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ORIGINAL PAPER

# A reduction in the butyrate producing species *Roseburia* spp. and *Faecalibacterium prausnitzii* is associated with chronic kidney disease progression

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**Abstract** The human gut microbiota plays an important role in human health and might also be implicated in kidney disease. The interest in butyrate producing bacteria has recently increased and is a poorly understood faecal condition in chronic kidney disease (CKD). Therefore, we evaluated differences of the butyrate producing species *Roseburia* spp. and *Faecalibacterium prausnitzii* in the faeces of Chinese patients with CKD. A case–control study was carried out for 65 CKD patients and 20 healthy controls. Differences were quantitatively validated using quantitative real-time polymerase chain reaction (qPCR). Spearman rank correlation was used to analyse the correlation between gut microbiota and clinical variables. *Roseburia* spp. and *F. prausnitzii* were significantly different in CKD patients and controls ($p = 0.001$; $p = 0.025$, respectively) and reduced more markedly in end stage renal disease ($p = 0.000$; $p = 0.003$, respectively) and microinflammation ($p = 0.004$; $p = 0.001$, respectively). *Roseburia* spp. and *F. prausnitzii* were negatively associated with C-reactive protein in plasma ($r = -0.493$, $p = 0.00$; $r = -0.528$, $p = 0.000$; respectively) and Cystatin C ($r = -0.321$, $p = 0.006$; $r = -0.445$, $p = 0.000$; respectively). They were positively associated with eGFR ($r = 0.347$, $p = 0.002$; $r = 0.416$, $p = 0.000$; respectively). The negative correlation between *Roseburia* spp., *F. prausnitzii* and CRP and renal function suggested that the depletion of butyrate producing bacteria may contribute to CKD-associated inflammation and CKD progression. *Roseburia* spp. and *F. prausnitzii* may thus serve as 'microbiomarkers'.

**Keywords** Gut microbiota · Chronic kidney disease · Butyrate · C-reactive protein

## Introduction

Chronic kidney disease (CKD) has widespread prevalence, afflicting millions of people worldwide. In China, 119.5 million adults have CKD, making it an important public health problem (Nugent et al. 2011; Zhang et al. 2012). Chronic renal failure induced oxidative stress and systemic inflammation play a major role in progression of CKD and its numerous complications including cardiovascular disease, anemia, cachexia and others (Cachofeiro et al. 2008; Vaziri 2004). More attention has been

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paid to the relationship between the intestinal microbiota and microinflammation in CKD recently. Moreover some studies have suggested a pathogenic role of the gut microbiota in kidney disease (Anders et al. 2013). Bacterial structural components and metabolites have been identified as potential microbial by-products capable of initiating proinflammatory cytokine/chemokine cascades seen in the setting of CKD and end-stage renal disease (ESRD). Serum levels of IL-6, IL-1, and TNF-α were elevated in patients with renal failure (Herbelin et al. 1991; Pereira et al. 1994). Circulating endotoxin derived from intestinal bacteria was associated with systemic inflammation, cardiac injury and reduced survival (McIntyre et al. 2011). Bacterial translocation occurs in ESRD patients and was associated with microinflammation (Wang et al. 2012), which was more severe in hemodialysis patients (Shi et al. 2014). Recent studies have demonstrated marked disintegration of the colonic epithelial barrier structure and significant alteration of the colonic bacterial flora in humans and animals with advanced CKD, suggesting a major role in the pathogenesis of inflammation and uremic toxicity accelerated cardiovascular disease and numerous other CKD-associated complications (Mafra et al. 2014; Vaziri 2012). Accumulation of gut derived uremic toxins (such as lipopolysaccharides, indoxyl sulfate, p-cresyl sulfate, ammonia, amines and trimethylamine oxide) contribute to the systemic inflammation, cardiovascular disease and numerous other CKD associated complications (Mafra et al. 2014; Sabatino et al. 2015; Wang et al. 2011). Indoxyl sulfate and p-cresyl sulfate were related to elevated levels of selected inflammatory markers (serum IL-6, TNF-alpha and IFN-gamma) in CKD patients (Rossi et al. 2014) and predict progression of CKD (Wu et al. 2011). Butyrate produced from microbial fermentation has a protective role in colonic disease, and appears to decrease the inflammatory response (Pryde et al. 2002; Rose et al. 2007). Smith et al. found that short chain fatty acids (SCFAs) regulate the size and function of the colonic Treg pool and the differentiation of Treg cells, which plays a major role in limiting the inflammatory response to foreign antigens and protecting against colitis (Furusawa et al. 2013; Smith et al. 2013). ESRD is compounded by the depletion and dysfunction of regulatory T lymphocytes and microinflammation (Hendrikx et al. 2009).

