Alteration of the gut microbiota in Chinese population with chronic kidney disease

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We evaluated differences in the compositions of faecal microbiota between 52 end stage renal disease (ESRD) patients and 60 healthy controls in southern China using quantitative real-time polymerase chain reaction (qPCR) and high-throughput sequencing (16S ribosomal RNA V4-6 region) methods. The absolute quantification of total bacteria was significantly reduced in ESRD patients (p < 0.01). In three enterotypes, Prevotella was enriched in the healthy group whereas Bacteroides were prevalent in the ESRD group (LDA score > 4.5). 11 bacterial taxa were significantly overrepresented in samples from ESRD and 22 bacterial taxa were overrepresented in samples from healthy controls. The butyrate producing bacteria, Roseburia, Faecalibacterium, Clostridium, Coprococcus and Prevotella were reduced in the ESRD group (LDA values > 2.0). Canonical correspondence analysis (CCA) indicated that Cystatin C (CysC), creatinine and eGFR appeared to be the most important environmental parameters to influence the overall microbial communities. In qPCR analysis, The butyrate producing species Roseburia spp., Faecalibacterium prausnitzii, Prevotella and Universal bacteria, were negatively related to CRP and CysC. Total bacteria in faeces were reduced in patients with ESRD compared to that in healthy individuals. The enterotypes change from Prevotella to Bacteroides in ESRD patients. The gut microbiota was associated with the inflammatory state and renal function of chronic kidney disease.

The human gut is immensely populated with microorganisms, predominantly anaerobic bacteria. This internalized 'Microbial organ', which are not encoded in the host genome, consists of at least 10^13 citizens and 500–1,000 different species whose collective genomes are estimated to contain 100 times more genes than our own human genome1, 2. This microbial community forms a natural defense barrier and influences nutrition (vitamin K and vitamin B12 synthesis, Energy regulation)3, 4, physiology5, immune function (mucosal immune responses, inflammatory signaling through toll-like receptors, NF-kB, etc and adaptive Immunity through CD4 T-regulatory cell populations (Tregs))6, 7, and metabolism (short-chain fatty acids, bile acids, choline, indole, lipids and others)3, 4, 8. Various clinical and animal studies suggest that our gut microbial environment plays a critical role in both maintenance of health and disease pathogenesis, such as in obesity9, diabetes10, 11, non-alcoholic fatty liver disease (NAFLD)10, IBD12, cardiovascular disease13, cancers14, and refractory Clostridium difficile infection15.

Chronic kidney disease (CKD) is widespread, afflicting millions of people worldwide. In China, approximately 119.5 million adults have CKD, making it an important public health problem16, 17. CKD patients have enteric bacterial overgrowth and harbor. A greatly increased microbial flora comprising both anaerobes (10^7 bacteria/mL) and aerobes (10^6 bacteria/mL) in the duodenum and jejunum, which is quantitatively comparable to those in blind loop. However the composition of these does not differ significantly between the two groups18. An fecal analysis revealed a disturbed composition of microbiota characterized by an overgrowth of aerobic bacteria in hemodialysis patients19. Recent studies have revealed profound alterations of gut microbiota in patients and animals with CKD. Vaziri et al. demonstrated via 16S rRNA genePhyloChip analysis that uremia profoundly alters...
location and uremic toxicity as possible sources contributed to the chronic inflammation noted in uremia. Patients were diagnosed in accordance with this guideline by professional kidney internal medicine physicians.

T lymphocytes. CKD impairs the barrier function and alters microbial flora of the intestine. Bacterial translocation, lipopolysaccharides, indoxyl sulphate (IS), p-cresyl sulphate (PCS), amines, ammonia, and trimethylamine oxide contribute to the systemic inflammation, cardiovascular disease and numerous other CKD associated complications. IS and PCS were associated with elevated levels of selected inflammatory markers (serum IL-6, TNF-alpha and IFN-gamma) and an antioxidant in CKD patients and predict progression of CKD. Butyrate produced from microbial fermentation is important for energy metabolism and normal development of colonic epithelial cells, mainly has a protective role in relation to colonic disease, and appears to decrease the inflammatory response. Smith et al. found that short chain fatty acids (SCFAs) regulate the size and function of the colonic Treg pool, which play a major role in the pathogenesis of systemic inflammation, maintaining immunological self-tolerance, limiting the inflammatory response to foreign antigens and protecting against colitis. Butyrate regulates the differentiation of Treg cells. ESRD is compounded by the depletion and dysfunction of regulatory T lymphocytes. CKD impairs the barrier function and alters microbial flora of the intestine. Bacterial translocation and uremic toxicity as possible sources contributed to the chronic inflammation noted in uremia.

