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ORIGINAL ARTICLE - GASTROENTEROLOGY (CLINICAL)

Washed microbiota transplantation improved the level of serum vitamin D in ulcerative colitis

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Declaration of conflict of interest: Faming Zhang conceived the concept of GenFMTer and transendoscopic enteral tubing and devices related to them. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contribution: HZ, BC, YX, and QW designed the study and wrote the manuscript. HZ, YX, QW, and SZ collected the sample and analyzed the clinical data. HZ, PL, BH, and HL analyzed data and offered technological support. BC, CM, and FZ revised the manuscript. BC and FZ conceived the study and obtained funding. All authors approved the final version to be submitted.

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Abstract

Background and Aim: Vitamin D (VD) deficiency was reported to correlate with ulcerative colitis (UC) activity, which might be closely related to gut microbiota dysbiosis. This study aims to investigate the effects of washed microbiota transplantation (WMT) on VD metabolism in UC.

Methods: The serum levels of 25-hdroxyvitamin D [25(OH)D] in 121 patients with UC and 53 healthy controls (HC) were detected. Subsequently, a non-randomized control trial (non-RCT) was conducted. Patients with UC were non-randomly assigned to undergo WMT ($n = 28$) *vs*. conventional treatment (5-aminosalicylic acid, 5-ASA, $n = 10$). Serum levels of 25(OH)D, fecal microbiota, and the expression of vitamin D receptor (VDR) in patients with UC were evaluated with a 3-month follow-up.

Results: Serum VD levels collected in the clinic practice indicated that patients with UC had significantly lower VD levels than HC ($P < 0.001$). In the non-RCT, serum 25(OH)D level and VDR expression significantly increased ($P = 0.011$, 0.026, respectively) in the WMT group, while no noticeable changes were observed in the non-WMT group. Microbiome profiling revealed that the increase in VD levels after WMT was positively associated with the abundances of *Adlercreutzia_equolifaciens*, *Ruminococcus_obeum*, and *Dorea* but negatively correlated with *Escherichia*.

Conclusions: The study suggested that WMT increases the levels of VD with characteristic changes of specific microbiota, which indicated the association between the VD and the activity of UC might be regulated by gut microbiota.

Introduction

Vitamin D (VD) deficiency has been reported to be associated with the risk of chronic inflammatory diseases, such as ulcerative colitis (UC).[1,2] Increasing serum VD can ameliorate the activity and severity of UC, indicating the potential therapeutic role of VD for patients with UC.[3,4] A study demonstrated that vitamin D changes the microbiota composition, increasing beneficial bacteria, such as *Ruminococcaceae*, *Akkermansia*, *Faecalibacterium*, and *Coprococcus*, and decreasing *Firmicutes*.[5] VD and vitamin D receptor (VDR) are essential in maintaining the integrity and function of the gastrointestinal barrier.[6] These findings indicate the association between VD metabolism and gut microbiota.[7] The dysbiosis of gut microbiota contributes to the pathogenesis of UC,[8] which raises research interest in the relationship among VD, gut microbiota, and UC. Fecal microbiota transplantation (FMT) is an effective method to reconstruct gut microbiota that induces clinical improvement and remission in patients with UC according to randomized controlled trials and our long-term real-world studies.[9–12] FMT is the permitted medical therapy for *Clostridioides difficile* infection and other gut microbiota

dysbiosis-related diseases, including UC, based on Chinese policy.[13] The improved methodology of FMT based on the automatic washing process and the related delivering consideration was coined as washed microbiota transplantation (WMT) by the consensus statement released by the FMT-standardization Study Group in 2019, which is the new generation of FMT.[14,15]

However, whether WMT can regulate the metabolism of VD during gut microbiota reconstruction in patients with UC is still unknown. This study investigated the VD profile and VDR expression under WMT intervention and evaluated the relationships among lifestyle behaviors, gut microbiota composition, and disease severity. Our findings may help to demonstrate the mechanism of WMT in treating UC.

