Effects of Sodium-Glucose Cotransporter-2 Inhibitors on Modulating Protein-Bound Uremic Toxins and Gut Microbiota in Predialysis CKD Patients

Matched Case-Control Study

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Key Points

- A reduction of indoxyl sulfate, p-cresyl sulfate, and several short-chain fatty acids was seen in sodium-glucose cotransporter-2 inhibitor-treated CKD patients.
- Variations in gut microbiota composition are correlated with levels of gut-derived uremic toxins in sodium-glucose cotransporter-2 inhibitor-treated CKD patients.

Abstract

Background The intricate interplay between CKD and intestinal microbiota has gained increasing attention, with gut dysbiosis being implicated in uremic toxin accumulation and CKD progression. Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are now transforming CKD management but pose uncertain effects on shaping gut microbiota. This study aimed to elucidate the effect of SGLT2i on perturbations of gut microbial composition and metabolic responses in patients with CKD.

Methods Analysis of fecal microbiota and targeted profiling of serum short-chain fatty acids and gut-derived uremic toxins were conducted in a matched case-control study, including 60 patients with CKD (treated: $n=30$; untreated: $n=30$) and 30 non-CKD controls.

Results Gut microbial composition differed significantly among the three study groups. Patients with CKD receiving SGLT2i exhibited distinctive taxonomic profiles, such as enrichment of *Bacteroides stercoris* and *Bacteroides coprocola.* Surveys of metabolomic profiles revealed a reduction of two uremic solutes, indoxyl sulfate and p-cresyl sulfate (pCS), and several short-chain fatty acids (formic, acetic, propionic, valeric, and 2-methylbutanoic acid) in SGLT2i-treated CKD patients. Co-occurrence analysis demonstrated a set of intestinal microbes that is positively or negatively correlated with the levels of pCS, and the abundance of these pCS-associated intestinal microorganisms was correlated with the levels of indoxyl sulfate and isovaleric acids in the same and opposite direction, respectively. Further functional prediction indicated attenuated pathways related to protein and carbohydrate metabolism.

Conclusions Treatment with SGLT2i in patients with CKD is associated with distinct gut microbial composition and metabolite profiles, suggesting potential modulation of gut dysbiosis and metabolic pathways. Further studies are warranted to elucidate the clinical implications of these findings in CKD management.

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Introduction

The comprehension of the bidirectional relationship between CKD and gut microbiota continues to advance. It is known that dysbiosis in renal insufficiency may contribute to heightened levels of uremic toxins, compromised intestinal barrier function, and dysregulated immune responses, ultimately influencing CKD progression.[1] Recent studies have emphasized the significance of specific gut metagenomic and metabolomic markers in distinguishing various severities of CKD.[2,3] Initially, through 16S rRNA sequencing, certain genera (such as *Escherichia_Shigella*, *Dialister*, *Lachnospiraceae_ND3007_group*, *Pseudobutyrivibrio*, *Roseburia*, *Paraprevotella*, and *Ruminiclostridium*) have been identified to be closely linked to different stages of CKD.[2,4–6] In addition, shotgun metagenomic sequencing has unveiled compositional and functional alterations of the fecal microbiota in association with metabolic and immune dysregulation of the host across the CKD severity spectrum.[7,8] These findings underscore the correlation between gut dysbiosis and CKD progression.

Individuals with CKD face an elevated risk of adverse renal and cardiovascular outcomes.[9] Sodium-glucose cotransporter-2 inhibitors (SGLT2i) have shown promise in mitigating cardiovascular risk and slowing the decline of renal function in patients with CKD.[10–13] SGLT2i render hemodynamic, metabolic, and direct cellular effects to slow the progression of kidney damage and to improve overall renal outcomes in patients with CKD.[14] Many pleiotropic effects have been proposed for SGLT2i in different organs, independent of glucose handling in the renal tubule. The effects of SGLT2i on the intestinal tract are unknown. An experimental model exhibited compositional and functional changes of gut microbiota after administration of canagliflozin in mice with renal failure and was associated with lower plasma levels of uremic toxins.[15] Dapagliflozin and empagliflozin have also been found to modulate microbiota in a type 2 diabetic rat model.[16–18] On the other hand, the effects of SGLT2i on the gut microbiome composition and host metabolic adaptation in patients with CKD remain largely unexploited. This study aims to evaluate the effects of SGLT2i on the alteration in gut microbiota communities and associated metabolic responses in patients with CKD.