The aim of this study was to evaluate and quantify differences in the composition of gut microbiota in ESRD patients in southern China.

**Materials and Methods**

**Study subjects.** CKD definitions and classifications in this study are in accordance with the 2002 clinical practice guideline, end stage renal disease (ESRD) was defined as the estimated glomerular filtration rate (eGFR) less than 15 ml/min/1.73 m² for 3 months, irrespective of the presence or absence of kidney damage. All ESRD patients were diagnosed in accordance with this guideline by professional kidney internal medicine physicians. All methods, including the collection of blood and faecal samples, were performed in accordance with the relevant guidelines and regulations. All the people have signed the informed consent. The study was reviewed and approved by the Medical Ethics Committee of the Southern Medical University, Guangzhou, China. Fresh faecal samples collected in sterile containers from 52 ESRD patients and 60 healthy volunteers (controls) were used for quantitative PCR (qPCR), of these, samples from 27 ESRD patients and 26 healthy volunteers underwent Pyrosequencing. The underlying cause of 21 ESRD patients was chronic glomerulonephritis, 11 was hypertensive nephropathy, 6 was obstructive nephropathy, 3 was polycystic kidney disease, 2 was systemic lupus erythematosus, 2 was chronic pyelonephritis, 7 was unclear. Only two of the ESRD patients have received hemodialysis therapy through deep venous catheterization for once before the enrollment because of the acute hyperkalemia. The rest patients have never been treated with dialysis. All ESRD patients have never been treated with dialysis or without a regular dialysis. Exclusion criteria included treatment with antibiotics, probiotics/prebiotics and other laxatives in the 4 weeks preceding sample collection. We also excluded cholecystectomy, colectomy or intestinal disease and diabetes and hyperlipidemia from our data. Clinical datas of all the subjects were shown in Table 1.

| characteristics | ESRD (n = 52) | controls (n = 60) | p value |
|-----------------|--------------|------------------|---------|
| Age (years)     | 51.58 ± 18.33 | 52.53 ± 13.98    | 0.746   |
| sex, male (female) | 29 (23)      | 25 (35)          | 0.184   |
| BMI (kg/m²)     | 22.52 ± 2.74  | 21.64 ± 3.25     | 0.098   |
| CysC (mg/L)     | 6.74 ± 3.84   | 0.91 ± 0.14      | 0.000** |
| BUN (mmol/L)    | 26.65 ± 10.38 | 5.24 ± 1.54      | 0.000** |
| Scr (µmol/L)    | 654.36 ± 174.86 | 76.57 ± 26.89   | 0.000** |
| eGFR (ml/min/1.73 m²) | 6.86 ± 2.87 | 98.03 ± 27.32 | 0.000** |
| CRP (mg/L)      | 19.20 ± 40.64 | 1.93 ± 2.58      | 0.005** |
| LPS (EU/mL)     | 0.11 ± 0.05   | 0.08 ± 0.04      | 0.033*  |
| glucose (mmol/L) | 5.45 ± 1.46   | 4.78 ± 0.60      | 0.089   |
| TG (mmol/L)     | 1.71 ± 1.31   | 1.61 ± 1.21      | 0.669   |
| CHOL (mmol/L)   | 4.80 ± 1.49   | 4.61 ± 0.69      | 0.831   |
| VLDL (mmol/L)   | 0.79 ± 0.51   | 0.61 ± 0.45      | 0.134   |
| LDL (mmol/L)    | 2.63 ± 1.15   | 2.76 ± 0.59      | 0.749   |
| HDL (mmol/L)    | 1.38 ± 0.41   | 1.54 ± 0.41      | 0.194   |
| Lpa (mg/L)      | 0.44 ± 0.29   | 0.39 ± 0.21      | 0.066   |
| ApoE (mg/L)     | 43.17 ± 20.27 | 39.67 ± 27.00    | 0.697   |
| ApoA, B (mg/L)  | 1.33 ± 0.32   | 1.45 ± 0.43      | 0.146   |