CKD results in a marked alteration of the gut microbiota. The patients have a greatly increased bacteria load, comprising both anaerobes ($10^7$ bacteria/mL) and aerobes ($10^6$ bacteria/mL) in the duodenum and jejunum (Simenhoff et al. 1978). A faecal analysis revealed an overgrowth of aerobic bacteria in hemodialysis patients (Hida et al. 1996). Recently, Vaziri et al. demonstrated using 16S rRNA genePhyloChip analysis that uremia profoundly alters the intestinal microbiota (Vaziri et al. 2013) and the expansion of indoxyl sulfate, p-cresol sulfate, and urea-derived ammonia, and contraction of beneficial SCFA may contribute to uremic toxicity and inflammation (Wong et al. 2014). The typical butyrate producing bacteria *Roseburia* spp. and *Faecalibacterium prausnitzii* were reduced in inflammatory bowel disease contributing to the pathogenesis of ulcerative colitis and Crohn's disease (Cao et al. 2014; Machiels et al. 2014; Takahashi et al. 2016). A reduction of *F. prausnitzii* on resected ileal Crohn's mucosa was associated with endoscopic recurrence at 6 months, and live *F. prausnitzii* or its supernatant (a microbial anti-inflammatory molecule markedly reduced the severity of trinitrobenzenesulfonic acid-induced colitis, suggesting potential for use as new probiotics (Quevrain et al. 2016; Sokol et al. 2008). Understanding of the distribution of *Roseburia* spp. and *F. prausnitzii* in CKD is limited, and so the aim of this study was to explore and quantify differences in these species in CKD patients from southern China.

## Materials and methods

### Study subjects

CKD definitions and classifications used in this study were in accordance with the 2012 clinical practice guideline (Stevens and Levin 2013). Fresh faecal samples collected in sterile containers from 65 CKD patients (CKD1: 15; CKD2:10; CKD3:10; CKD4: 10; and CKD5 [ESRD]: 20) and 20 healthy volunteers (controls) were used for quantitative PCR (qPCR). All ESRD inpatients had never been treated with dialysis or were without a regular dialysis. Exclusion criteria included treatment with antibiotics, probiotics/prebiotics and other laxatives in the 4 weeks preceding sample collection, and excluded diabetic and hyperlipidemia patients.

**Table 1** Clinical parameters among CKD and Healthy controls

| Characteristics | CKD (n = 65) | Controls (n = 20) | *p* value |
|---|---|---|---|
| Age (years) | 43.45 ± 16.90 | 43.05 ± 9.88 | 0.921 |
| Sex, male (female) | 30 (35) | 6 (14) | 0.301 |
| Body mass index (BMI) | 21.94 ± 2.96 | 21.16 ± 2.85 | 0.296 |
| CRP (mg/L)** | 8.91 ± 17.38 | 0.84 ± 0.81 | 0.002 |
| Glucose (mmol/L) | 5.78 ± 3.17 | 4.85 ± 0.35 | 0.686 |
| TG (mmol/L) | 1.71 ± 1.39 | 1.79 ± 2.09 | 0.910 |
| CHOL (mmol/L) | 4.99 ± 1.71 | 4.68 ± 0.77 | 0.694 |
| CysC (mg/L)** | 3.19 ± 2.66 | 0.91 ± 0.09 | 0.000 |
| BUN (mmol/L)** | 14.31 ± 12.51 | 4.72 ± 1.39 | 0.000 |
| Creatinine (μmol/L)** | 201.81 ± 216.55 | 74.60 ± 11.59 | 0.000 |
| eGFR (ml/min/1.73 $m^2$)** | 55.61 ± 52.55 | 104.99 ± 19.82 | 0.000 |

In this experiment, patients with diabetes and hyperlipidemia were excluded

*CKD* chronic kidney disease, CRP C-reactive protein, TG triglyceride, CHOL cholesterol, CysC Cystatin C, *BUN* blood urea nitrogen, *eGFR* estimated glomerular filtration rate

* $p < 0.05$, ** $p < 0.01$

## Assessment of clinical parameters

One tube fasting venous blood samples (2–5 mL) were collected. Samples were centrifuged for 10 min, at 3000 g/min, at 4 °C. The supernatant was collected, packed in 200 μl EP tubes and frozen at −80 °C. A modified kinetic Jaffé method was used to measure serum creatinine, and blood urea nitrogen (BUN). 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. Plasma cholesterol and triglycerides levels were determined using enzymatic methods. Anthropometric and biochemical variables of the control group and CKD patients are shown in Table 1.