Materials and methods

Study participants. This study recruited patients with UC aged 16–65 from May 2017 to July 2021 at the Second Affiliated Hospital of Nanjing Medical University, based on our registered clinical trials (NCT01790061), which included two steps: clinical discovery and clinical research. All subjects provided written informed consent in accordance with *Declaration of Helsinki*. In the clinical discovery, we detected the serum levels of 25-hydroxyvitamin D [25(OH)D] in 121 patients with UC and 53 healthy controls (HC). In addition, we collected the potential influence factors for the 25(OH)D levels, including gender, body mass index (BMI), the extent and severity of disease, Mayo score, ultraviolet (UV) radiation, daily sunlight exposure, and use of VD supplements. In the clinical research, we conducted a non-randomized control trial (non-RCT) to compare the effects of WMT modalities on serum VD levels in patients with UC and explore intestinal VDR expression and fecal microbiota changes.

In the non-RCT, patients were non-randomly assigned to the WMT group and the non-WMT group (5-aminosalicylic acid, 5-ASA). The allocation of patients was mainly determined by our clinician expert panel's discussions, and to some extent, patients' wishes were also taken into account. Participants completed a 3-month follow-up and received serum 25(OH)D level measurement at admission and the follow-up endpoint. The inclusion criteria included (i) aged 16–65 years old; (ii) diagnosed with UC for at least 6 months based on clinical symptoms and endoscopy; and (iii) able to tolerate colonoscopy. The exclusion criteria included (i) previous immunosuppressive drugs use within 6 weeks before recruitment, steroid hormone use within 4 weeks before recruitment, or antibiotic use within 1 week before recruitment; (ii) history of surgery and WMT treatment within 3 months before recruitment; and (iii) enterocutaneous fistula, infection, abnormal thyroid and parathyroid function, tumor, or pregnancy.

Donor screening and WMT procedure. Healthy adults and adolescents (preferably aged 6–24 years old) are informed of the risks and benefits of becoming a donor and sign an informed consent form (adolescents are required to sign an informed consent form by both themselves and their guardian) and then undergo a questionnaire, a face-to-face screening, and a laboratory test to qualify as a donor.[15] The main criteria for review include age, physiology, pathology, psychology, integrity, time, environment, and recipient status.[14,16] Donor screening success rate of 3.1% (32/1036).[13] Donors will still undergo periodic laboratory screening to determine eligibility.

Our previous reports described the original FMT preparation method in detail.[17] After April 2014, the methodology of FMT was coined as WMT, which is based on the automatic microfiltration machine (GenFMTer, FMT Medical, Nanjing, China).[15] Fresh stool from the donor was microfiltered, centrifuged, washed, discarded, and diluted in this machine, which was performed in a biosafety level 3 laboratory. Washed microbiota suspension from donors will be delivered to patients' intestine within 1 h. The delivery route in this study for microbiota transplantation was a colonic transendoscopic enteral tube (TET) (diameter 2.7 mm, FMT Medical, Nanjing, China),[14] which has high acceptance and high satisfaction.[18]

Primary and secondary outcomes. The primary outcome was the change in serum 25(OH)D levels after receiving two kinds of treatment. The secondary outcomes were the intestinal VDR expression and fecal microbiota changes after WMT. In addition, the potential influence factors for the 25(OH)D levels in patients with UC were analyzed.

Serum 25(OH)D levels and lifestyle behaviors. Serum 25(OH)D reflects the storage of serum VD in serum and is used to assess VD sufficiency.[19] According to the 2011 US Institute of Medicine report (on VD), serum 25(OH)D levels ≥20 ng/mL were considered adequate, while 25(OH)D levels <20 ng/L were defined as VD deficiency.[20] We defined the threshold for the 25(OH)D level increase as 1.2-fold after treatment compared to the baseline (IVD). Otherwise, it was considered as no increase in VD (NIVD). Daily sunlight exposure time, the average value of the UV radiation 20 days before sample collection, and the history of VD supplement within the past 30 days of all patients were recorded. The Atmospheric Sub-Center of the Chinese Ecosystem Research Network (CERN) calculated the daily UV radiation. Efficient models for estimating UV radiation under various sky conditions were developed based on the measurements of UV radiation from CERN. The empirical estimation models were introduced by analyzing the dependence of UV irradiation on the clearness index, the solar elevation angle, and sunshine durations. These estimation models were combined with a hybrid model to obtain the dataset of daily UV radiation at routine weather stations belonging to the China Meteorological Administration (CMA).[21,22] This dataset was not publicly published before the present report.