Table 1. Clinical parameters among ESRD patients and healthy controls. In this experiment, patients with diabetes and hyperlipidemia were excluded. Abbreviations: CKD, Chronic kidney disease; BMI, body mass index; Scr, serum creatinine; CysC, Cystatin C; BUN, Blood Urea Nitrogen; eGFR, estimated glomerular filtration rate. CRP, C-reactive protein; LPS, Lipopolysaccharide; TG, Triglyceride; CHOL, Cholesterol; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high density lipoprotein; Lpa, lipoproteins a; ApoE, apolipoprotein E; ApoA, B, apolipoprotein A, B *p < 0.05, **p < 0.01, mean ± SD.
mined using enzymatic methods. (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) cholesterol levels were determined with the enzymatic assay. Plasma cholesterol (CHOL); triglycerides (TG); and high-density lipoprotein (HDL) were measured by immunoturbidimetric assays. Lipopolysaccharide (LPS) was detected with the chromogenic end-point Limulus amebocyte Lysate (LAL) assay. Proteinuria was determined by the immunoturbidimetric method to measure the creatinine. A modified kinetic Jaffé method was used to measure blood urea nitrogen (BUN). Glomerular filtration rate (eGFR) values. Cystatin C (CysC) and C-reactive protein (CRP) were measured using the CKD Epidemiology Collaboration (CKD-EPI) equation to measure estimated glomerular filtration rate (eGFR) values. Cystatin C (CysC) and C-reactive protein (CRP) were measured by immunoturbidimetric assays. Lipopolysaccharide (LPS) was detected with the chromogenic end-point Limulus Amoebocyte Lysate (LAL) assay. Plasma cholesterol (CHOL); triglycerides (TG); and high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) cholesterol levels were determined using enzymatic methods.

### Assessment of clinical parameters.
Fasting venous blood samples were collected in the morning, and centrifuged at 3000 g/min, at 4°C for 10 min. The recovered supernatants was separated in 200 μl tubes and immediately frozen at –80°C. We used the enzymatic method (isotope dilution mass spectrometry, IDMS reference method) to measure the creatinine. A modified kinetic Jaffé method was used to measure blood urea nitrogen (BUN) and the CKD Epidemiology Collaboration (CKD-EPI) equation was used to measure estimated glomerular filtration rate (eGFR) values. Cystatin C (CysC) and C-reactive protein (CRP) were measured by immunoturbidimetric assays. Lipopolysaccharide (LPS) was detected with the chromogenic end-point Limulus Amoebocyte Lysate (LAL) assay. Plasma cholesterol (CHOL); triglycerides (TG); and high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) cholesterol levels were determined using enzymatic methods.

### Sampling and DNA extraction.
Fresh stools were collected one day after enrollment and frozen at –80°C, patients who did not have a bowel movement were excluded. Two tubes were collected and filled at least 1/3. According to the TIANamp Stool DNA Kit (TIANGEN Biotech, Beijing, China) manufacturer steps to extract the fecal DNA. All DNA samples were stored at –80°C until further processing.

### Pyrosequencing and bioinformatics analysis.
Isolated fecal DNA was used as a template for amplification of the 16S rRNA V4-6 region using the universal primer V4F (5'-GTGCCAGCMGCGRGGTAA-3') and V6R (5'-ACAGCGATGTGCACT-3'). 20 μl reaction mixture: 10 μl TaKaRa Premix Taq, 2 μl template DNA, 0.5 μl 10 μM barcode forward primer, 0.5 μl 10 μM reverse primer, and 7 μl double-distilled H2O. The PCR cycle conditions: an initial denaturation at 94°C for 5 min, 25 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products were sequenced using Illumina GAII (Illumina, San Diego, CA, USA) at the Beijing Genomic Institute (Shenzhen, China). Sequencing results were clustered by Illumina paired barcoded - sequencing (end) (BIPE) process for preliminary analysis, the rest of the sequence were screened by UCHIME and removed the suspected chimeric sequence. All reads were sorted into different samples according to their barcodes. Then the two stage clustering (TSC) was used for clustering to extract the OUT in order to to distinguish the high abundance and low abundance sequences. Principal coordinates analysis (PCoA) based on UniFrac distance was performed with QiIME. The linear discriminant analysis (LDA) with effect size measurements (LEfSe) were used to identify indicator bacterial groups specialized within the two groups.

