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# Washed microbiota transplantation improves renal function in patients with renal dysfunction: a retrospective cohort study

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## Abstract

**Background** Changes in the gut microbiota composition is a hallmark of chronic kidney disease (CKD), and interventions targeting the gut microbiota present a potent approach for CKD treatment. This study aimed to evaluate the efficacy and safety of washed microbiota transplantation (WMT), a modified faecal microbiota transplantation method, on the renal activity of patients with renal dysfunction.

**Methods** A comparative analysis of gut microbiota profiles was conducted in patients with renal dysfunction and healthy controls. Furthermore, the efficacy of WMT on renal parameters in patients with renal dysfunction was evaluated, and the changes in gut microbiota and urinary metabolites after WMT treatment were analysed.

**Results** Principal coordinate analysis revealed a significant difference in microbial community structure between patients with renal dysfunction and healthy controls ($P = 0.01$). Patients with renal dysfunction who underwent WMT exhibited significant improvement in serum creatinine, estimated glomerular filtration rate, and blood urea nitrogen (all $P < 0.05$) compared with those who did not undergo WMT. The incidence of adverse events associated with WMT treatment was low (2.91%). After WMT, the Shannon index of gut microbiota and the abundance of several probiotic bacteria significantly increased in patients with renal dysfunction, aligning their gut microbiome profiles more closely with those of healthy donors (all $P < 0.05$). Additionally, the urine of patients after WMT demonstrated relatively higher levels of three toxic metabolites, namely hippuric acid, cinnamoylglycine, and indole (all $P < 0.05$).

**Conclusions** WMT is a safe and effective method for improving renal function in patients with renal dysfunction by modulating the gut microbiota and promoting toxic metabolite excretion.

**Keywords** Faecal microbiota transplantation, Chronic kidney disease, Microbiome analysis, Metabolomics analysis, Gut microbiota, Renal insufficiency

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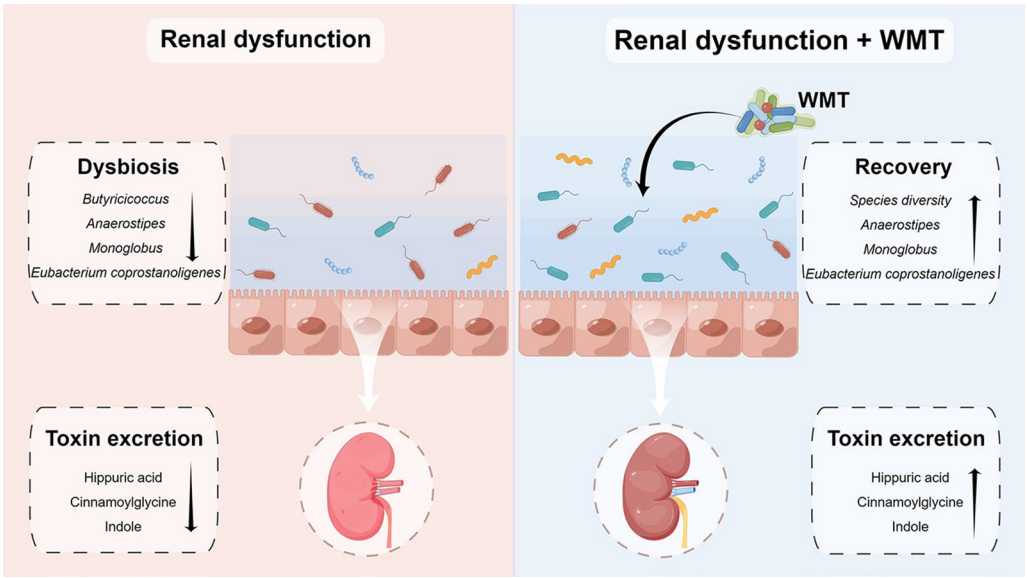
**Graphical Abstract**




## Background

Chronic kidney disease (CKD), affecting approximately 10% of the global population [1, 2], is expected to become the fifth leading cause of death by 2040 [3]. CKD results in a progressive decline in kidney function culminating in end-stage renal disease (ESRD), requiring renal replacement therapy (RRT) for patient survival. The current count of over 2.5 million patients with CKD undergoing RRT is predicted to double, reaching 5.4 million by 2030 [4]. Regrettably, existing therapies offer limited efficacy and only slow disease progression [5]. Consequently, an urgent imperative exists to develop novel approaches that can arrest or reverse the decline in renal function.

Accumulating evidence underscores the involvement of gut microbiota in kidney disease pathophysiology, and a conjectured gut-kidney axis has been proposed [6, 7]. Profound disparities in gut microbiome composition between patients with CKD and healthy controls have been documented [8, 9]. Additionally, animal studies have demonstrated that probiotics, as gut microbiota modulators, can significantly improve renal function in CKD mice [10, 11]. However, in human patients with CKD, probiotics can only delay the decline in renal function rather than effect a cure or reversal [10, 12]. Given the complexity of bacteria-host interactions, a single-species microbiota-targeted intervention might prove insufficient to improve the outcomes of all patients with CKD [13].

Faecal microbiota transplantation (FMT), involving the transfer of multispecies gut microbiota from a healthy donor to a recipient, has proven effective in treating conditions such as *Clostridioides difficile* infection, inflammatory bowel disease, and metabolic disorders [14]. Recent clinical studies have shown FMT's potential benefits for hypertension, systemic lupus erythematosus, and hyperuricaemia [15–17], which were considered causative factors of CKD. Furthermore, CKD mice treated with healthy-donor gut microbiota exhibited less severe kidney histopathology and lower serum creatinine (SCr) levels compared with those treated with gut microbiota from patients with ESRD [18]. While individual case reports exist [19], no cohort study has addressed whether FMT can improve renal function in patients with renal dysfunction.

Challenges including intricate sample preparation and the high incidence of adverse events (AEs) restrict FMT's application [20]. Washed microbiota transplantation (WMT), using an automated purification system distinct from traditional FMT, significantly reduces AEs [21]. This study evaluated WMT's efficacy and safety in improving renal activity among patients with renal dysfunction.

## Methods

### Study design and patients

This retrospective, single-centre, cohort study adhered to the Declaration of Helsinki and obtained approval from the Ethics Committee of the First Affiliated Hospital of

Guangdong Pharmaceutical University (approval number: 2021–123). Written informed consent was obtained from all patients, except in cases where a legal representative consented on behalf of those unable to do so.

The study encompassed consecutive adult inpatients (≥ 18 years of age) who underwent WMT and attended at least one follow-up visit at the Department of Gastroenterology, First Affiliated Hospital of Guangdong Pharmaceutical University from 1 January 2017 to 30 June 2021. Additionally, a control group of patients with renal dysfunction, who did not undergo WMT within the same timeframe, was recruited to assess the effect of WMT on renal parameters. The control group was nearly 1:1 matched for sex and age. The exclusion criteria were as follows: (1) acute gastrointestinal infection within 1 month; (2) antibiotic usage within 3 months (except for those who underwent WMT for antibiotic-associated diarrhoea); (3) pregnancy; (4) ongoing RRT (renal transplantation or dialysis) or substantial renal-affecting medication usage (e.g., diuretics or glucocorticoids); and (5) missing medical data. Sample size estimation was performed using online software (Power and Sample Size Calculators; HyLown Consulting LLC, Atlanta, GA, USA).

#### Donor selection and WMT procedure

Healthy donors were initially screened using a questionnaire followed by blood and stool tests to rule out communicable diseases, as previously described [15].

A total of 500 mL of 0.9% saline (NaCl) and 100 g of stool sample were homogenised and microfiltered through an automated microbiota purification system (GenFMTer; FMT Medical, Nanjing, China) to prepare the washed microbiota suspension. The faecal microbiota suspension was centrifuged (1100 × *g* for 3 min at room temperature), and the precipitate was washed with 0.9% NaCl. This process was repeated twice more, each time involving centrifugation and washing. Eventually, 100 mL NaCl was added to resuspend the microbiota precipitate, yielding the final washed microbiota suspension [15].