## Sampling and DNA extraction

Faecal samples were collected 1 day after admission and patients who did not have a bowel movement were excluded. Immediately after collection, faecal samples were stored at −80 °C until they were analysed. DNA extraction from stools was performed with TIANamp Stool DNA Kits (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. All DNA samples were stored at −80 °C until further processing.

## Quantitative real-time PCR (qPCR)

All qPCR primer sets that were used are listed in Table 2 (Hedin et al. 2014). qPCR assays were performed in 96-well optical plates on a LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Basel, Switzerland). The reaction mixtures contained: 10 μl SYBR Premix Ex Taq (Tli RNaseH Plus) (2 × Conc.) [TaKaRa Ex Taq HS, dNTP Mixture, $Mg^{2+}$, Tli RNaseH, SYBR Green I], 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 $H_2O$. The PCR cycle conditions were: an initial denaturation at 95 °C for 5 min, 25 cycles of 95 °C for 30 s, annealing for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. The copy number of the target DNA was determined by comparison with serially diluted standards ($10^1$–$10^7$ copies of plasmid DNA containing the respective amplicon for each set of primers) run on the same plate. Bacterial quantity was expressed as $\log_{10}$ bacteria per gram of stool. The gel-based PCR assay was conducted as follows: Premix Taq™(TaKaRa Taq™ Version 2.0) 25 μl, template DNA < 500 ng, Primer F 0.2–1.0 μM (final conc.), Primer R 0.2–1.0 μM (final conc.), double-distilled $H_2O$ up to 50 μl. The PCR conditions were consistent with those for qPCR. The amplified products were loaded in an agarose gel (2.0 %) containing 0.5 g/ml ethidium bromide and the

**Table 2** Primers used for qPCR in this study

| Target bacteria | Primer | Sequence (5′–3′) | Annealing temperature (°C) | Product size (bp) | Reference |
|---|---|---|---|---|---|
| *Roseburia* spp. | Ros-F | GCGGTRCGGCAAGTCTGA | 60 | 81 | (Hedin et al. (2014) |
| | Ros-R | CCTCCGACACTCTAGTMCGAC | | | |
| *Faecalibacterium prausnitzii* | Fae-F | GGAGGAAGAAGGTCTTCGG | 60 | 248 | (Hedin et al. (2014) |
| | Fae-R | AATTCCGCCTACCTCTGCACT | | | |

electrophoresis was conducted in TAE buffer. After the electrophoresis the DNA bands were visualised by UV transillumination.

### Statistical analysis

The gender composition was tested by Chi-square. One-Way ANOVA followed by LSD methods was applied for comparison of continuous variables. Spearman rank correlations were calculated to estimate the linear correlations between variables. 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

Compared with healthy controls (Table 1), CysC, BUN and creatinine of CKD patients was significantly higher, and eGFR was reduced ($p<0.001$). Levels of the plasma inflammatory biomarker CRP differed significantly among CKD patients and controls ($p = 0.002$). There was no significant differences in age, sex, body mass index (BMI), triglyceride, cholesterol and glucose levels ($p>0.05$). To determine the characteristics of the gut microbiota based on kidney function, we excluded the influences of BMI, blood lipids, and blood glucose.