Sample collection and metagenomic sequencing. Fresh stool samples were collected and stored at −80°C for microbiome analysis until extraction. The total gut microbiota genomic DNA was extracted from stool samples using the QIAamp PowerFecal DNA Kit (Qiagen, Germany) and sequenced on the Illumina NovaSeq PE 150 platform. The raw sequencing data were subjected to quality control by KneadData (v0.7.2) utilizing Trimmomatic 0.36 software to filter and remove low-quality reads or reads mapped to the host genome. After quality control, 292 840 587 and 240 123 692 metagenomic sequences were obtained in the two groups of samples, respectively. Microbial taxonomic

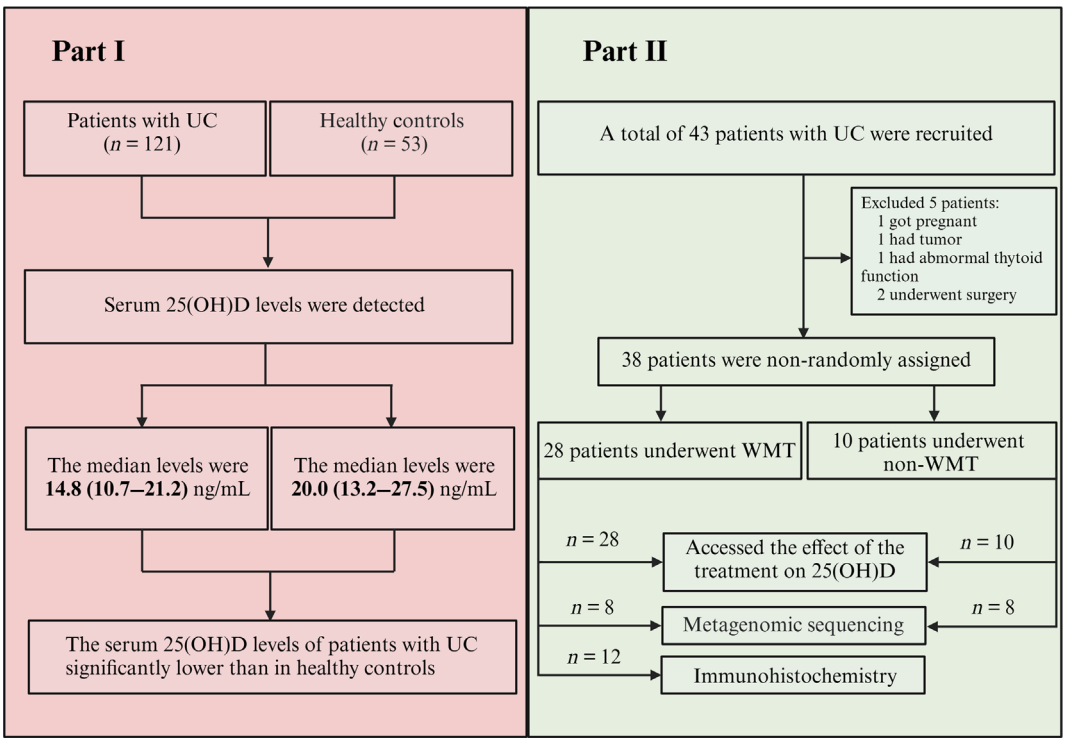

Figure 1 The flowchart of the study. 25(OH)D, 25-hydroxyvitamin D; Part I, clinical discovery; Part II, clinical research; UC, ulcerative colitis; WMT, washed microbiota transplantation.

and functional annotations were derived utilizing MetaPhlAn2 and HUMAnN2, as reported before.[23] The linear discriminant analysis (LDA) score of significantly abundant species marker in each group was calculated by linear discriminant analysis effect size (LEfSe) using a cutoff of LDA > 2.0.[24]

All metagenomic sequences have been deposited at the National Center for Biotechnology Information (NCBI) under the BioProject ID PRJNA929645 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA929645/).