The WMT procedure involved administering the washed microbiota suspension (120 mL per day for 3 consecutive days) to patients via a transendoscopic enteral tube (for the lower gastrointestinal tract) or a nasojejunal tube (for the upper gastrointestinal tract), according to each patient's specific conditions and preference. Patients received microbial suspensions from healthy donors, allocated at random.

#### Data collection

Electronic medical records provided the following clinical information: demographic details, body mass index, smoking and alcohol habits, history of comorbidities (e.g., hypertension and type 2 diabetes), history of RRT, medication usage, indication for WMT (organic or functional disease), route of WMT delivery (lower or upper gastrointestinal tract), AEs associated with WMT, and laboratory parameters, including SCr, blood urea nitrogen (BUN), serum uric acid (UA), haemoglobin, serum sodium, serum potassium, serum calcium, serum phosphorus, triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-c).

#### Definitions

The estimated glomerular filtration rate (eGFR) was calculated as follows: eGFR (mL/min/1.73 $m^2$) = $186 \times SCr^{-1.154} \times age^{-0.203} \times$ (0.742 if female). Normal renal function was defined as eGFR of ≥ 90 mL/min/1.73 $m^2$, while renal dysfunction was defined as eGFR of < 90 mL/min/1.73 $m^2$ (CKD stages 2–5) [22]. Alcoholism was defined as weekly alcohol consumption of > 210 g for males and > 140 g for females [23]. Organic diseases encompassed conditions resulting in structural changes to the organs or tissues (e.g., inflammatory bowel disease and chronic liver disease), while functional diseases referred to those lacking structural changes (e.g., functional bowel disorders and gut dysbiosis). WMT-related AEs, including abdominal pain, diarrhoea, and fever, were assessed by physicians based on clinical judgment. The effect of WMT on renal parameters was determined as follows: △renal parameter = renal parameter after WMT—renal parameter at baseline.

#### Sample collection

Patient stool, urine, and blood samples were collected 2 days before each WMT session (baseline and approximately 1 month, 2 months, and 6 months after the first WMT). Stool samples from healthy donors used for WMT were also collected for sequencing. The stool samples were contained within stool collection tubes with a deoxyribonucleic acid (DNA) stabiliser (Invitek, Germany). All samples were stored at -80℃ until sequencing.

#### Microbiome analysis

DNA extraction and sequencing were conducted by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China), as previously described [15]. Briefly, DNA was extracted from each stool sample using the E.Z.N.A.® soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). DNA concentration was assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Amplification of bacterial 16S ribosomal ribonucleic acid (rRNA) gene V3–V4 regions was achieved through the 338F and 806R primer sets, and amplicon integrity was verified via agarose gel electrophoresis. Paired-end sequencing was performed using the Illumina

MiSeq platform. Raw sequencing reads were deposited in the National Centre for Biotechnology Information Sequence Read Archive under the Accession numbers PRJNA790000.

Paired-end sequences were combined using FLASh (version 1.2.11), and subsequent quality filtering was performed using fastp (version 0.19.6). The remaining sequencing data underwent DADA2-based denoising to generate amplicon sequence variants (ASVs) in QIIME2 (version 2020.2). Taxonomic assignment for the ASVs was performed using QIIME2 and the SILVA 16S rRNA database. Sequencing data analyses were performed using the Majorbio Cloud Platform (www.majorbio.com).

#### Metabolomics analysis

For liquid chromatography-mass spectrometry (LC–MS), frozen urine samples were thawed on ice and vortexed. Each urine sample (100 μL) was combined with methanol (300 μL) and 1 μg/mL of L-2-chlorophenyl alanine (Bidepharm, Shanghai, China) as an internal standard for protein precipitation. The mixture was sonicated in an ice-water bath for 10 min, followed by incubation at − 20 ℃ for 1 h and centrifugation at 14,000 × *g* at 4 ℃ for 15 min. The supernatant (100 μL) was transferred to a glass vial for LC–MS analysis. A quality control sample was prepared by combining 20 μL supernatant from each sample.

LC–MS analysis employed a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific), with all samples analysed in positive and negative ionisation modes. The positive mode mobile phase comprised water with 0.1% formic acid (A) and acetonitrile (B), while the negative mode mobile phase comprised water with 5 mM acetic acid (A) and acetonitrile (B). The column temperature was maintained at 35 ℃, with an injection volume of 3 μL. The gradient elution program was run as follows: 0 min, 1% B; 8 min, 99% B; and 10.1 min, 1% B, at a flow rate of 0.4 mL/min. Electrospray ionisation source parameters included sheath gas flow at 45 L/min, auxiliary gas flow at 15 L/min, sweep gas flow at 0 L/min, spray voltage at 4000 V (for positive mode) or − 3000 V (for negative mode), and capillary temperature at 400 ℃.

Thermo Fisher Scientific Compound Discoverer (version 3.1) facilitated metabolite annotation of LC–MS data, referencing the BioCyc, Human Metabolome, Kyoto Encyclopaedia of Genes and Genomes, MassBank, and National Institute of Standards and Technology databases. Metabolomics analyses and related graphs were generated using MetaboAnalyst 5.0 online tools (www.metaboanalyst.ca). Based on partial least squares discriminant analysis (PLS-DA) results, variable importance in projection (VIP) scores were calculated. Metabolites with VIP scores > 1.0 in the PLS-DA model and $P < 0.05$ in the Wilcoxon rank-sum test were identified as differential metabolites.

#### Statistical analysis

Statistical analysis was performed using SPSS software (version 22.0; IBM, Armonk, NY, USA) and Prism (version 8; GraphPad, San Diego, CA, USA). Continuous data are presented as the mean and standard deviation for normally distributed variables and as a median and interquartile range for non-normally distributed variables. Categorical data are presented as frequencies and percentages. Between-group comparisons of continuous variables were performed using the Student's *t*-test and the Wilcoxon rank-sum test, while categorical variables were analysed using the chi-square test and Fisher's exact test. For one-sample comparisons (between time points), the one-sample *t*-test or Wilcoxon signed-rank test was used as appropriate. Statistical significance was determined by a two-tailed *P*-value of < 0.05.

## Results

#### Demographic characteristics of patients and healthy donors

Initially, 527 patients who underwent WMT were enrolled, and 253 met the final analysis criteria. Of these patients, 86 had renal dysfunction while 168 did not. Among those with renal dysfunction, 76 were in CKD G2, nine in CKD G3, and one in CKD G4. A control group comprising 86 sex- and age-matched patients with renal dysfunction who did not undergo WMT was also included. Additionally, 25 healthy donors passed the donor screening. The demographic and clinical characteristics of patients and healthy donors are summarised in Additional file 3: Table S1.

The most prevalent indication for WMT was functional bowel disorders (n = 147), followed by inflammatory bowel disease (n = 32) and chronic liver disease (n = 20; Additional file 4: Table S2). The median intervals between the first and second WMT, second and third WMT, and third and fourth WMT were 36.89 (31.85, 52.00) days, 42.89 (34.03, 64.11), and 97.02 (79.00, 125.03) days, respectively (Fig. 1a).

