressure; UPCR, urine protein-creatinine ratio. †Sample size for CRP is 12 for CAD and 45 for no CAD in the entire cohort.

Supplementary Table 2: Comparing mean SCFA concentrations by CKD stage. Values are mean ± standard deviation.

Short Chain Fatty Acid	CKD Stages 1,2 Mean ± SD	CKD Stage 3 Mean ± SD	CKD Stage 4 Mean ± SD	CKD Stage 5 Mean ± SD	P value
Acetate(µm/L)	228.9 ± 44.5	211.6 ± 41.7	203.6 ± 35.8	195.2 ± 29.6	<0.01
Propionate(µm/L)	96.3 ± 3.4	95.4 ± 3.6	95.8 ± 3.1	95.9 ± 3.1	0.61
Butyrate(µm/L)	65.5 ± 0.7	65.5 ± 0.7	65.4 ± 0.5	65.6 ± 0.5	0.78
Isovalerate(µm/L)	80.8 ± 5.4	79.1 ± 4.4	78.4 ± 2.3	80.2 ± 6.6	0.05
Valerate(µm/L)	28.4 ± 0.8	29.0 ± 1.4	29.0 ± 1.4	29.5 ± 1.4*	0.045
Caproate(µm/L)	25.5 ± 1.2	26.2 ± 1.8	26 ± 1.4	26.7 ± 1.7	0.07

*p=0.02, Dunnett post hoc corrected for multiple comparisons when compared with CKD stage 2.
CKD, chronic kidney disease; SCFA, short-chain fatty acid; SD, standard deviation.

Supplementary Table 3: Comparing mean concentrations of SCFAs by stages of CKD in the "Training" and "Validation" sets

SCFA		CKD Stages 1,2	CKD Stage 3	CKD Stage 4	CKD Stage 5	P value
Acetate	Training	235.7 ± 42.6	216.4 ± 42.2	204.6 ± 39.2	199 ± 26.5	0.04
	Validation	219.5 ± 46.7	206.9 ± 41.1	202.6 ± 33.3	188.1 ± 36.2	0.36
Propionate	Training	96.3 ± 3.5	95.3 ± 3.8	96.2 ± 3.0	95.4 ± 3.5	0.59
	Validation	96.2 ± 3.5	95.6 ± 3.4	95.5 ± 3.1	97 ± 2.2	0.68
Butyrate	Training	65.5 ± 0.5	65.6 ± 0.7	65.4 ± 0.5	65.6 ± 0.6	0.38
	Validation	65.6 ± 0.9	65.4 ± 0.7	65.5 ± 0.6	65.5 ± 0.3	0.90
Isovalerate	Training	81.3 ± 6.0	80.2 ± 5.6	78.1 ± 2.4	82.2 ± 7.8	0.09
	Validation	80.2 ± 4.8	78.1 ± 2.4	79.0 ± 2.1	77.1 ± 1.0	0.04
Valerate	Training	28.3 ± 0.8	29.1 ± 1.7	28.8 ± 0.8	29.8 ± 1.7	0.04
	Validation	28.6 ± 0.9	29.0 ± 1.1	29.2 ± 1.7	29.0 ± 0.5	0.48
Caproate	Training	25.3 ± 1.1	26.5 ± 2.1	26.3 ± 1.5	27.2 ± 1.8	0.04
	Validation	25.8 ± 1.2	25.9 ± 1.3	25.7 ± 1.2	25.8 ± 0.9	0.93

Values are mean ± standard deviation in micromoles/liter. CKD, chronic kidney disease; SCFA, short chain fatty acid

Supplementary Table 4: Comparing the odds ratio of study outcomes in unadjusted, eGFR-adjusted, and fully adjusted models. Odds ratios are compared by each 1 μmol/L alteration in plasma valerate in the training set, and by each 1 standard alteration in the standardized probabilistic risk score (developed from the coefficients of the logistic regression models of the training set) in the validation set. Covariates of the fully adjusted model consisted of age, diabetes, hypertension, use of statins, glomerular etiology, and eGFR.

	B	S.E.	P value	OR	95% CI for OR
Training set:					
Unadjusted:					
CAD (Training)	0.595	0.198	0.003	1.812	1.229 to 2.672
CVD outcomes (Training)	0.495	0.196	0.01	1.640	1.117 to 2.408
eGFR adjusted					
CAD (Training)	0.547	0.199	0.006	1.728	1.169 to 2.555
CVD outcomes (Training)	0.400	0.199	0.04	1.493	1.011 to 2.203
Fully adjusted					
CAD (Training)	0.462	0.234	0.049	1.587	1.003 to 2.513
CVD outcomes (Training)	0.522	0.242	0.03	1.686	1.048 to 2.711
Validation set:					
Unadjusted:					
CAD (Validation)	0.645	0.226	0.004	1.905	1.224 to 2.965
CVD outcomes (Validation)	0.509	0.218	0.02	1.663	1.085 to 2.550
eGFR adjusted					
CAD (Validation)	0.604	0.220	0.006	1.830	1.188 to 2.819
CVD outcomes (Validation)	0.521	0.212	0.01	1.685	1.111 to 2.553
Fully adjusted					
CAD (Validation)	0.586	0.214	0.006	1.797	1.181 to 2.732
CVD outcomes (Validation)	0.725	0.217	0.001	2.065	1.351 to 3.158

CAD, coronary artery disease; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; OR, odds ratio; SE, standard error