Immunohistochemistry. For immunohistochemistry (IHC), the intestinal mucosa was sampled from the terminal ileum and sigmoid colon lesion 20–30 cm from the anus. The samples were fixed in 10% paraformaldehyde solution, embedded in paraffin within 12–24 h, and then sectioned. According to standard protocols, primary antibody VDR (sc13133; Santa Cruz, dilution 1:400) and secondary antibodies were used to stain the sections. Negative controls were stained with secondary antibodies only. Under the microscope, yellow granules appearing in the cytoplasm indicated positive cells. Three observers blindly scored images of representative areas for each sample. An average score represented the final score. A 4-point scale ranging from 0 to 3 was used to score staining intensity. In this scale, a score of 0 corresponded to the lightest or absence of staining of VDR, whereas 3 corresponded to the strongest staining.

Statistical analyses. Comparisons between two groups were assessed by means of $\chi 2$ contingency tables or Wilcoxon–Mann–Whitney *U* tests or Student's *t*-test as

Table 1 Characteristics of the patients in two cohorts

Characteristics	WMT (*n* = 28)	Non-WMT (*n* = 10)	*P* value
Male, *n* (%)	14 (50.0)	8 (80.0)	0.143
Age, years (mean ± SD)	42.36 ± 2.58	40.9 ± 3.17	0.760
BMI (kg/m^2) (mean ± SD)	20.25 ± 0.59	21.75 ± 1.36	0.247
Mayo score (mean ± SD)	8.14 ± 0.436	9.5 ± 0.48	0.093
Extent of disease, *n*			0.842
E1, proctitis	3	0	
E2, left-sided colitis	5	2	
E3, pancolitis	20	8	
Disease severity, *n*			0.435
Mild	5	0	
Moderate	17	7	
Severe	6	3	
Serum 25(OH)D (ng/mL) (mean ± SD)	14.15 ± 1.03	14.97 ± 2.17	0.706
VD deficiency (<20 ng/mL) (*n*/*N*)	24 (24/28)	7 (7/10)	0.351
Sunlight exposure, *n*			0.201
<0.5 h/d	5	1	
0.5–1 h/d	13	2	
>1 h/d	10	7	
UV radiation (MJ/m^{2}d) (mean ± SD)	0.344 ± 0.02	0.453 ± 0.83	0.071
VD supplementary, n (%)	9 (24.3)	1 (10.0)	0.236

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; SD, standard deviation; UV, ultraviolet; VD, vitamin D; WMT, washed microbiota transplantation.

appropriate. Spearman correlation analysis was used for variable correlation analysis, and $P < 0.05$ was considered significantly correlated. A P value threshold of < 0.3 was used in univariate analysis to include putative risk factors into the multivariate model, and multivariate analyses were performed by binary logistic regression models for significant associations. The tests above were achieved using IBM SPSS Statistics version 23.0. P values < 0.05 were considered statistically significant. All statistical tests in our study were performed two-sided.

Results

Patient characteristics. In the clinical discovery, we collected the serum levels of 25(OH)D in 121 patients with UC and 53 HC. Table S1 showed the characteristics of the two groups. The median 25(OH)D of the HC and UC were 20.00 ng/mL (IQR, 13.23–27.52) and 14.77 (IQR, 10.69–21.17), respectively (Table S1). VD deficiency was observed in 45.28% (24/53) of the HC group and 75.2% (91/121) of the UC group, with a significant difference between the two groups ($P < 0.001$). Additionally, we recorded the basic information and clinical characteristics of the 121 patients with UC, which was shown in Table S2.