#### Gut microbiota profiles in patients with renal dysfunction and healthy donors

Gut microbiota profiles were compared between patients with renal dysfunction and healthy donors. The phylum-level relative abundances of gut microbes in patients with renal dysfunction and healthy donors are presented in Additional file 1: Fig. S1a. Although no differences were observed in genus-level richness and diversity (Additional file 1: Fig. S1b, Fig. 1b), principal coordinate analysis (PCoA) and nonmetric multidimensional scaling

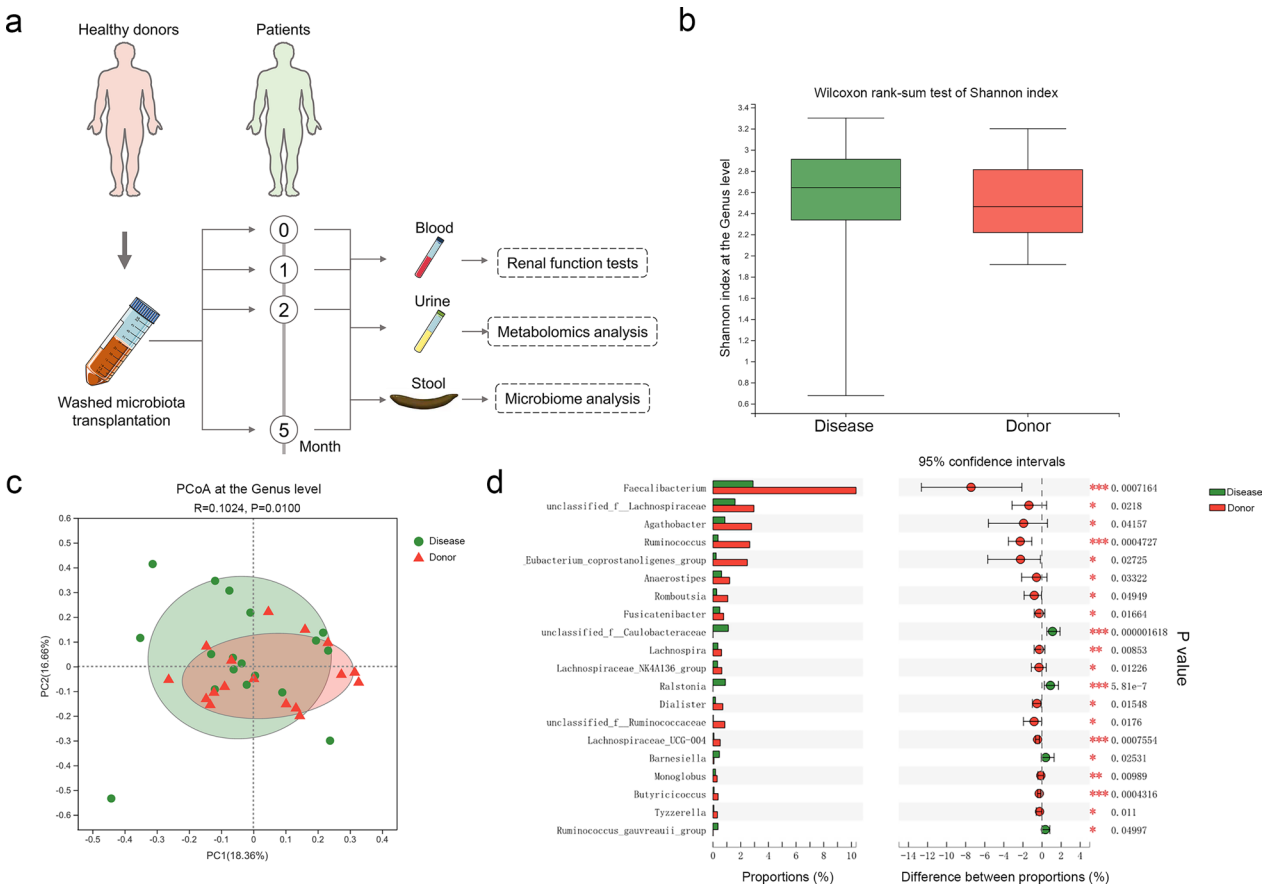



**Fig. 1** Gut microbiota profiles of patients with renal dysfunction and healthy donors. **a** Study design; **b** Shannon's diversity index at the genus level; **c** Principal coordinate analysis of microbiota composition at the genus level; **d** Wilcoxon rank-sum test bar plot of relative abundances of the top 20 differential genera. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

(NMDS) analysis based on β-diversity showed a significantly different microbial community structure between the two groups (Fig. 1c, Additional file 1: Fig. S1c). Compared to healthy donors, patients with renal dysfunction had notable changes in genus-level relative abundances (Fig. 1d, Additional file 1: Fig. S1d). This encompassed reduced relative abundances of *Eubacterium coprostanoligenes*, *Anaerostipes*, *Monoglobus,* and *Butyricicoccus* (all $P<0.05$).

### Effects of WMT on renal function in patients with or without renal dysfunction

Given the distinctive gut microbiota profiles between patients with renal dysfunction and healthy controls, the study evaluated the influence of gut microbiota remodelling through WMT on renal activity in patients with renal dysfunction. Notably, SCr levels after the first (△SCr: − 9.29 ± 14.31, $P<0.01$), second (△SCr: − 3.12 ± 8.42, $P=0.038$), and third (△SCr: − 8.00 [− 22.50, − 0.50], $P=0.004$) WMT were significantly lower than the levels before WMT, and the eGFR levels after the first (△eGFR: 8.54 [1.02, 23.38], $P<0.001$), second (△eGFR: 3.58 ± 9.26, $P=0.031$), and third (△eGFR: 16.72 ± 17.03, $P=0.004$) WMT were significantly higher than the levels before WMT (Fig. 2a). Additionally, BUN levels after the first WMT (△BUN: − 0.41 [− 1.34, 0.58], $P=0.023$) and serum UA levels after the third WMT (△UA: − 44.86 ± 39.65, $P=0.024$) were significantly lower than levels before WMT (Fig. 2a). Furthermore, patients with renal dysfunction who underwent WMT exhibited marked improvements in SCr, eGFR, and BUN compared with those who did not undergo WMT (Fig. 2b).

The effects of WMT on renal function in patients without renal dysfunction were also assessed. No significant effects of WMT on renal parameters were observed in these patients, except for a decrease in serum UA after the third WMT (Fig. 3).

### Clinical factors associated with the effects of WMT on renal function

Subsequently, potential factors influencing the effects of WMT on renal function were assessed. Among the