Methods

Study Design and Patient Settings

A total of 90 patients (60 patients with nondialysis CKD and 30 age-matched and sex-matched non-CKD controls) were recruited from Chang Gung Memorial Hospital in Keelung, Taiwan. The CKD was diagnosed based on an eGFR, calculated by using the 2021 CKD Epidemiology Collaboration equation, of <60 ml/min per 1.73 m^2 or a urine protein-to-creatinine ratio exceeding 150 mg/g on two separate occasions. Among the patients with CKD, 30 were treated with SGLT2i (dapagliflozin 10 mg/d or empagliflozin 10 mg/g) for a period of 3 months (CKD+SGLT2i group), while the remaining 30 patients did not receive SGLT2i (CKD group). Patients were excluded from the study if they were undergoing dialysis therapy, had undergone renal transplant, or had cardiovascular disease, malignancy, liver cirrhosis, active infection, previous intestinal operation, prior use of SGLT2i, or concurrent use of probiotics, prebiotics, or antibiotics. Since specific dysbiosis of intestinal microbiome has been associated with diabetes[19] and hypertension,[20] two of the most common complications of CKD, subjects with matched age, sex, and status of diabetes mellitus and hypertension among study groups were enrolled to exclude potential confounding effects. Participants were instructed to not to consume any supplements containing probiotics, such as yogurt, within 7 days before sample collection. Fecal and plasma samples were collected after a 3-month period and stored at −80°C until analysis. A minimum of 28 subjects per group was determined to provide a study power of 0.9 and an α-error probability of 0.05 in a 3-group design (non-CKD control, CKD, and CKD+SGLT2i groups), on the basis of an effect size of 40% and a significance level of 0.05 under two-tailed analysis. A total of 84 patients were justified by the sample size calculation statement. To account for potential inadequate sampling or loss to follow-up of participants, an estimated sample size of 90 patients was deemed sufficient to meet the assumptions of the study. The Institutional Review Board at Chang Gung Memorial Hospital (202101942B0) approved this study, and informed consent was obtained from all participants before all procedures begin.

Metabolomics Profiling of Gut-Derived Metabolites

Targeted metabolomic profiling has been described in detail in our previous publications.[3,21] In brief, 250 μl of internal standard solution containing 10% H_2SO_4 and 20 mg/L of 2-methylvaleric acid was combined with 150 μl of serum samples. This combination was used for the profiling of ten short-chain fatty acids (SCFAs) through gas chromatography-mass spectrometry analysis using an Agilent 7890B gas chromatograph system coupled with an Agilent 5977B mass spectrometer. Circulating p-cresyl sulfate (pCS) and indoxyl sulfate (IS), in both free and protein-bound fractions, were analyzed using the Waters Xevo TQS liquid chromatography-MS/MS system, which incorporates an HSS T3, 1.8 μm, 2.1× 100 mm column. The mass spectrometry conditions were configured in negative electrospray ionization mode with multiple reaction monitoring (electrospray ionization negative mode with multiple reaction monitoring). The ion source temperature was maintained at 150°C with a capillary voltage of 1.5 kV. Desolvation gas flow was set at 650 L/h, while the desolvation gas temperature was set to 500°C. Cone gas flow was set at 150 L/h. The multiple reaction monitoring transitions were as follows: m/z 212.03→80.24 and 212.03→132.08 for IS, m/z 216.03→80.24 for IS −d4, m/z 187.03→107.08 and 187.03→80.12 for pCS, and m/z 194.1→114.16 for pCS −d7. All transitions were optimized using IntelliStart software.

Fecal 16S rRNA Gene Sequencing and Data Analysis

Bacterial DNA extraction was performed using the FastDNA SPIN Kit for feces (MP Biomedical, LLC). 16S rRNA gene sequencing, data processing, and analysis were conducted, as described previously.[2,21] In brief, we used PCR to amplify the variable regions 3–4 (V3–V4) of the 16S

rRNA gene, which was subsequently sequenced on an Illumina HiSeq 2500 platform. Sequencing reads were processed and clustered into operational taxonomic units at 97% sequence identity using UPARSE.[22] Taxonomy classification was achieved using the SILVA database.[23] α-diversity was assessed using the Chao1 index, while β-diversity was evaluated using nonmetric dimensional scaling. Functional composition of metagenomes was predicted from 16S rRNA data by the Tax4Fun software.[24] To predict functional profile of the microbial community, the taxonomic abundance transformed from the SILVA-based 16S rRNA and normalized by the 16S rRNA copy number acquired from the National Center for Biotechnology Information annotations was applied to incorporate the precomputed functional profiles of Kyoto Encyclopedia of Genes and Genomes pathways. The Kyoto Encyclopedia of Genes and Genomes analysis was only focused on "Metabolism" pathways.

Statistical Analysis

Categorical variables were presented as frequency and percentage, and comparisons were made using the Fisher exact test. Continuous clinical indices were expressed as means±SD or median (interquartile range, and comparisons were conducted using either the Student *t* test or Kruskal-Wallis test. Normality of numerical variables was assessed using the Kolmogorov-Smirnov method. The Kruskal-Wallis test was used to analyze the Chao1 index. Analysis of similarities of UniFrac parameters was conducted to assess discrimination in community composition between groups. Significant differences in the relative abundance of taxa among the three groups were assessed using the Kruskal-Wallis test, with *post hoc* comparisons between two groups conducted using the Dunn test. In addition, linear discriminant analysis of effect size (LEfSe) analysis was performed to further identify statistically significant species for each group. The connection of microbes to host metabolites was assessed by Spearman rank correlation, and the importance was corrected by using the Benjamini-Hochberg procedure. Differences in the relative abundance of predicted microbial genes related to metabolism between groups were evaluated using the Student *t* test. Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 (SPSS, Inc., US). All tests were two-tailed, and a P value < 0.05 was considered statistically significant.

Results

Subject Characteristics

Baseline characteristics of the participants (n=90) enrolled in the study are presented in Table 1. The mean age of the subjects was 67.8±9.6 years, and 52 (57.7%) of them were male. No significant difference was observed between the CKD and CKD+SGLT2i groups concerning age, sex, diabetes, hypertension, gout, hyperlipidemia, systolic BP, body mass index, renal functions, and urine protein-creatinine ratio.