### Quantitative real-time PCR (qPCR).
The bacteria selected for qPCR are wellknown bacteria in gut. Escherichia coli (E. coli) belongs to Proteobacteria. Bacteroides fragilis group belong to Bacteroidetes. Bifidobacterium belong to Actinobacteria. Enterococcus spp., Lactobacillus group and Clostridium coccoides group belong to Firmicutes. Based on the sequencing data, Roseburia spp., Faecalibacterium prausnitzii and Prevotella which are typical butyrate producing bacteria were decreased in ESRD patients, so we chose it. All qPCR primer are listed in Table 2. 480 Real-Time PCR System (Roche Diagnostics, Basel, Switzerland). All assays were carried out in duplicate. The reaction mixtures consisted of 10 μl TaKaRa Premix Taq, 2 μl template DNA, 0.4 μl 10 μM barcode forward primer, 0.4 μl 10 μM reverse primer, and 7.2 μl double-distilled H2O. The copy number of target DNA was determined by serially diluting standards (10^1 to 10^7 copies of plasmid DNA containing the respective amplicon for each set of primers) running on the same plate. Bacterial quantity was expressed as log_{10} bacteria per gram of stool.

| Target Bacteria                        | Primer | Sequence (5 to 3)       | Annealing (°C) | Product | Reference |
|----------------------------------------|--------|-------------------------|----------------|---------|-----------|
| Universal bacteria                     | Univ-F | AGAGTTTGTATCAGGCTTCAAG  | 55             | 540     | 34        |
|                                        | Univ-R | ACCGGACTCTGCTGGGAC      |                |         |           |
| E. coli                                | E. col-F | GTTAAATCTTTGGCTCATTTGA  | 55             | 340     | 35        |
|                                        | E. col-R | ACCAGGGTATCTAAATCC      |                |         |           |
| Bacteroides fragilis group             | Bfra-F | ATAGCCCTTGGAAAGRAAGAT   | 50             | 501     | 36        |
|                                        | Bfra-R | CCGATATCAACTGCAATTTTA   |                |         |           |
| Enterococcus spp.                      | Ente-F | CCGTATGTTGAGTCGCCATT    | 61             | 144     | 37        |
|                                        | Ente-R | ACTCTGTTGATCTCCGATT     |                |         |           |
| Lactobacillus group                    | Lact-f | AGAGCTAGGGAACTTCACCA    | 58             | 341     | 37        |
|                                        | Lact-R | CACCGCTACACTCATGGAGG    |                |         |           |
| Bifidobacterium                        | Bifid-F | CTCCTGGGAAACGGGTG       | 55             | 549−563 | 36        |
|                                        | Bifid-R | GTGTTCTTGGCCGATACATCA   |                |         |           |
| Clostridium cocoides group             | Conc-F | AATGAGCGGTCTGACTAAG     | 50             | 438−441 | 36        |
|                                        | Conc-R | CTTTGAGTTTCATCTTGCGA    |                |         |           |
| Faecalibacterium prausnitzii           | Fae-F  | GGAGGAAGAGGAGTCCTCGG    | 60             | 248     | 38        |
|                                        | Fae-R  | AATCCGGCTACCTGCGACT     |                |         |           |
| Roseburia spp.                         | Ros-F  | GCCGTRCGCGGAAAGTCCTGA   | 60             | 81      | 38        |
|                                        | Ros-R  | CCTCGGACACTGTAGMCAG     |                |         |           |
| Prevotella                             | Pre-F  | GAAGGTCCCCCCAGATTG      | 103            | 60      | 38        |
|                                        | Pre-R  | CGCACKTTGGCTGGTTCAAG    |                |         |           |

Table 2. Primers used for qPCR in this study.
Statistical analysis. Enumeration data are tested by chi-square. Independent-samples T test was used to analyse the quantitative data. Spearman rank correlation were calculated to estimate the linear correlations between variables. Wilcoxon test, Kruskal-Wallis, LDA, Monte Carlo test CCA were used to analyse the sequencing data. Canonical correspondence analysis (CCA) was performed to measure physiological properties that have the most significant influence on microbial communities. Statistical analyses were performed with the statistical software package SPSS13.0 (SPSS Inc., Chicago, IL, USA). P < 0.05 was considered indicative of statistical significance.