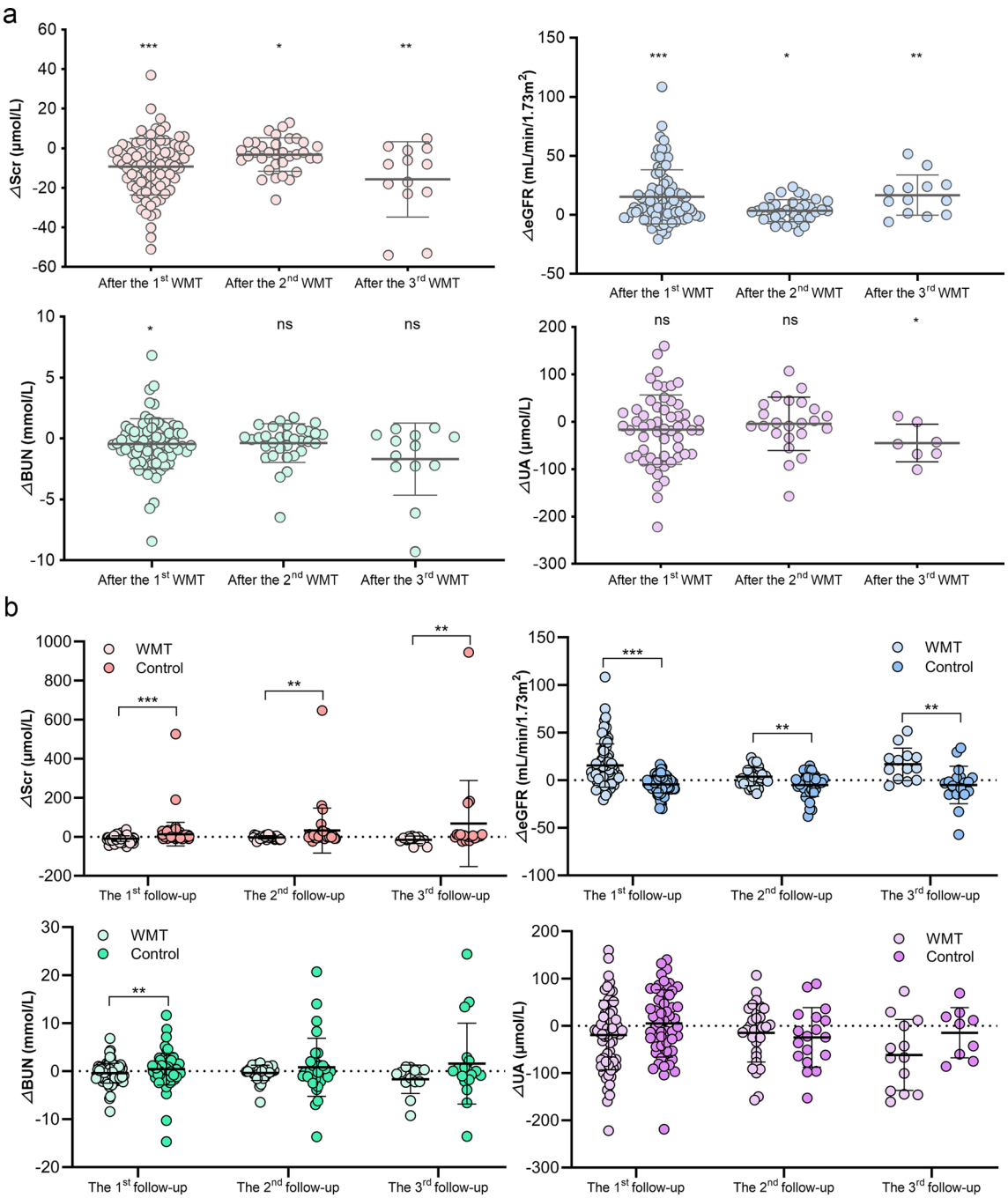



**Fig. 2** Effects of WMT on renal parameters in patients with renal dysfunction. **a** Changes in the levels of SCr, eGFR, BUN, and UA in patients with renal dysfunction before and after WMT. **b** Comparison of the changes of renal parameters between patients with renal dysfunction who did and did not undergo WMT. △renal parameter = renal parameter after WMT—renal parameter at baseline. BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; UA, uric acid; WMT, washed microbiota transplantation. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, no significance

patients with renal dysfunction, 56 underwent WMT through the lower gastrointestinal tract, while 30 underwent WMT through the upper gastrointestinal tract. At a significance level of 0.10, the former group displayed greater improvements in SCr after the second WMT (△SCr: − 5.45 ± 8.88 vs. − 0.21 ± 6.65, $P = 0.052$), eGFR after the second (△eGFR: 5.95 ± 8.99 vs. 0.19 ± 8.86, $P = 0.073$) and third (△eGFR: 22.22 ± 17.07 vs.

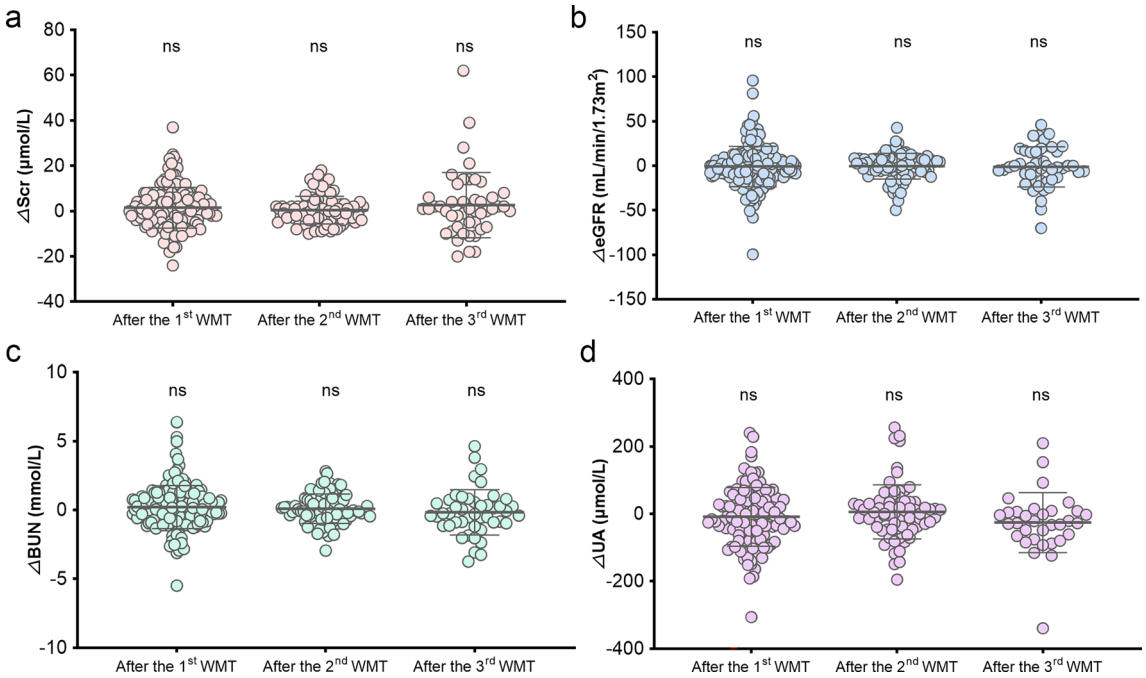



**Fig. 3** Effects of WMT on renal parameters in patients without renal dysfunction. The effects of WMT on SCr (**a**), eGFR (**b**), BUN (**c**), and UA (**d**) in patients without renal dysfunction. △renal parameter = renal parameter after WMT—renal parameter at baseline. BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; UA, uric acid; WMT, washed microbiota transplantation. $^{*}P < 0.05$; ns, no significance

4.36 ± 9.45, $P = 0.079$) WMT, BUN after the third WMT (△BUN: − 2.20 [− 4.22, 0.03] vs. 0.47 [− 0.41, 0.86], $P = 0.050$), and serum UA after the third WMT (△UA: − 65.20 ± 23.00 vs.6.00 ± 8.49, $P = 0.010$; Fig. 4) compared with the latter group.

Among the patients with renal dysfunction, 60 and 26 underwent WMT for functional and organic diseases, respectively. However, no significant differences were observed in the effects of WMT on renal function parameters (SCr, eGFR, BUN, and UA) between these two groups (Fig. 5a). Given hypertensive nephropathy as the primary cause of renal dysfunction, a comparison was drawn between the effects of WMT on renal function parameters in patients with renal dysfunction caused by hypertensive nephrology (n = 31) and those resulting from other aetiologies (n = 55). However, minimal significant differences in most renal function parameters were observed between patients with hypertensive nephropathy and those with other aetiologies (Fig. 5b).

### Effects of WMT on renal disease-related parameters in patients with renal dysfunction

Given that patients with renal dysfunction experience a wide array of complications, including electrolyte disturbances, dyslipidaemia, and anaemia, the impact of WMT on renal disease-related parameters in patients with renal dysfunction was also analysed. The total cholesterol, LDL-c and haemoglobin levels demonstrated signs of improvement after WMT, while other parameters did not exhibit significant changes after treatment (Additional file 5: Table S3).

### AEs of WMT

As safety remains a primary concern in WMT, WMT-related AEs were examined. Among 86 patients with renal dysfunction undergoing 206 WMT procedures, the AE incidence was 2.91%. The most prevalent WMT-related AE was diarrhoea (two WMT procedures, 0.97%), followed by bloating (one WMT procedure, 0.49%), fever (one WMT procedure, 0.49%), vomiting (one WMT procedure, 0.49%), and anal pain (one WMT procedure, 0.49%). Notably, the bloating experienced by one patient resolved spontaneously, while AEs in the remaining five patients improved after symptomatic treatment. No serious AEs were observed.