Alterations in Microbial Composition and Diversity in Patients with CKD Undergoing SGLT2i Therapy

To characterize the gut microbial composition among the three groups, we conducted a taxonomic analysis focusing on the top ten genera. Our analysis revealed a notable prevalence of bacteroides (18.2% of the overall sequence reads) in the CKD+SGLT2i group, surpassing that of both the CKD group (14.4%) and the normal controls (10.6%; Figure 1A). We observed a subtle reduction of the α-diversity in the CKD+SGLT2i group as compared with the CKD group and individuals with normal renal function (Figure 1B). Furthermore, assessments of sample-to-sample

Table 1. Baseline characteristics of study population (n=90)

Variables	All Subjects (n=90)	Normal (n=30)	CKD (n=30)	CKD+SGLT2i (n=30)	P Value
Age, mean (SD)	67.81±9.66	66.27±7.71	71.97±9.91	65.2±10.04	0.612
Male, *n* (%)	52 (57.7)	20 (66.7)	17 (56.7)	15 (50)	0.605
Diabetes, *n* (%)	53 (58.9)	8 (26.7)	20 (66.6)	25 (83.3)	0.136
Hypertension, *n* (%)	58 (64.4)	15 (50)	24 (80)	19 (63.3)	0.152
Gout, *n* (%)	33 (36.7)	10 (33.3)	14 (46.7)	9 (30)	0.067
Hyperlipidemia, *n* (%)	42 (46.7)	13 (43.3)	13 (43.3)	16 (53.3)	0.303
Systolic pressure, mm Hg	133.98±14.24	124.44±8.96	138.8±14.64	135±17.135	0.988
Body mass index, kg/m^2	26.28±4.48	23.20±1.27	25.42±3.81	27.62±4.98	0.135
Serum creatinine, mg/dl	1.42±0.82	0.93±0.20	1.86±1.17	1.47±0.46	0.382
eGFR, ml/min per m^2, MDRD	55.25±22.95	75.8±11.88	43.71±24.11	46.26±15.34	0.190
eGFR, ml/min per m^2, CKD-EPI	55.69±24.30	77.45±12.58	43.47±25.53	46.17±16.25	0.380
Hemoglobin, g/dl	13.18±1.84	13.51±1.46	12.52±2.04	13.51±1.87	0.059
Serum albumin, mg/dl	4.47±0.29	4.56±0.24	4.33±0.29	4.52±0.32	0.052
Serum calcium, mg/dl	9.39±1.06	9.29±1.79	9.4±0.33	9.5±0.47	0.455
Serum phosphate, mg/dl	3.61±0.64	3.41±0.49	3.75±0.68	3.65±0.71	0.867
Serum potassium, mEq/L	4.46±0.49	4.35±0.47	4.63±0.51	4.40±0.47	0.060
Serum uric acid, mg/dl	5.68±1.51	5.66±1.46	6.03±1.41	5.36±1.61	0.092
Urine protein-creatinine ratio, mg/g	187 (235)	53.5 (71)	232 (365)	258 (241)	0.534

Data are expressed in mean (SD) or median (interquartile range). Estimation of P value between CKD versus CKD+SGLT2i by using median test. CKD-EPI, GFR estimation by CKD Epidemiology Collaboration study equation; MDRD, GFR estimation by Modification of Diet in Renal Disease study equation; SGLT2i, sodium-glucose cotransporter-2 inhibitor.