Based on the clinical discovery, we conducted a non-RCT to explore the relationship between serum VD levels and gut microbes in patients with UC. In the non-RCT, a total of 38 patients with UC were non-randomly assigned, among whom 28 were assigned to the WMT group and 10 were assigned to the non-WMT group. Figure 1 showed the flowchart of the study. We performed a post hoc power analysis calculation based on the mean and standard deviation of the difference in VD changes before and after treatment for each patient in the two groups. It showed that the power analysis was sufficient (95.7%) to detect a difference (2.3643 *vs* 0.2020) between the two groups with a statistical significance of samples (28 in WMT *vs* 10 in non-WMT). There was no significant difference in the extent of the disease or Mayo score between the WMT and non-WMT groups. The detailed comparison of characteristics was shown in Table 1.

Serum 25(OH)D levels are associated with UC severity. A significant decrease in serum 25(OH)D level was found in patients with UC compared with the matched HC

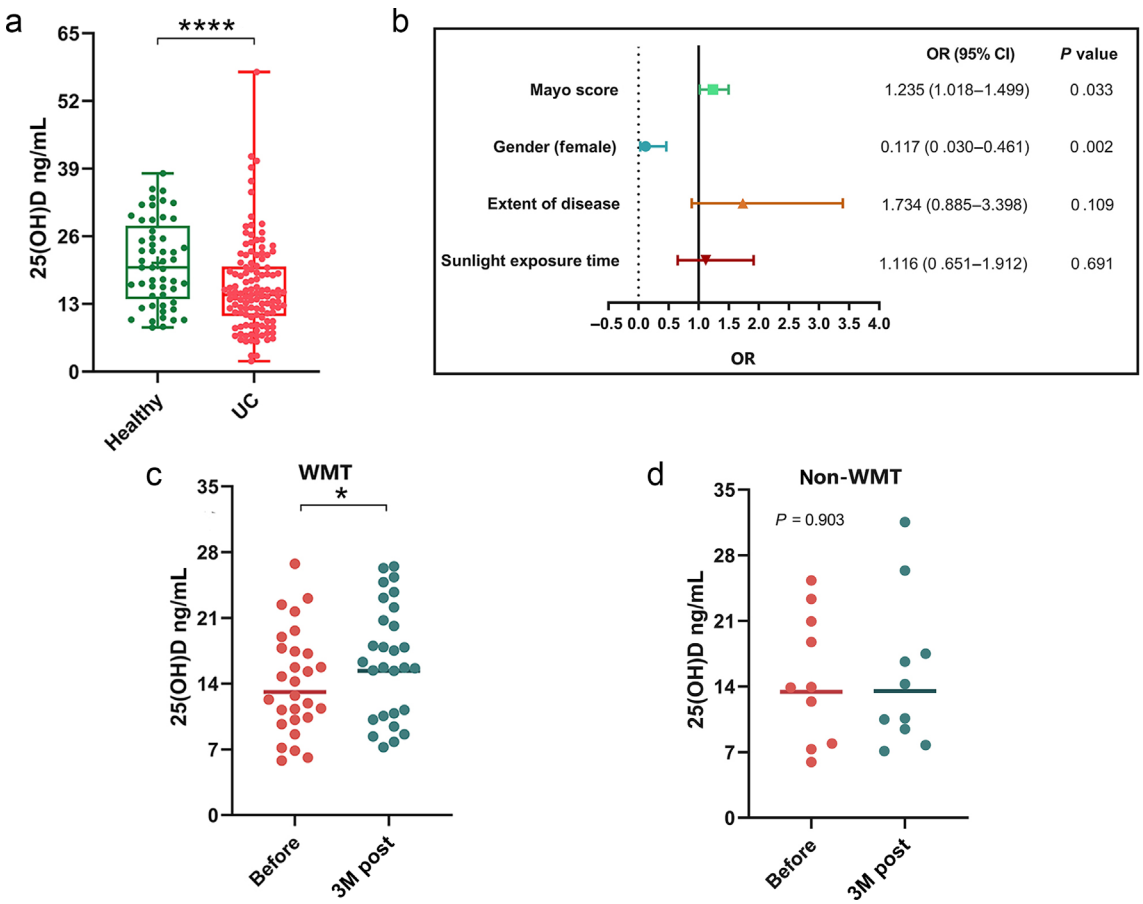

Figure 2 (a) The serum 25(OH)D level before and after treatment. Significant difference in 25(OH)D level between patients with UC and healthy controls (HC) as analyzed by Wilcoxon–Mann–Whitney *U* tests. (b) The risk factors of VD deficiency identified in binary difference logistic regression analysis: for covariate Mayo score, OR = 1.235, 95% CI: 1.018–1.499; for covariate gender (female), OR = 0.117, 95% CI: 0.030–0.461. Comparison of serum 25(OH)D levels before and after treatment in the WMT (c, $n = 28$) and non-WMT (d, $n = 10$) groups using paired Student's *t*-test. Significance levels: $^{*}P < 0.05$, $^{****}P < 0.001$. 25(OH)D, 25-hydroxyvitamin D; UC, ulcerative colitis; VD, vitamin D; WMT, washed microbiota transplantation.

($P < 0.001$) (Fig. 2a). Univariate analysis of vitamin D status identified potential influencing factors (Table S3). Subsequent univariate and multivariate analyses revealed women had a risk of VD deficiency 7.45 times higher than men ($P = 0.002$). Additionally, each one-point increase in the Mayo score was associated with a 23.5% increase in the risk of vitamin D deficiency ($P = 0.033$), indicating a correlation between serum VD level and UC severity (Fig. 2b).

Increased serum 25(OH)D levels after WMT. In the non-RCT, the serum 25(OH)D levels in the WMT group were