### Gut microbiota profiles in patients with renal dysfunction before and after WMT

Gut microbiota profiles of patients with renal dysfunction were compared before and after WMT to further investigate the potential mechanism by which WMT improves renal function. A total of 26 stool samples (collected at baseline [n = 13], 1 month [n = 9], 2 months [n = 2], and 6 months [n = 2] after the first WMT) were included for

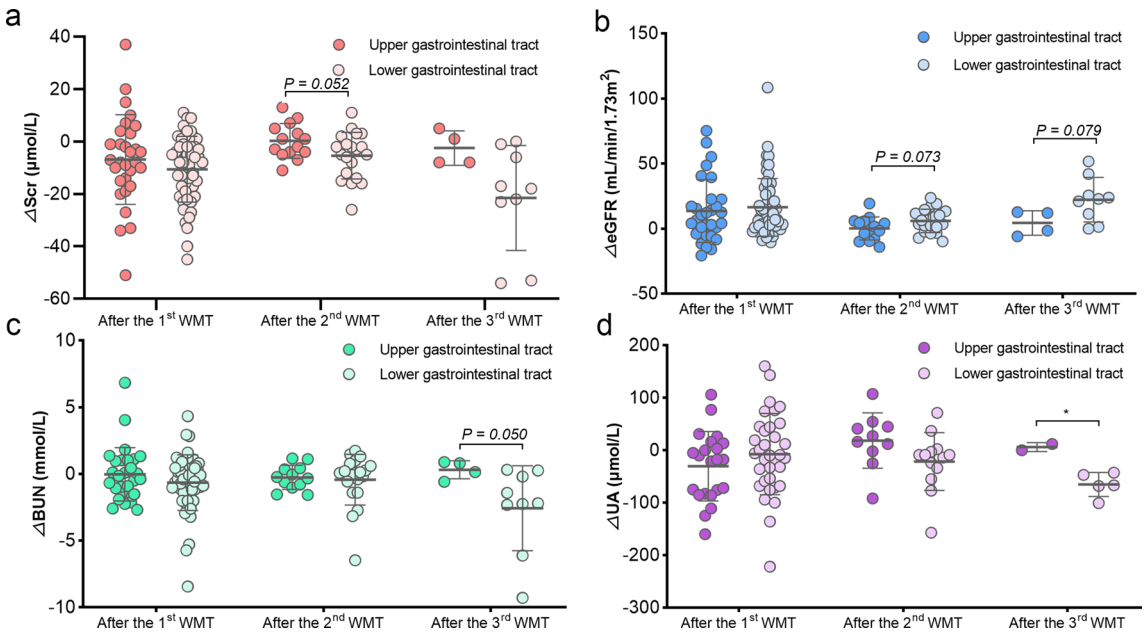



**Fig. 4** Association between WMT delivery routines and the effects of WMT on renal function. The effects of WMT on SCr (**a**), eGFR (**b**), BUN (**c**), and UA (**d**) in patients with renal dysfunction who underwent WMT through the upper or lower gastrointestinal tract. Δrenal parameter = renal parameter after WMT—renal parameter at baseline. BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; UA, uric acid; WMT, washed microbiota transplantation. $^{*}P < 0.05$

gut microbial analysis. The phylum-level relative abundances of gut microbes in patients with renal dysfunction before and after WMT are presented in Additional file 2: Fig. S2a. The Shannon index (2.32 ± 0.77 vs. 3.09 ± 0.34, $P = 0.002$; Fig. 6a) at the genus level was significantly higher and the Simpson index was significantly lower (0.24 ± 0.22 vs.0.09 ± 0.04, $P = 0.004$; Additional file 2: Fig. S2b) after WMT, while no significant differences were observed in the abundance-based coverage estimator and Chao indices (Additional file 2: Fig. S2b). Genus-level PCoA (R = 0.139, $P = 0.001$; Fig. 6b) and NMDS analysis (stress: 0.264, R = 0.139, $P = 0.001$; Additional file 2: Fig. S2c) demonstrated that the gut microbiota profile of patients with renal dysfunction after WMT tended to resemble that of healthy donors. Notably, several gut genera, including *Eubacterium coprostanoligenes*, *Anaerostipes*, *Monoglobus*, and *Dorea*, exhibited significant enrichment after WMT, having initially been significantly reduced in patients with renal dysfunction. Simultaneously, other genera, including *Hungatella,* were significantly decreased after WMT (Fig. 6c, Additional file 2: Fig. S2d). As presented in Fig. 6d, the relative abundances of several genera correlated with renal parameters in patients with renal dysfunction. For instance, the *Eubacterium coprostanoligenes* group, *Senegalimassilia*, and *Coriobacteriales incertae sedis* abundances were positively correlated with eGFR levels.

### Urine metabolic profiles in patients with renal dysfunction before and after WMT

Urine metabolomic profiles from 13 patients with renal dysfunction before and after WMT (with available samples at baseline [n = 13], 1 month [n = 12], 2 months [n = 8], and 6 months [n = 2] after the first WMT) were subjected to metabolomics analysis. As demonstrated by the distinct separation in the PLS-DA score plot (Fig. 7a), points representing pre- and post-WMT stages were distinctly separated. VIP scores, derived from PLS-DA outcomes, led to the identification of the top 15 metabolites ranked by VIP scores, as presented in Fig. 7b. Moreover, a heatmap visualised the abundance of the top 25 metabolites based on VIP scores before and after WMT (Fig. 7c). Among these, 16 metabolites with VIP scores > 1.0 and $P < 0.05$ were identified as differential metabolites (Additional file 6: Table S4). More importantly, the relative abundances of three toxic metabolites, namely hippuric acid, cinnamoylglycine, and indole, associated with CKD progression [24–27], were elevated in the urine of patients after WMT (all $P < 0.05$). Using the Small Molecule Pathway Database metabolite set enrichment analysis revealed that pathways such as "homocysteine degradation", "sulphate/sulphite metabolism", "methionine metabolism" and "glycine and serine metabolism" experienced notable alterations in patients with renal dysfunction after WMT (Fig. 7d).