Results

Patients and controls. CysC, BUN, and creatinine was significantly higher, and eGFR was reduced in ESRD patients compared to healthy controls. Levels of the plasma inflammatory biomarker CRP differed significantly between ESRD patients and controls (p = 0.005). LPS was increased in ESRD patients (p = 0.033). The ethnic background of all the participants were Han nationally Chinese. All the ESRD patients had been treated with phosphate binders, oral iron supplements or intravenous iron compounds, antihypertensive drugs. 12 of the ESRD patients had been treated with calcium supplements and Vitamin D. There were no significant differences in age, sex, body mass index (BMI), glucose, TG, CHOL, VLDL, LDL, HDL, Lpa, Lipoprotein a (Lpa), apolipoprotein E (ApoE), and apolipoprotein A, B (ApoA, B) (Table 1).

Diversity and phylum/subfamily -level taxonomic distribution of gut microbiota in ESRD patients. Diversity concerns both taxon richness and evenness, and our results demonstrated that the diversity was similar (p > 0.05) as assessed by chao1, observed_species, Shannon, simpson diversity indexes. PCoA based on the UniFrac metric did not reveal a separation trend of healthy controls and ESRD patients (Fig. 1).

Bacteroidetes was the most abundant phylum in both healthy individuals and CKD patients, accounting for 41.76%, 40.23% of the total valid reads respectively. Firmicutes was the second most abundant phylum in all samples with an average relative abundance of 41.43%, 38.01% respectively. The other dominant phyla were Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia and Others (Fig. 2A). Based on the average relative abundance, 21 genera were dominant (>1%) at the genus level. Bacteroides, Escherichia/Shigella, Subdoligranulum, Fusobacterium, etc were enriched in ESRD patients. Prevotella, Roseburia, Faecalibacterium, Clostridium, Coprococcus etc produced butyrate26, Dorea was the other predominant SCFA-producing genera 40. All of these species were reduced in ESRD patients, indicating that bacteria producing SCFAs especially butyrate were decreased in ESRD patients.

A reduction in SCFAs producing bacteria as a prominent feature of ESRD patients. LEfSe showed so much biomarkers for ESRD patients and controls subjects (Fig. 3) (LDA score > 2.0, p < 0.05). 11 species enriched in ESRD patients, and 22 in controls. According to Wong J, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, Bacteroidaceae with p-Cresol production enzymes enriched in ESRD patients. Desulfovibrionaceae, Bacteroidaceae, Alcaligenaceae, Pseudomonadaceae, and Pasteurellaceae produced urease. Bacteroidaceae relative abundance was higher, the others were much lower in ESRD patients than the controls group in this study. Microbes of the genus Prevotella, Roseburia, Faecalibacterium, Clostridium, Coprococcus can produce butyrate26, Dorea was the other predominant SCFA-producing genera 40. All of these species were reduced in ESRD patients, indicating that bacteria producing SCFAs especially butyrate were decreased in ESRD patients.
patients. Bacteroides (enterotype 1), Prevotella (enterotype 2) and Ruminococcus (enterotype 3) were three main enterotypes of human gut microbiota. In this study, from healthy people to ESRD patients, the enterotype changes from Prevotella (enterotype 2) to Bacteroides (enterotype 1).
Canonical correspondence analysis (CCA). Microbial community may be more correlated with indigenous environmental parameters. Analyzing the dynamic changes of microbial communities with geochemical factors will reveal the correlation between environmental parameters and microbial community. Therefore, CCA analysis was used to reveal how microbes can adapt to the changes of physiochemical environments. A correlation between the important environmental parameters and microbial community was discerned by CCA analysis as shown in Fig. 4. Sixteen environmental parameters and the dominant genera (>1%) in each sample were selected to determine their correlation. The length of an environmental parameter arrow indicated the strength of the environmental parameter to the overall microbial communities. As such, CysC ($r^2 = 0.1689$, $p = 0.020$), creatinine ($r^2 = 0.1593$, $p = 0.008$) and eGFR ($r^2 = 0.1255$, $p = 0.041$) concentrations appear to be the most important environmental parameters (Monte Carlo test). For instance, Enterobacter, Bacteroides, Fusobacterium, Escherichia and Klebsiella, which were positively correlated with CysC, creatinine (Scr) as shown in Fig. 4, and dominant in ESRD patients. Whereas Faecalibacterium, Akkermansia, Prevotella, Roseburia, Coprococcus and Clostridium were positively correlated with eGFR, and dominant in controls. Therefore, it is fair to propose that CKD played an active role in shaping the indigenous microbial communities.