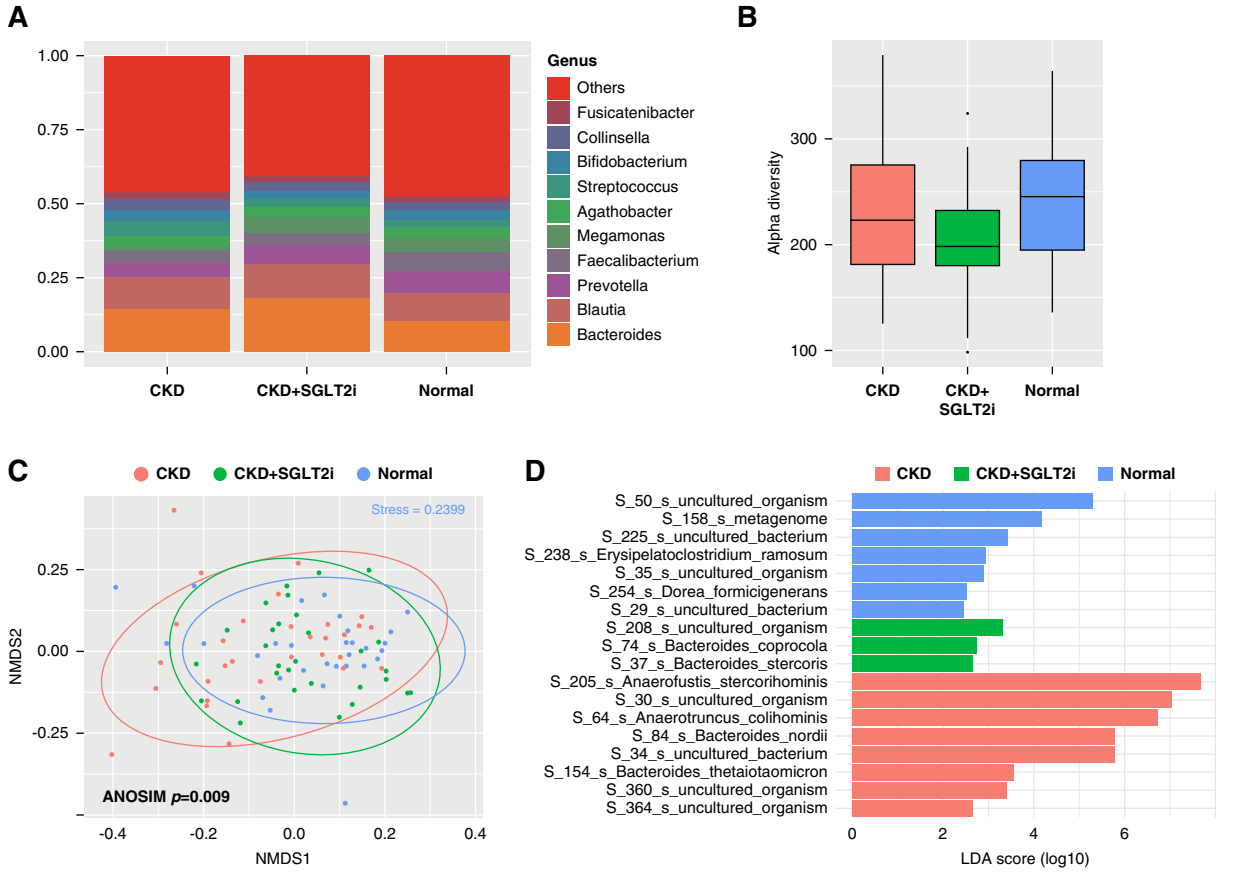

Figure 1. Comparisons of gut microbiota composition and diversity in normal renal function controls and patients with CKD receiving SGLT2i or not. (A) Distribution of top ten genera among groups. (B) α-diversity (Chao1). (C) NMDS ordination on the basis of weighted UniFrac parameters of intestinal microbial communities among groups. Significant sample-to-sample dissimilarities refer to ANOSIM ($P < 0.001$) test for discrimination in community composition among groups. (D) Bacterial taxa that best characterize each group were determine by applying LEfSe on OTU tables. ANOSIM, analysis of similarity; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis of effect size; NMDS, nonmetric dimensional scaling; OTU, operational taxonomic units; SGLT2i, sodium-glucose cotransporter-2 inhibitor.

Table 2. Variations of gut microbiota (at genus and species level) associated with SGLT2i treatment

Gut Microbiota		RA (%) Non-CKD	RA (%) CKD+SGLT2i	RA (%) CKD	*P* Value[a]
Family	**Genus**				
Erysipelotrichaceae	*Escherichia-Shigella* ↑	0.0159	0.0239	0.0108	0.0021
Clostridiaceae	*Clostridium_sensu_stricto_1* ↓	0.0099	0.0020	0.0047	0.0008
Ruminococcaceae	*UCG-005* ↓	0.0012	0.0011	0.0031	0.0045
Peptostreptococcaceae	*Romboutsia* ↓	0.0130	0.0035	0.0082	0.0043
Eggerthellaceae	*Raoultibacter* ↓	0.0001	0.0001	0.0002	0.0035
Clostridiaceae	*Intestinibacter* ↓	0.0027	0.0010	0.0035	0.0022
Family/genus	**Species**				
Bacteroidaceae/ Bacteroides	*Bacteroides_stercoris* ↑	0.0075	0.0388	0.0095	0.0001
Bacteroidaceae/ Bacteroides	*Bacteroides_coprocola* ↑	0.0136	0.0228	0.0013	0.0052
Bacteroidaceae/ Bacteroides	*Bacteroides_thetaiotaomicron* ↓	0.0025	0.0046	0.0122	0.0258

Relative abundances were expressed in %. 1 denotes 100%. RA, relative abundances; SGLT2i, sodium-glucose cotransporter-2 inhibitors.
↑ and ↓ denote an increase or decrease of bacterial abundance associated with SGLT2i.
[a]Dunn test (CKD+SGLT2i versus CKD).