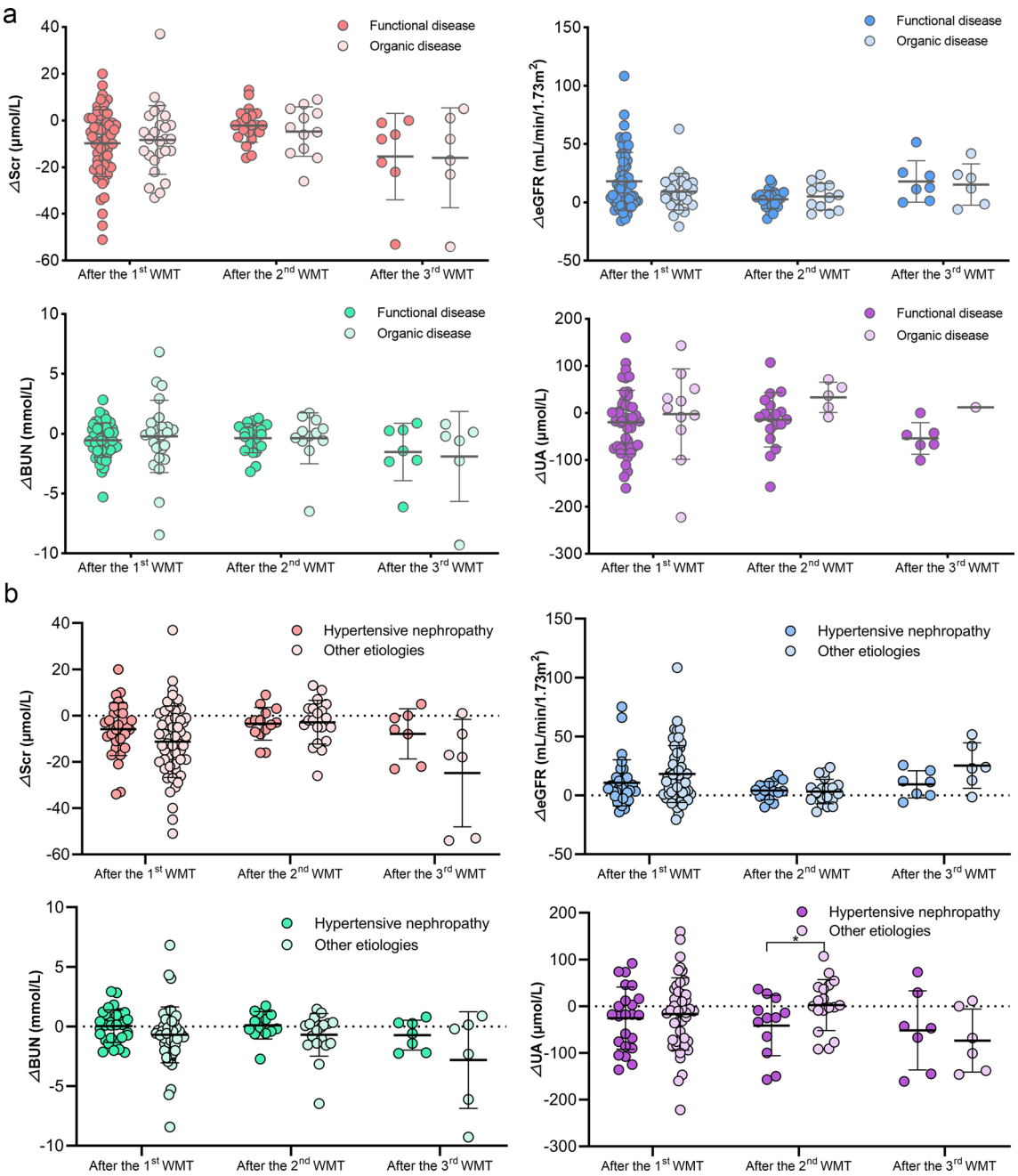



**Fig. 5** Clinical factors associated with effects of WMT on renal function. **a** Effects of WMT on renal parameters in patients with renal dysfunction who underwent WMT for organic or functional disease; **b** Effects of WMT on renal parameters in patients with renal dysfunction caused by hypertensive nephrology or other aetiologies. Δrenal parameter = renal parameter after WMT—renal parameter at baseline. BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; UA, uric acid; WMT, washed microbiota transplantation. $^{*}P < 0.05$

## Discussion

This study investigated the efficacy, safety, and underlying mechanism of WMT in enhancing renal activity among patients with renal dysfunction. The findings revealed that WMT resulted in a significant improvement in renal activity for patients with renal dysfunction, while not significantly affecting those without renal dysfunction. In addition, WMT exhibited favourable tolerability and safety, with a low AE incidence (2.91%). After WMT administration, an increase in gut microbiota diversity and the abundance of specific probiotic bacteria were observed in patients with renal dysfunction.

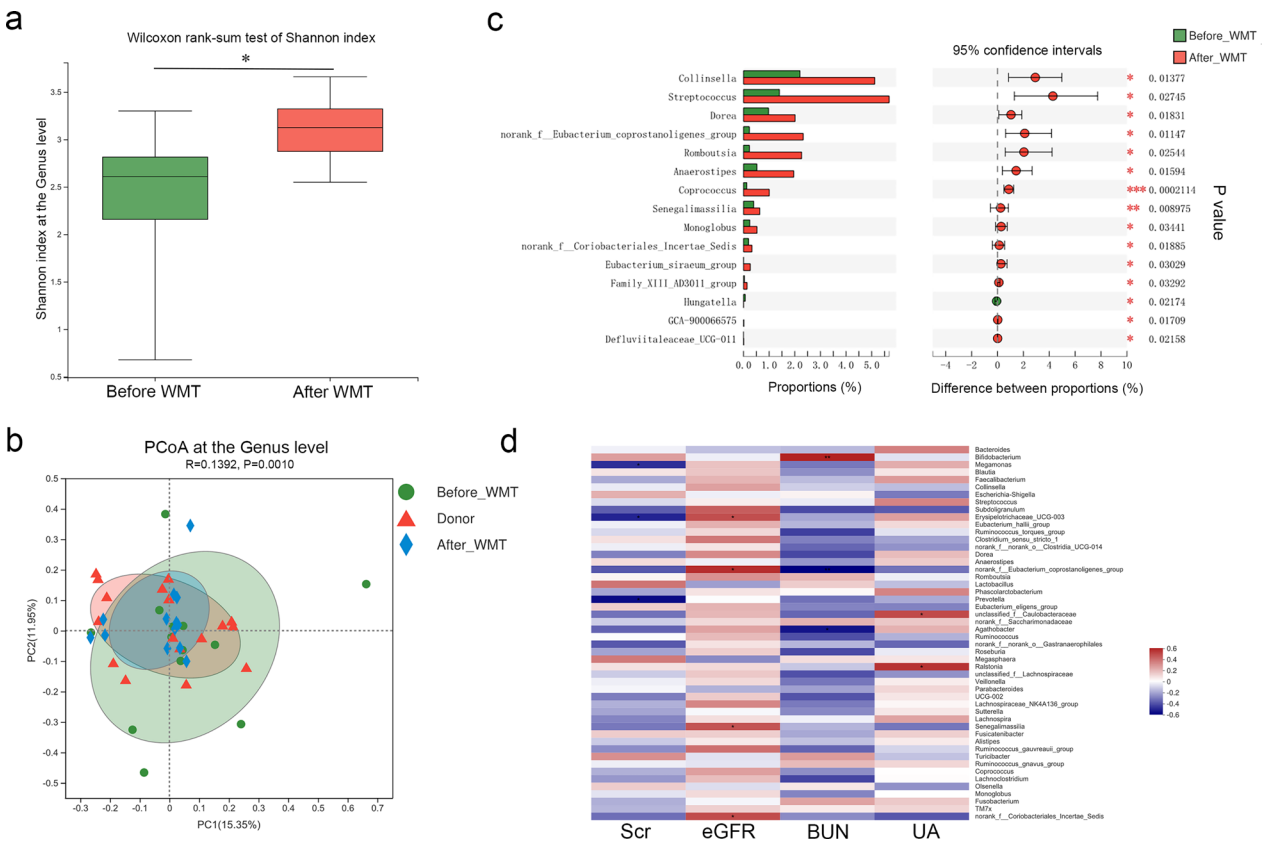



**Fig. 6** Gut microbiota profiles in patients with renal dysfunction before and after WMT. **a** Shannon's diversity index at the genus level; **b** Principal coordinate analysis (PCoA) of microbiota composition at the genus level; **c** Wilcoxon rank-sum test bar plot of relative abundances of the top 15 differential genera; **d** Heatmap of the correlations of genus-level abundances and renal parameters. BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; SCr, serum creatinine; UA, uric acid; WMT, washed microbiota transplantation. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Furthermore, their gut microbiota profiles demonstrated a close resemblance to those of healthy donors, and enhanced removal of toxic metabolites through the urine was evident. This suggests that WMT might improve renal function through gut microbiota regulation and improved toxin excretion (Fig. 8). To the best of our knowledge, this is the first clinical study demonstrating the efficacy and safety of WMT in improving renal function in humans.