### Quantification of butyrate producing species in faeces by qPCR

qPCR was used to assess changes in bacterial population abundance in faecal samples. *Roseburia* spp. and *F. prausnitzii* were significantly different in CKD patients and controls ($F = 4.743$, $p = 0.001$; $F = 2.735$, $p = 0.025$). *Roseburia* spp. were significantly reduced in CKD1, CKD2, CKD3, CKD4 and CKD5 category patients compared with controls ($p = 0.034$, $p = 0.036$, $p = 0.048$, $p = 0.003$, $p = 0.000$, respectively) and *Roseburia* spp. in CKD5 patients were reduced compared to CKD1 (mean 5.80 vs. 7.56, $p = 0.023$). *F. prausnitzii* levels in CKD5 patients was reduced compared to controls (mean 5.20 vs. 7.27, $p = 0.003$), CKD1 (mean 5.20 vs. 7.33, $p = 0.004$) and CKD2 (mean 5.20 vs. 6.97, $p = 0.032$). *Roseburia* spp. and *F. prausnitzii* levels were found to gradually decrease with the progression of renal disease (Fig. 1). All patients were divided into two groups: those with CRP greater than or equal to 5 mg/L and less than 5 mg/L. We found that *Roseburia* spp. (mean 5.56 vs. 7.63, $p = 0.004$) and *F. prausnitzii*(mean 4.78 vs. 6.84, $p = 0.001$) were decreased in the CRP $\geq$5 mg/L group compared to CRP<5 mg/L (Fig. 2).

### Butyrate producing species are negatively related to microinflammation and renal function of CKD patients

*Roseburia* spp. and *F. prausnitzii* levels were negatively related to CRP ($r = -0.493$, $p = 0.00$; $r = -0.528$, $p = 0.000$) (Fig. 3). These findings strengthen and further support the importance and necessity of bacteria producing butyrate in modulating inflammation in CKD patients. In addition, levels of *Roseburia* spp. and *F. prausnitzii* were negatively correlated with CysC levels ($r = -0.321$, $p = 0.006$; $r = -0.445$, $p = 0.000$; respectively); the inverse tendency was observed with regard to eGFR ($r = 0.347$, $p = 0.002$; $r = 0.416$, $p = 0.000$; respectively). This suggested that a reduction in the butyrate producing species *Roseburia* spp. and *F. prausnitzii* were involved in CKD progression. Levels of these

bacteria were not related to triglyceride, cholesterol, BUN and creatinine levels ($|r| < 0.3$) (Table 3).

## Discussion

The microbial community has a beneficial role during normal homeostasis, immune system function, physiology, organ development and morphogenesis, and host metabolism. The interactions between the host and the microbiota are key requirements for host health. Many things can affect the gut microbiota, including urea, uric acid, oxalate, diet, phosphate binding products, antibiotics, etc.(Sommer and Backhed 2013; Vaziri et al. 2015). We thus excluded patients treated with antibiotics, probiotics/prebiotics and other laxatives in the 4 weeks preceding sample collection, and excluded diabetic and hyperlipidemia patients. To determine the characteristics of the gut microbiota based on kidney function, we excluded the influences of BMI, blood lipids, blood glucose and medicine. No significant differences in blood lipid and blood glucose levels were found between CKD patients and controls consistent with a previous study by McIntyre et al. (2011).

SCFAs (propionate, acetate, and butyrate) are a by-product of the fermentation of non-absorbable complex carbohydrates, reaching concentrations of 50–130 mM in the proximal colon, the formation of these SCFAs by saccharolytic microbes is complex (Cummings et al. 1987). Cultural and molecular studies have indicated that the main butyrate-producing bacteria found in human faeces are phylogenetically diverse. *Ruminococcus, Coprococcus, Eubacterium hallii* and *Eubacterium rectale/Roseburia* spp. belong to *Clostridiales cluster XIVa* (*Clostridium coccoides*); *F. prausnitzii,* and

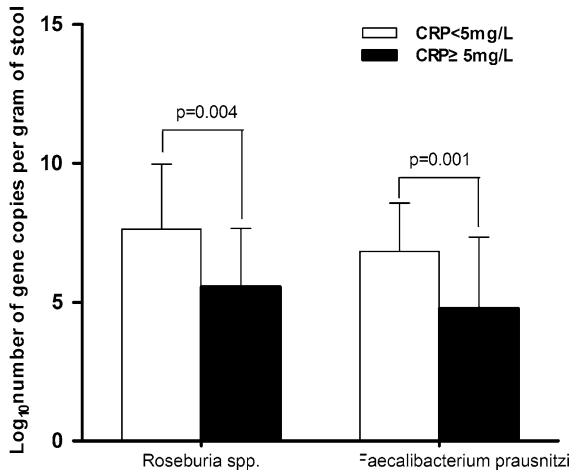


**Fig. 2** Butyrate producing species *Roseburia* spp. and *Faecalibacterium prausnitzii* were significantly decreased in the microinflammatory state of CKD. The difference of species among all samples between two levels of CRP (CRP <5 mg/L and CRP ≥5 mg/L)