Quantification of well known species in faeces by qPCR. qPCR was used to assess changes in bacterial absolute quantity in faecal samples from the two groups (Fig. 5). Bacterial copy number values were converted into logarithmic values before analysis. Quantities of total gene copies of Universal bacteria, *E. coli*, Bifidobacterium, Bacteroides fragilis group, Enterococcus spp., Clostridium cocoides group, Faecalibacterium prausnitzii, Roseburia spp. and Prevotella were significantly decreased in ESRD patients compared with controls ($p = 0.000$, $p = 0.001$, $p = 0.000$, $p = 0.000$, $p = 0.028$, $p = 0.000$, $p = 0.000$, respectively). However, the numbers of beneficial microorganisms from the Lactobacillus group were similar between two groups ($p = 0.395$). In ESRD patients, universal bacteria were decreased, and the butyrate producing species Clostridium cocoides group, Faecalibacterium prausnitzii, Roseburia spp. and Prevotella were also reduced, consistent with the sequencing results.

Butyrate producing gut microbes are negatively related to microinflammation and renal function via qPCR. Among these, the butyrate producing species Roseburia spp., Faecalibacterium prausnitzii, Prevotella and Universal bacteria, were negatively related to inflammation index CRP ($r = -0.452$, $p = 0.001$; $r = -0.431$, $p = 0.002$; $r = -0.480$, $p = 0.000$; and $r = -0.438$, $p = 0.000$; respectively) (Table 3). In addition, levels of Roseburia spp., Faecalibacterium prausnitzii, Clostridium cocoides group, Prevotella and Universal bacteria, were negatively correlated with CysC level sensitive index to evaluate glomerular filtration rate ($r = -0.414$,...
These findings strengthened and further proved the importance and necessity of butyrate producing bacteria in CKD patients. These data further clarified that the structural dynamics of the bacterial community in the intestinal tract played an important role in CKD progression, especially the beneficial effects on systemic inflammation or renal function of CKD patients. These data further clarified that the structural dynamics of the bacterial community in the intestinal tract played an important role in CKD progression, especially the beneficial effects on systemic inflammation or renal function of CKD patients.

Discussion
This report represents the first investigation of faecal microbiota diversity and quantity among Chinese CKD patients that employ high-throughput sequencing and qPCR analyses. We supplemented the intestinal bacteria data of CKD patients. In the analysis of sequencing data, we did not find any diversity differences between CKD patients and controls, which suggests that the diversity of the bacterial community was not destroyed critically, it was not like microbe-scarce scenario. Bacteroidetes (~40%), Firmicutes (~40%) and Proteobacteria (~10%) were the predominant phyla in both healthy individuals and CKD patients, consistent with reports from previous studies among cohorts from Western countries, Africa and Asia. Although Bacteroidetes and Firmicutes were the two most abundant phyla constituting the vast majority of gut microbiota in this study, an interesting variation among different human populations was observed.