Current research highlights that patients with CKD have an altered intestinal microbiota [28]. Consistent with previous studies [29, 30], this study reported that the β-diversity of the microbial community was significantly different between patients with renal dysfunction and healthy donors. However, unlike studies finding marked α-diversity variations in mild CKD (CKD stages 1 and 2) compared with patients without CKD, no significant differences in gut microbiota richness or diversity were observed in our study. This aligns with another study suggesting comparable α-diversity in these two patient groups [31]. Furthermore, the study uncovered substantial reductions in the relative abundances of several genera, such as *Eubacterium coprostanoligenes*, *Anaerostipes*, *Monoglobus,* and *Butyricicoccus*, in patients with renal dysfunction compared with healthy donors, which is consistent with previous reports [29, 32–34].

Recent studies have shed new light on the pathogenetic roles of the gut microbiota in kidney diseases, with interventions targeting it (e.g., diet, probiotics, and FMT) holding promise for CKD treatment [13]. Notably, Zhu et al. observed that *Lactobacillus casei* Zhang administration ameliorated gut dysbiosis and slowed disease progression, yet failed to arrest or reverse renal function decline [10]. Likewise, Wang et al. demonstrated that healthy donor gut microbiota administration effectively lowered SCr and urea levels, mitigating kidney pathology in CKD mice compared with those receiving microbiota from patients with ESRD, thereby suggesting the potential for FMT to reverse kidney disease progression [18]. In our study, WMT targeted gut microbiota not only arrested but reversed renal function decline among patients with renal dysfunction, suggesting that

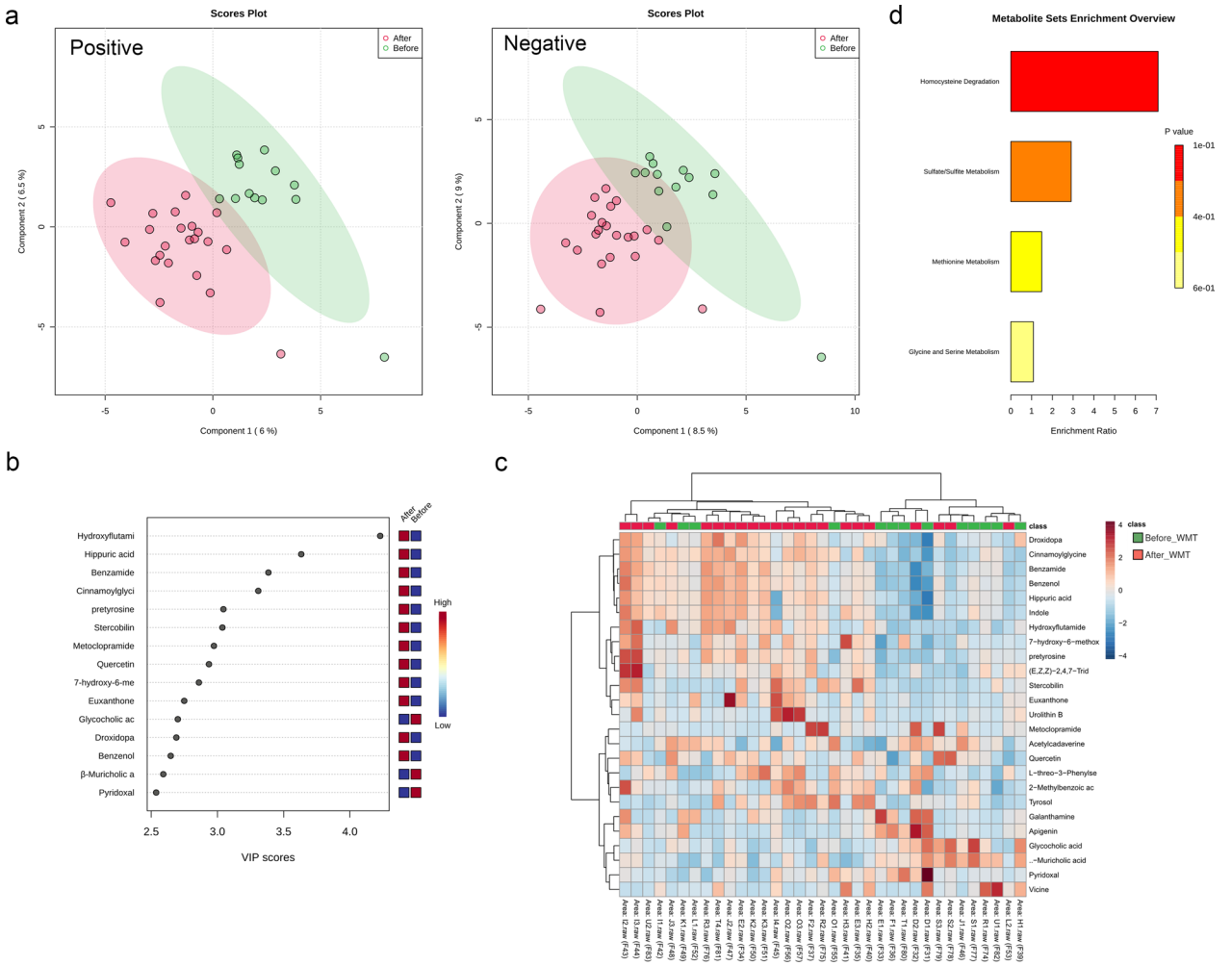



**Fig. 7** Urine metabolic profiles in patients with renal dysfunction before and after WMT. **a** Partial least squares discriminant analysis (PLS-DA) score plots of the metabolites; **b** Important metabolites identified by PLS-DA based on variable importance in projection (VIP) scores; **c** Heatmap of the abundances of the top 25 metabolites based on the VIP scores; **d** Metabolite set enrichment analysis

manipulating gut microbiota might be a novel treatment strategy for CKD.

Several mechanisms could explain these findings. First, patients with CKD exhibit dysbiosis, a change in microbiota composition and structure, with decreased probiotic bacteria and increased pathogenic bacteria [35, 36]. After WMT, the abundances of several probiotic genera, such as *Dorea* and *Anaerostipes*, often reduced in kidney disease [37, 38], increased, while the abundance of potential pathogens such as *Hungatella*, which is significantly increased in patients with CKD [39], markedly decreased in patients with renal dysfunction after receiving WMT. Second, CKD-associated harmful microbiota generates trimethylamine-N-oxide, implicated in uremic toxin accumulation by activating the renin–angiotensin–aldosterone system [40]. Our study evidenced decreased toxic microbiota abundance after WMT, coupled with increased urinary toxin excretion. Therefore, WMT might promote toxin excretion by reducing trimethylamine-N-oxide production, subsequently improving the renin–angiotensin–aldosterone system [19]. Third, uraemia alters the gut biochemical environment, resulting in intestinal mucosal injury (leaky gut), common in CKD. This promotes lipopolysaccharide translocation and serum proinflammatory cytokine production, such as interleukin (IL)-6 and tumour necrosis factor (TNF)-α, exacerbating kidney injury [41, 42]. FMT has been shown to restore intestinal barrier function, lowering serum lipopolysaccharide, IL-6, and TNF-α levels [43], suggesting that WMT may improve renal function by enhancing intestinal barrier integrity and reducing systemic inflammation.