*Eubacterium* spp. belong to the *Clostridiales cluster IV (Clostridium leptum). Clostridiales cluster XIVa* and *Clostridiales cluster IV* are normally the two most abundant groups of human faecal bacteria that produce butyrate (Kumari et al. 2013; Louis and Flint 2009; Pryde et al. 2002). Butyrate is involved in the differentiation of Treg cells in vitro and in vivo, and ameliorated the development of colitis (Furusawa et al. 2013). ESRD is simultaneously associated with upregulation of ROS production, reduced CD4/CD8 T cell ratio, depletion of naïve, regulatory and central memory T cells (Vaziri et al. 2012). The presence of persistent inflammation magnifies the risk of poor outcome, via mechanisms related to exacerbation of both wasting and vascular calcification processes and self-enhancement of the inflammatory cascade (Carrero and Stenvinkel 2009). High dietary total fiber

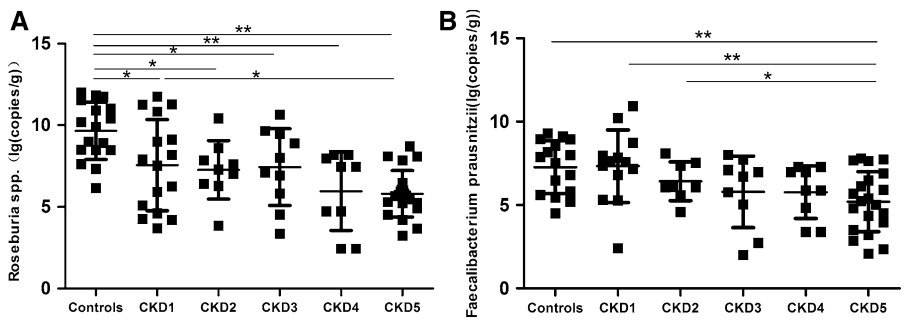


**Fig. 1** Quantification of *Roseburia* spp. and *Faecalibacterium prausnitzii* in controls and different stages of CKD by qPCR expressed as $\log_{10}$ bacteria per gram of stool. $*p < 0.05$, $**p < 0.01$