Distribution of a number of genera could be differentiated between ESRD patients and controls. The SCFAs (propionate, acetate, and butyrate) are a by-product of the fermentation of non-absorbable complex carbohydrates. Firmicutes-Clostridiales-Lachnospiraceae-Dorea producing SCFAs were diminished in ESRD patients. Members of Prevotellaceae possess phosphotransbutyrylase and butyrate kinase, and Prevotella can produce SCFAs. In this study, both Prevotella and Prevotellaceae were reduced in ESRD patients. The human colonic butyrate (Short-chain fatty acids) producers are Gram-positive firmicutes, but are phylogenetically diverse. Clostridiales cluster the XIVa (Clostridium cocoides) including Ruminococcus, Coprococcus, Eubacterium hallii (E. hallii), Eubacterium rectale/Roseburia spp. and Clostridiales cluster IV (Clostridium leptum) including Faecalibacterium prausnitzii, and Eubacterium spp. are normally the two most abundant groups of human faecal bacteria that produce butyrate. Roseburia, Coprococcus, and Faecalibacterium belong to Firmicutes-Clostridiales. All of them are typically producing butyrate bacteria and were particularly and significantly more abundant in healthy controls and decreased in ESRD patients and consistent with previous studies. The qPCR analysis of Roseburia spp. and Faecalibacterium prausnitzii showed a similar trend in ESRD. Butyrate gets involved in the adjustment of body reaction to inflammation. Systemic inflammation in patients with end-stage renal disease (ESRD) is mediated by activation of the innate immune system. The presence of persistent inflammation magnifies the risk of poor outcome, and is a risk factor for cardiovascular disease (CVD), via mechanisms related to exacerbation of both wasting and vascular calcification processes and self-enhancement of the inflammatory cascade. High dietary total fiber intake is associated with lower risk of inflammation and mortality in kidney disease. Interestingly, CRP was increased in ESRD compared with that in controls. Spearman rank correlation analysis demonstrated that the absolute abundance of Roseburia spp., Faecalibacterium prausnitzii, Prevotella and Universal bacteria were negatively associated with CRP level and renal function indexes. These data indicate that bacteria producing butyrate as biomarkers may involve in the pathological process of CKD. Recently, Andrade-Oliveira V proved that SCFAs can reduce inflammation in acute kidney injury (AKI), which supports our inferences.
Reduced quantity of fecal microbiota were found in ESRD patients on qPCR analysis. This means that the absolute quantity of total faecal microbiota was decreased in CKD patients. In general, Universal bacteria, E. coli, Bifidobacterium, Bacteroides fragilis group, Enterococcus spp., Clostridium cocoides group, Faecalibacterium prausnizti, Roseburia spp. and Prevotella were decreased in ESRD. Bifidobacterium, Roseburia and Clostridium cocoides can produce SCFAs. Bacteroides fragilis and Clostridium spp. can protect against dextran sulfate sodium (DSS) or trinitrobenzenesulfonic acid-induced colitis[26,31]. This suggests that CKD status may influence the absolute quantity of the microbiome, which may result from accumulation of uremic toxins, inflammation and malnutrition and needs further investigation. This reduction in beneficial bacteria may play an important role in the pathogenic processes of CKD.

LPS is derived from the cell wall of gram negative bacteria, and the increase of the gamma Proteobacteria is also effective in increasing the LPS level in circulation. The degree of circulating endotoxemia might be related to the severity of systemic inflammation and features of atherosclerosis in peritoneal dialysis (PD) patients[24]. LPS may accelerate activation of neutrophils and macrophages/monocytes, which further explain the persistent inflammation of ESRD[55]. Although most CKD patients presented signs of fluid overload that was associated with endotoxaemia, there was no association between endotoxaemia and systemic inflammation, suggesting the endotoxaemia may not be the main determinant of the inflammatory status in CKD patients[56]. So the correlation between LPS and inflammation is unclear. In this study, LPS was elevated in ESRD patients, but we didn't find the correlation between LPS and bacterial amounts. To determine the characteristics of gut microbiota based on kidney function, we excluded the influences of body mass index (BMI), blood lipids, and blood glucose. No significant differences in blood lipid and blood glucose levels were found between CKD patients and controls consistent with that reported in previous study by McIntyre, C. W.[57]. Further research is needed in this area to provide more conclusive evidence while taking into account the relationships of gut flora with human diet, environment and habits.

Table 3. Correlation analysis of CRP, CysC, BUN, creatinine, eGFR values and the species count determined by qPCR. Abbreviations: CysC, Cystatin C; BUN, Blood Urea Nitrogen; eGFR, estimated glomerular filtration rate. Spearman rank correlation were used to evaluate statistical importance: r: correlation coefficient. *p < 0.05, **p < 0.01.
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Author Contributions
Shuanghong Jiang wrote the main manuscript text, Shuanghong Jiang, Shan Xie, Dan Lv, Pu Wang, Hanchang He, Ting Zhang, Youlian Zhou, and Qianyun Lin collected clinical datas and analysis. Hongwei Zhou, Jianping Jiang, Jing Nie, Fanfan Hou, and Ye Chen further summarized and concluded the whole subject. All authors reviewed the manuscript.

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