Patients with renal dysfunction who underwent WMT through the lower gastrointestinal tract (with

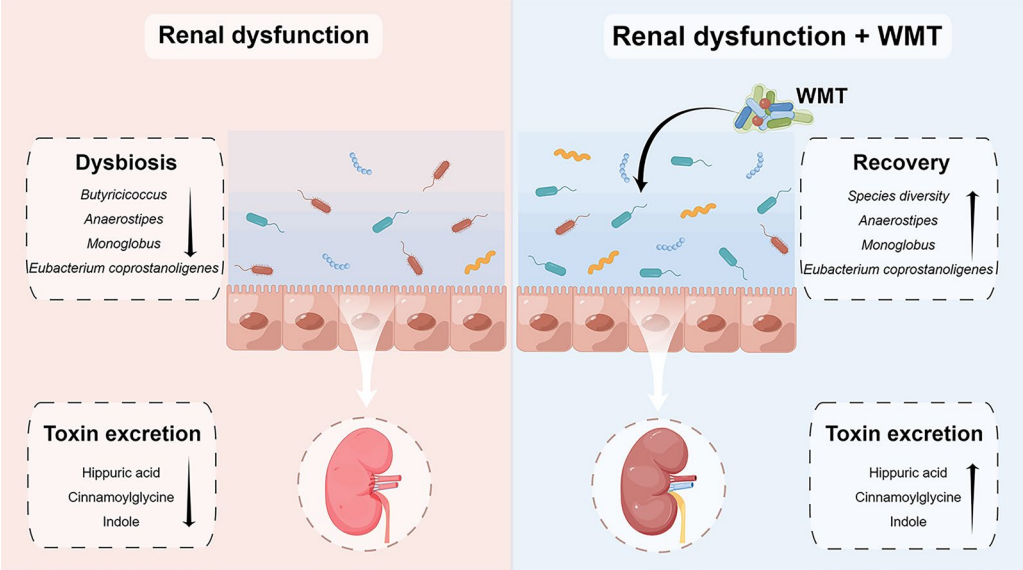



**Fig. 8** Graphical abstract. WMT, washed microbiota transplantation

the faecal microbiota suspension reaching the large intestine) experienced more substantial renal function improvement compared with those who received WMT through the upper gastrointestinal tract. Consistent with research on Parkinson's disease [44] and hypertension [15], colonic FMT demonstrated superiority over nasointestinal FMT. There are two possible explanations for these results. First, location-specific microbes tend to colonise homologous gut regions, suggesting that microbes from the large intestine are more likely to colonise the large intestine than the small intestine [45]. Thus, large-intestine-derived microbes in faecal suspension, when delivered to the large intestine via the lower gastrointestinal tract, might improve microbiota colonisation. Second, patients who received colonic WMT underwent bowel preparation, which potentially facilitated microbiota colonisation, thereby enhancing the therapeutic effect.

Electrolyte abnormalities, dyslipidaemia, and anaemia are common systemic complications of CKD [46]. This study suggested a trend of improvement in blood lipids (total cholesterol and LDL-c) and haemoglobin among patients with renal dysfunction after WMT, indicating the potential of WMT to ameliorate CKD-related metabolic abnormalities and anaemia. Similar observations are seen in clinical studies where FMT increased insulin sensitivity in patients with metabolic syndrome and increased haemoglobin in those with anaemia caused by chronic disease by modulating the intestinal microbiota composition and metabolism [47, 48]. However, whether WMT can improve other CKD-related parameters and complications, such as mineral bone disorder and endocrine dysfunction, remains to be investigated.

This study observed a significant reduction in the abundances of *Eubacterium coprostanoligenes*, *Anaerostipes,* and *Monoglobus* in faecal samples from patients with renal, consistent with findings in patients with immunoglobulin A nephropathy and renal failure [18, 33]. Furthermore, the abundances of *Eubacterium coprostanoligenes*, *Senegalimassilia,* and *Coriobacteriales incertae sedis*, were positively correlated with eGFR levels, indicating their protective role against renal disease progression. Interestingly, WMT led to the abundance of these five genera in patients with renal dysfunction. Further investigation is warranted to assess the therapeutic potential of these genera in CKD management.

Several limitations of our study warrant consideration. First, its retrospective design and small sample size led to a limited number of samples from patients with renal dysfunction. Additionally, the use of DNA stabilising buffer in stool samples posed challenges for metabolomics analysis. Second, several potential confounders, such as protein, water, and salt intake, medication use, and underlying cause of renal dysfunction, which might influence renal disease progression, were incompletely recorded. Third, the relatively short follow-up duration with only 40% and 15% of the patients completing 3 months and 6 months follow-up after WMT, respectively, precludes assessing long-term outcomes of patients with renal dysfunction. Future

prospective studies, featuring larger samples and longer follow-up durations are essential to validate these findings.

## Conclusions

In conclusion, WMT proves both safe and effective in improving renal function among patients with renal dysfunction by modulating the gut microbiota and promoting toxic metabolite excretion. These findings suggest that targeting the gut microbiota using WMT offers a promising novel approach for treating CKD.

### Abbreviations

| | |
|---|---|
| AE | Adverse event |
| ASV | Amplicon sequence variant |
| BUN | Blood urea nitrogen |
| CKD | Chronic kidney disease |
| DNA | Deoxyribonucleic acid |
| eGFR | Estimated glomerular filtration rate |
| ESRD | End-stage renal disease |
| FMT | Faecal microbiota transplantation |
| IL | Interleukin |
| LC–MS | Liquid chromatography-mass spectrometry |
| LDL-c | Low-density lipoprotein cholesterol |
| NaCl | Saline |
| NMDS | Nonmetric multidimensional scaling |
| PCoA | Principal coordinate analysis |
| PLS-DA | Partial least squares discriminant analysis |
| rRNA | Ribosomal ribonucleic acid |
| RRT | Renal replacement therapy |
| SCr | Serum creatinine |
| TNF | Tumour necrosis factor |
| UA | Uric acid |
| VIP | Variable importance in projection |
| WMT | Washed microbiota transplantation |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-023-04570-0.

**Additional file 1: Figure S1.** Gut microbiota profiles of patients with renal dysfunction and healthy donors. **a** Circularised plot of the genus-level abundances in faecal samples; **b** abundance-based coverage estimator (ACE) and Chao and Simpson index at the genus level; **c** nonmetric multidimensional scaling (NMDS) analysis of microbiota composition at the genus level; **d** linear discriminant analysis effect size analysis of the differential genera in stool samples between patients with renal dysfunction and healthy donors.

**Additional file 2: Figure S2.** Gut microbiota profiles of patients with renal dysfunction before and after washed microbiota transplantation (WMT). **a** Bar graph of the genus-level abundances in faecal samples; **b** abundance-based coverage estimator (ACE), Chao and Simpson index at the genus level; **c** nonmetric multidimensional scaling (NMDS) analysis of microbiota composition at the genus level; **d** linear discriminant analysis effect size analysis of the differential genera in stool samples between patients before and after WMT.

**Additional file 3: Table S1.** Demographics and clinical characteristics of the enrolled patients.

**Additional file 4: Table S2.** Reasons for patients undergoing washed microbiota transplantation.

**Additional file 5: Table S3.** Effects of washed microbiota transplantation on renal disease-related parameters in patients with renal dysfunction.

**Additional file 6: Table S4.** Significantly altered metabolites in urine samples from patients before and after washed microbiota transplantation.

**Additional file 7: Table S5.** Untargeted metabolomics data based on the positive mode.

**Additional file 8: Table S6.** Untargeted metabolomics data based on the negative mode.

**Additional file 9: Table S7.** Clinical dataset.

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Not applicable.

### Author contributions

Conceptualisation: QW and XXH. Data curation: WJC, YPZ, YLC, and HLZ. Formal analysis: HJZ, WJC, and YPZ. Investigation: HJZ and XX. Methodology: HJZ, CX, and XX. Software: HJZ, WJC, YPZ, LX, and MC. Project administration: XX, QW, and XXH. Resources: HJZ, XX, QW, and XXH. Supervision: XXH and MZ. Validation: YPZ, XH, and YD. Writing—original draft: HJZ and XX. Writing—review and editing: HJZ, WJC, QW, and XXH. All authors read and approved the final manuscript.

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### Availability of data and materials

The data supporting the findings of this study are available at https://dataview.ncbi.nlm.nih.gov/object/PRJNA790000. Untargeted metabolomics data are provided in Additional files 7 and 8.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangdong Pharmaceutical University (Approval number: 2021-123). Written informed consent was obtained from all patients, except in cases where a legal representative consented on behalf of those unable to do so.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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## Publisher's Note