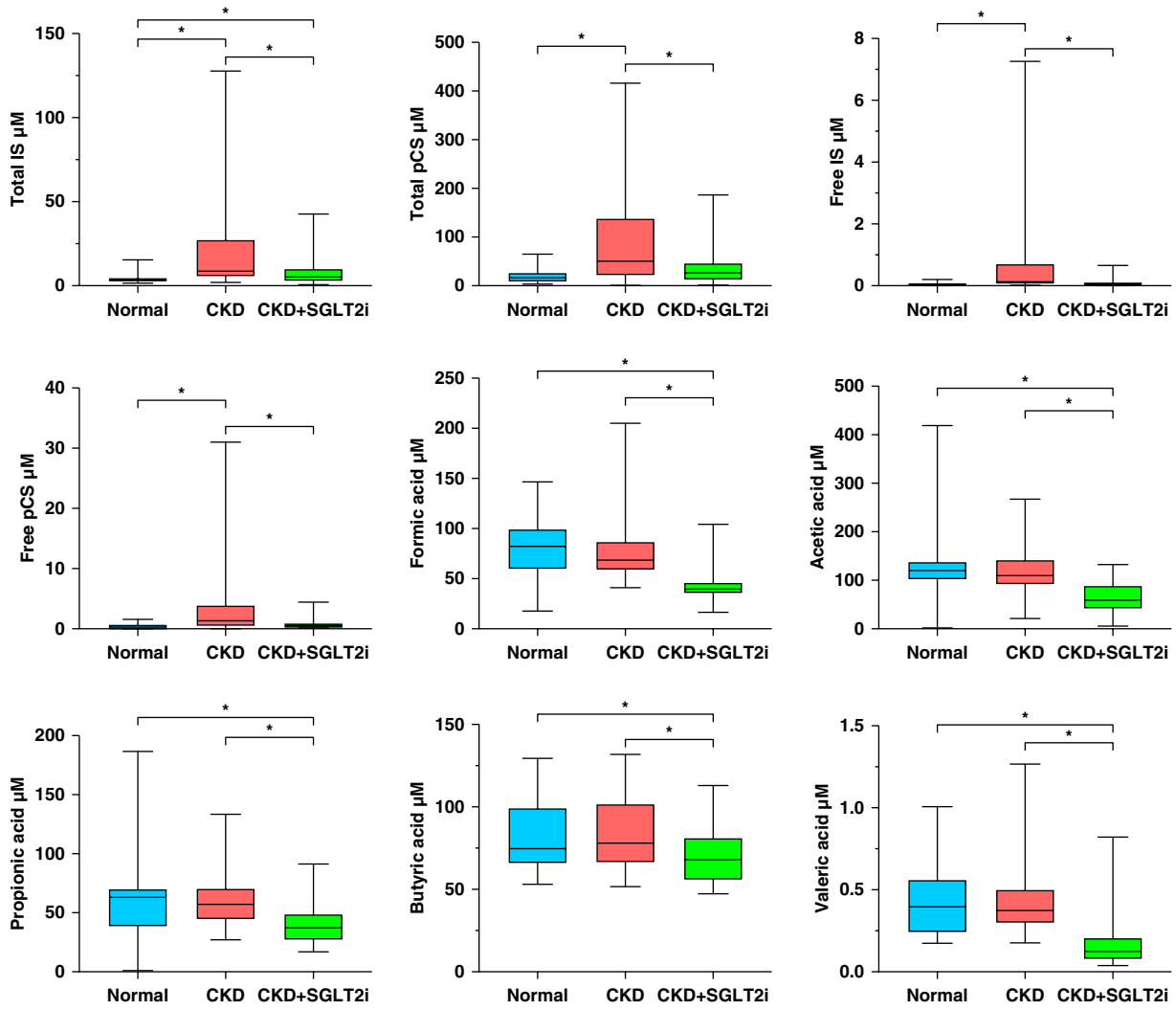

Figure 2. Altered circulating metabolite concentration associated with SGLT2i therapy in patients with CKD. Levels of metabolites among different groups were analyzed by the Wilcoxon rank-sum test. Box plot shows the median, the 25th, and the 75th percentile in each group. * $P < 0.05$. IS, indoxyl sulfate; pCS, p cresyl-sulfate.

dissimilarities in microbial communities indicated that patients with CKD receiving SGLT2i exhibited a less heterogeneous microbial community structure than the CKD group (analysis of similarity, $P < 0.009$) (Figure 1C).

Given the notable variations observed in the composition of the gut microbiome among the groups, we conducted a deeper investigation into the differences in the relative abundances (RA) of specific gut microorganisms at the genus and species level associated with SGLT2i therapy. Through using stringent criteria (abundance >0.1% and presence in >90% of samples), we identified six genera and three species exhibiting significant differences in RA among the three groups (Table 2). Specifically, in comparison with the CKD group, the CKD+SGLT2i group demonstrated elevated levels of *Escherichia-Shigella*, *Bacteroides_stercoris*, and *Bacteroides_coprocola*, along with reductions in the RA of *Clostridium_sensu_stricto_1*, *UCG-005*, *Romboutsia*, *Raoultibacter*, *Intestinibacter*, and *Bacteroides_thetaiotaomicron*. Furthermore, by taking both statistical significance and biologic consistency into consideration with LEfSe, we demonstrated that the CKD+SGLT2i group exhibited significant enrichments for *Bacteroides_stercoris* and *Bacteroides_coprocola* over the other groups (Figure 1D).

Differences in Targeted Metabolomics Profiles of Patients with CKD Receiving SGLT2i

Next, we conducted targeted metabolomic profiling of ten SCFAs (Supplemental Table 1) and two gut-producing protein bind uremic toxins to investigate the possible alterations in the levels of host-microbe–derived metabolites among patients with CKD receiving SGLT2i. We observed significant differences in the concentrations of free-form and total (free-form and protein-bound) IS and pCS among the three groups. The serum IS concentration was lower in the CKD+SGLT2i group than the CKD group (total IS, 1.126 versus 1.852 mg/L, $P = 0.011$; free IS, 0.013 versus 0.028 mg/L, $P = 0.004$). Similarly, the serum pCS concentration was decreased in the CKD+SGLT2i group than the CKD group (total pCS, 4.927 versus 9.513 mg/L, $P = 0.025$;

