**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/m2) | 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