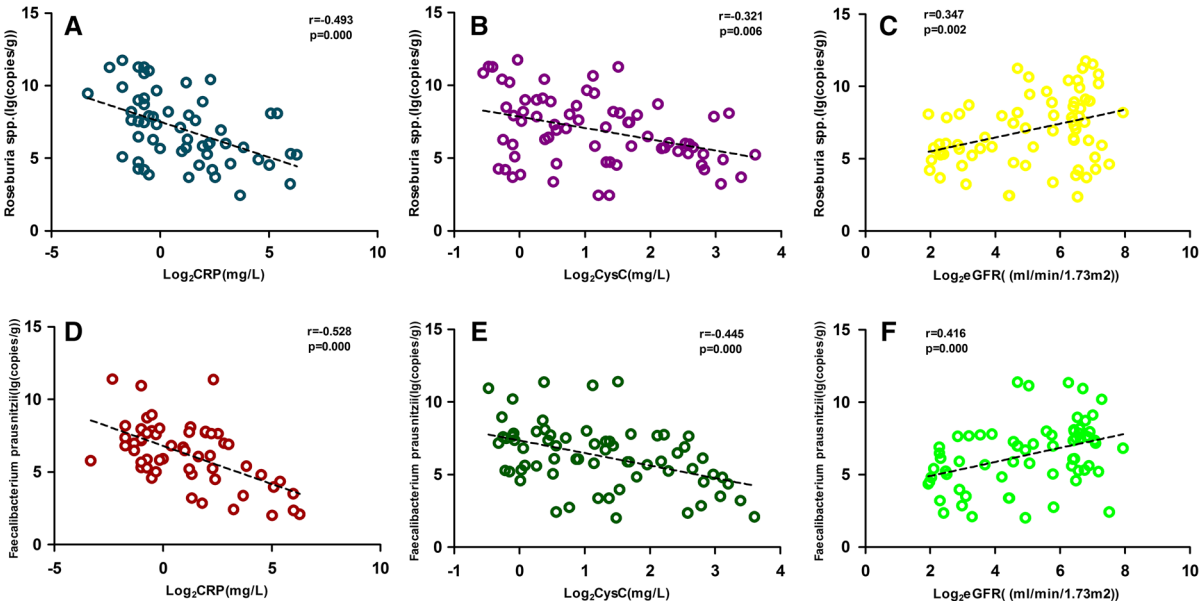


**Fig. 3** Correlation analysis of the butyrate producing bacteria and CRP, CysC and eGFR. **a–c** Correlation between CRP, CysC, eGFR and count of *Roseburia* spp. **d–f** Correlation between CRP, CysC, eGFR and count of *F. prausnitzii* in faecal samples determined by qPCR

**Table 3** Correlation analysis of CRP, CysC, BUN, creatinine, eGFR values and the species count determined by qPCR

| | CRP | CysC | BUN | Creatinine | eGFR |
|---|---|---|---|---|---|
| *Roseburia* spp. | | | | | |
| r | −0.493** | −0.321** | −0.279* | −0.094 | 0.347** |
| *p* | 0.000 | 0.006 | 0.016 | 0.426 | 0.002 |
| *Faecalibacterium prausnitzii* | | | | | |
| r | −0.528** | −0.445** | −0.279* | −0.048 | 0.416** |
| *p* | 0.000 | 0.000 | 0.019 | 0.692 | 0.000 |

Spearman rank correlation were used to evaluate statistical importance: *r* correlation coefficient

* $p < 0.05$, ** $p < 0.01$

intake is associated with decreased inflammation and mortality in kidney disease, and fiber is the source of SCFAs (Krishnamurthy et al. 2012). Interestingly, as compared with the CRP <5 mg/L group, *Roseburia* spp. and *F. prausnitzii* decreased in the CRP ≥5 mg/L group. Spearman rank correlation analysis demonstrated that the absolute abundance of *Roseburia* spp. and *F. prausnitzii* was negatively associated with CRP levels. These data indicate that bacteria producing butyrate are beneficial for inflammatory conditions in CKD patients. Recently, Andrade-Oliveira et al. (2015) demonstrated that SCFAs can reduce inflammation in acute kidney injury. A 15 kDa protein with anti-inflammatory properties is produced by *F. prausnitzii*, a commensal bacterium involved in Crohn's disease pathogenesis (Quevrain et al. 2016). These studies, in conjunction with our results, confirm that the reduction in the abundance of butyrate-forming species may contribute to the pathogenesis of inflammation in CKD patients. In this study, *Roseburia* spp. and *F. prausnitzii* were found to be particularly and significantly more abundant in healthy controls than CKD patients. The qPCR analysis of *Roseburia* spp. and *F. prausnitzii* showed a trend of gradually decreasing from CKD1 to ESRD. Reduced abundance of *Roseburia* spp. and *F. prausnitzii* were found in both early stage and end-stage patients by qPCR analysis, and the reduction tended to be more pronounced in patients with advanced kidney function deterioration. *Roseburia* spp. and *F.prausnitzii* were negatively associated with CysC levels and positively associated with eGFR. We suggest that in subjects with CKD, *Roseburia* spp. and *F.prausnitzii* not only contribute to the micro-inflammatory state but also CKD progression.

To conclude, the depletion of the butyrate producing bacteria *Roseburia* spp., *F. prausnitzii* likely contributes to CKD-associated inflammation and

CKD progression, which may thus serve as 'microbiomarkers' for CKD. This reduction in butyrate producing bacteria may play an impotant role in the pathogenic processes of CKD. Similarly in inflammatory bowel disease, *Faecalibacterium* and *Papillibacter* display consistent patterns with respect to disease status rather than geographical patterns and may thus serve as reliable 'microbiomarkers' (Rehman et al. 2016). To our knowledge, this report represents the first investigation of the levels of key faecal bacteria in different stages of CKD. It will be of interest to extend these findings with studies of the full microbial communities associated with CKD.

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**Compliance with ethical standards**

**Ethical approval** The study was reviewed and approved by the Medical Ethics Committee of the Southern Medical University, Guangzhou, China. The study was conducted according to the principles of the Declaration of Helsinki.

**Competing interests** The authors declare that they have no competing interests.

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