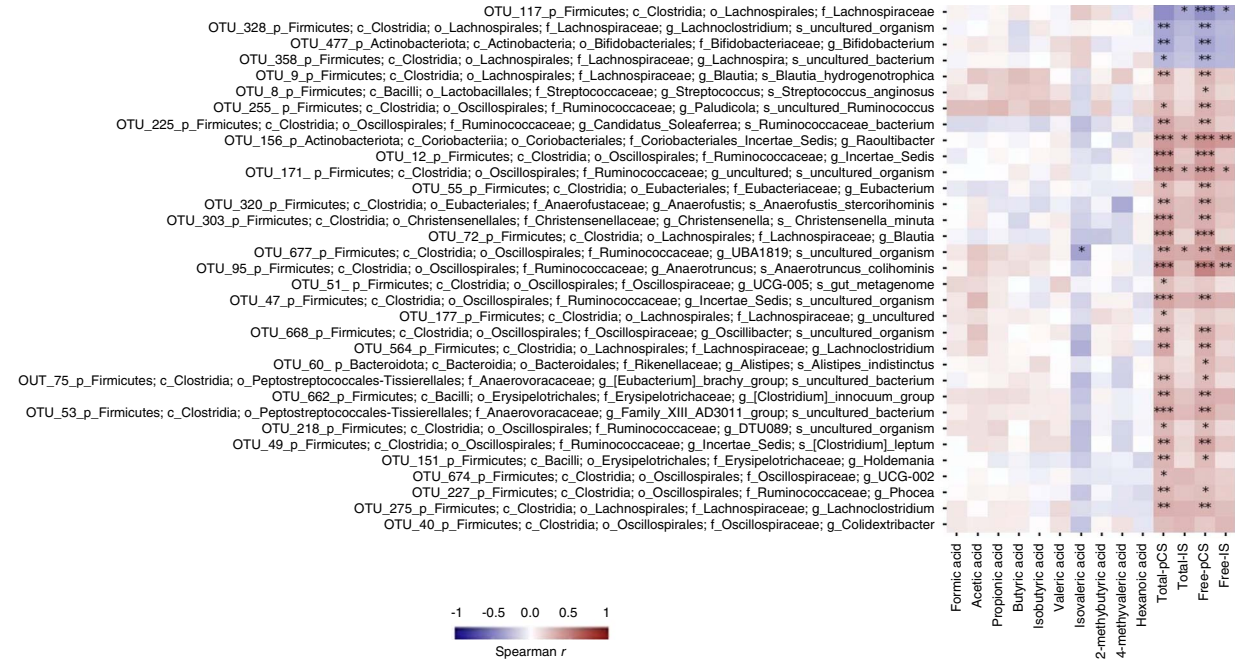

Figure 3. Potentially mechanistic associations of gut microbes with circulating metabolites. Spearman correlation of serum SCFAs and uremic toxins with gut microbes (*q<0.1, **q<0.05, ***q<0.01). Data are shown as bacterial taxa were detectable in at least 90% of samples. SCFA, short-chain fatty acid.

free pCS, 0.087 versus 0.251 mg/L, $P = 0.006$). In addition, significant differences were observed in the concentrations of five SCFAs (formic acid, acetic acid, propionic acid, valeric acid, and 2-methylbutanoic acid) among the three groups. The CKD+SGLT2i group displayed lower circulating levels of formic acid (39.515 versus 68.509 μM, $P < 0.001$), acetic acid (58.751 μM versus 109.826, $P < 0.001$), propionic acid (37.178 versus 57.052 μM, $P < 0.001$), valeric acid (0.124 versus 0.374 μM, $P < 0.001$), and 2-methylbutanoic acid (0.183 versus 0.238 μM, $P = 0.029$) compared with the CKD group (Figure 2).

Associations of Gut Microbial Taxa with Circulating Host-Microbe Cometabolites

To explore whether specific gut microorganisms are accountable for a mechanistic relationship relating the abundance of microbes and metabolites that are fluctuated during the progression and treatment of CKD, a large-scale association discovery of bacterial taxa with circulating SCFAs and uremic toxins was evaluated. We identified a set of intestinal microbes that is positively or negatively correlated with the levels of pCS (Figure 3), many of which consistently were selected as biomarkers by LEfSe (*e.g.*, *Anaerofustis stercorihominis* and *Anaerotruncus colihominis*), suggesting a potential involvement of such associations in CKD treatment. Although mostly statistically insignificant, the abundance of these pCS-associated intestinal microorganisms was correlated with the levels of IS in the same direction while associated with that of isovaleric acids in an opposite direction, implicating a connection of this SCFA with renal function. Of note, levels of *UBA1819* spp. were positively associated with the levels of pCS but anticorrelated with that of isovaleric acid. These data highlight a host-microbe-metabolite network in CKD intervention.

Functional Prediction of Gut Microbiota Associated with SGLT2i in Patients with CKD

To gain insight into the functional capability of fecal microbiota associated with SGLT2i, we used Tax4Fun to infer the functional profile of microbial communities.[24] In addition to the observed differences in key bacteria taxa, our analysis revealed differential enrichment of pathway modules relevant to protein and carbohydrate metabolism (digestion and absorption) between the CKD+SGLT2i and CKD groups (Figure 4). Intriguingly, we did not find differences in the microbial genes related to the metabolism of IS and pCS (including those involved in tyrosine, phenylalanine, and tryptophan metabolism) between CKD+SGLT2i and CKD groups.

Discussion

Our research findings reveal a significant correlation between the use of SGLT2i and altered gut microbiota composition in patients with CKD, suggesting a potential mechanism involving a reduction of uremic toxins, such as IS and pCS, through the modulation of protein digestion and absorption. In patients with CKD, high concentrations of urea and nitrogen waste products in the gut, together with greater bioavailability of amino acids and peptides related to malabsorption, facilitate outgrowth of proteolytic bacteria, which, in turn, leads to induction of uremic toxins.[25] Previous murine studies have suggested a possible role of SGLT2i in reducing plasma levels of IS and pCS by altering gut microbiota.[26,27] In agreement with these

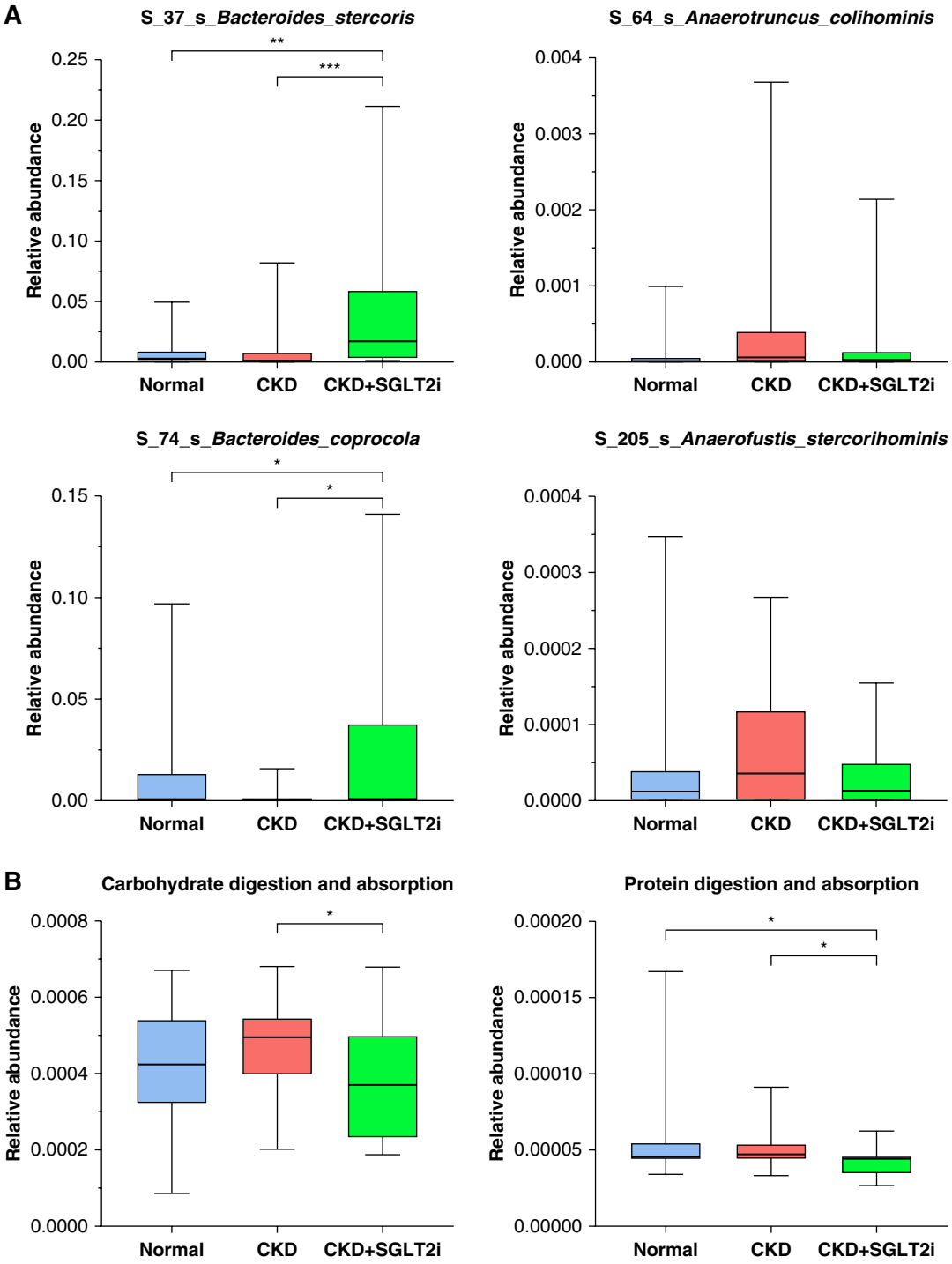

Figure 4. Functional adaptation of gut microbiota among group. (A). Relative abundance of key species. (B). Prediction of microbial gene pathway module among groups. Differences in RA were analyzed by the Wilcoxon rank-sum test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. RA, relative abundances.

findings from animal experiments, our observations from patients with CKD suggest that SGLT2i establish a favorable metabolic environment where protein malabsorption and overgrowth of proteolytic microbes are controlled. However, in contrast to other studies, we did not observe an increase in serum SCFA concentrations in patients with CKD receiving SGLT2i, indicating potential discrepancies in the effect of SGLT2i on altering gut microbiota from mice and humans.[27,28]

The nephroprotective mechanisms of SGLT2i are mainly attributable to the promotion of natriuresis, reduction in intraglomerular pressure, and mitigation of proximal

tubule glucotoxicity.[14,26,29] The role of SGLT2i played in modulating levels of uremic toxins in patients with CKD remains obscure. Our results suggest an additional mechanism wherein SGLT2i may provide renal protection in patients with CKD through attenuation of serum IS and pCS concentration, possible through decreased gut production secondary to the alterations in protein digestion and absorption of gut microbiota. Our findings were consistent with previous CKD murine research.[15–18,27,28,30–35] These studies consistently demonstrated alteration of microbiota architecture associated with the use of SGLT2i. Reduction of the *Bacillota/Bacteroidota* ratio (previously named *Firmicutes/Bacteroides* ratio) and the levels of *Oscillospira*, and *Oscillibacter*, and, enrichment of the abundance of *Bacteroides*, *Lactobacillaceae*, *Lachnospiraceae*, *Lactobacillus*, *Corynebacterium spp.*, and *Bifidobacterium spp* were reported.[15,18,30,32,33] An integrative omic study revealed that dapagliflozin in diabetic mice can induce a downregulation of apical transporters of the proximal tubules but also reduce phenylalanine and tryptophan fermenting bacteria, thereby reducing fecal and circulating indole, cresol, and phenol metabolites.[26]

Furthermore, SGLT2i have shown potential in restoring colonic tight junction integrity compromised due to renal dysfunction.[34] Clinically, discrepancies in the changes of microbiota were found in diabetic patients. van Bommel *et al.* did not find any changes in gut microbiota composition or α diversity of 44 patients randomized to receive either dapagliflozin or gliclazide treatment for 12 weeks.[36] Conversely, Deng *et al.* observed an elevation in the levels of SCFAs-producing bacteria, such as species from *Roseburia*, *Eubacterium*, and *Faecalibacterium*, and a reduction in harmful bacteria, including *Escherichia-Shigella*, *Bilophila*, and *Hungatella*, in 76 treatment-naïve patients with type 2 diabetes mellitus treated with empagliflozin or metformin.[37] Among these potential microbial markers, *Escherichia-Shigella* was recently identified as a risk factor for CKD,[38] although their levels were found to be elevated in patients with CKD with the treatment of SGLT2i. Kusunoki *et al.* reported a significant increase in the prevalence of *Ruminococci*, balance-regulating bacteria classified as SCFAs-producing bacteria, in 36 Japanese patients with type 2 diabetes mellitus treated with SGLT2i (luseogliflozin or dapagliflozin) for 3 months.[39] Wang *et al.* also found an increase in the relative abundance of SCFA-producing bacteria, particularly *Lachnospiraceae UCG 004*, *Bacteroides*, and *Lachnospiraceae NK4A136 group* in 21 treatment-naïve type 2 diabetes mellitus patients treated with canagliflozin.[40] Despite promising findings of gut microbiota modulation associated with SGLT2i, none of the abovementioned studies had addressed patients with CKD. We did not observe increases of SCFAs-producing bacteria in CKD patients receiving SGLT2i treatment. Because of significant gut dysbiosis and broad use of SGLT2i in the CKD population, further trials should be warranted to validate the findings of this study.

Using LEfSe analysis, we found a significant enrichment of *Bacteroides stercoris* in patients with CKD treated SGLT2i, as in type 2 diabetes mellitus patients treated with canagliflozin.[40] *Bacteroides stercoris* was positively correlated with the consumption of healthy food, such as fiber, grain products, and vegetables, but negatively correlated with diastolic BP.[41] This microbe exhibited antiobesity activity by restoring glucose sensitivity and reducing leptin and triglyceride levels.[42]

Taken together, the results of this study suggest that the administration of SGLT2i may ameliorate circulating protein-bound uremic toxins by decreasing pCS-producing bacteria. However, several limitations should be acknowledged. First, direct measurements of fecal or urine uremic toxins concentration, dietary protein intake, and residual renal function, as key determinants of uremic toxins production and clearance, were not available. Second, the medication history was incomplete. We did not consider the diet intake or use of phosphate binders and proton pump inhibitors as these may interfere the composition of gut microbiota. Moreover, the limited sample size impeded further subgroup analysis stratified by diabetes mellitus, and the differences observed in this pilot study were marginal as considering multiple testing. However, efforts were made to match common confounding factors to avoid bias. Comparable baseline renal function, nutrition, and electrolyte parameters minimized possible dietary imbalance between SGLT2i users and nonusers among patients with CKD. Oral carbonaceous adsorbents are often administered to patients with late-stage CKD and were less likely to be given to our study population. To the best of our knowledge, this is the first study investigating the effects of SGLT2i on gut microbiota and their functional adaptations in patients with CKD. Further prospective, longitudinal, randomized studies with extended intervention periods and comprehensive omic approaches may provide insights into the mechanisms underlying the therapeutic effects of SGLT2i on the synergies between intestinal host-microbiome metabolites in patients with CKD.

In conclusion, in this small-scale study, the administration of SGLT2i seemed to be associated with variations in gut microbiota composition and a reduction in serum IS and pCS levels. This effect was further supported by alterations in microbial gene function related to protein digestion and absorption in patients with CKD. Our findings not only connect a role of uremic toxins in modulating the gut homeostasis to CKD interventions with SGLT2i but also offer new therapeutic insights targeting the kidney-gut axis in renal patients.

Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at http://links.lww.com/KN9/B7.

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Data sharing Statement

All data are included in the manuscript and/or supporting information. Partial restrictions to the data and/or materials apply. All analytic data were incorporated into the article and the raw data underlying this article will be shared on reasonable request to the corresponding author.

Supplemental Material

This article contains the following supplemental material online at http://links.lww.com/KN9/B8.

Supplemental Table 1. Changes of serum SCFA and medium-chain fatty acid concentration associated with SGLT2i (mean±SD).

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