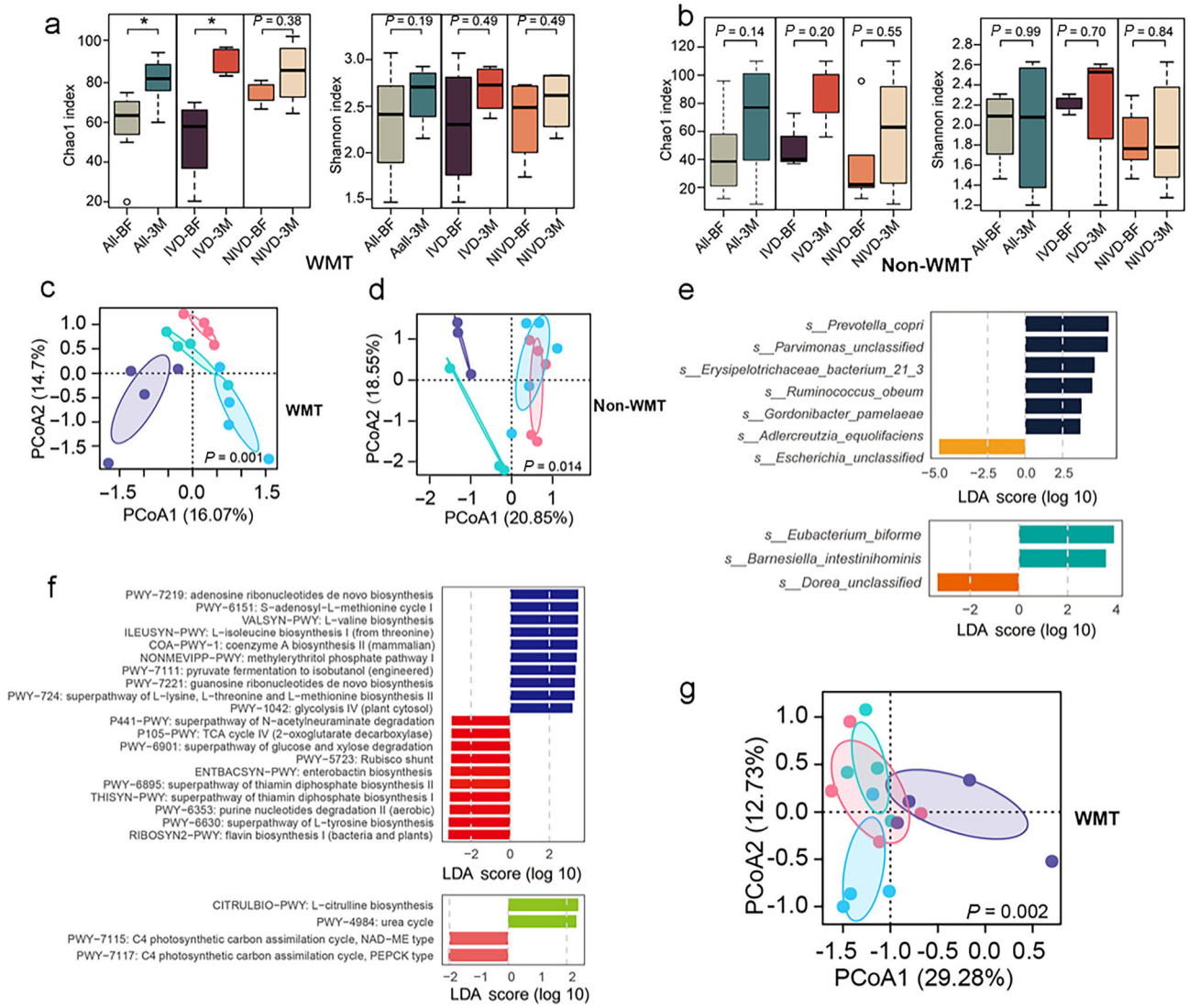

Figure 3 The taxonomic and function analyses of gut microbiota in IVD and NIVD groups. (a,b) Boxplot of α-diversity (Chao 1 index and Shannon index) in patients treated with WMT or non-WMT at the species level by Wilcoxon rank-sum tests (all, $P = 0.01$; IVD, $p = 0.029$). (c,d) β-Diversity (Bray–Curtis similarity index) analyses of gut microbiota in the four groups treated with WMT or non-WMT. Envfit analysis: the *P* values are 0.002 and 0.014, respectively. 95% confidence interval. (c) ●, IVD-BF; ●, IVD-3M; ●, NIVD-BF; ●, NIVD-3M. (d) ●, IVD-BF; ●, IVD-3M; ●, NIVD-BF; ●, NIVD-3M. (e) LEfSe analyses for the differential abundance of species before and 3 months after treatment. The left bars indicate taxa are enriched before treatment, and the right bars indicate taxa are enriched after treatment based on the shift of 25(OH)D in the WMT cohort (linear discriminant analysis (LDA) > 2), by Wilcoxon rank-sum tests. Top: ■, IVD-BF; ■, IVD-3M. Bottom: ■, NIVD-BF; ■, NIVD-3M. (f) LDA scores for the MetaCyc functions show different abundance before and after WMT in IVD and NIVD. Positive and negative LDA scores indicate the pathways are enriched after or before treatment. Only the top 10 in absolute value are shown by Wilcoxon rank-sum tests, LDA > 2. Top: ■, IVD-BF; ■, IVD-3M. Bottom: ■, NIVD-BF; ■, NIVD-3M. (g) Principal coordinate analysis (PCoA) of the microbial pathways of four groups treated with WMT, $P = 0.002$. ●, IVD-BF; ●, IVD-3M; ●, NIVD-BF; ●, NIVD-3M. 25(OH)D, 25-hydroxyvitamin D; 3M, 3 months after treatment; BF, before treatment; IVD, increase of VD; NIVD, no increase of VD; WMT, washed microbiota transplantation.

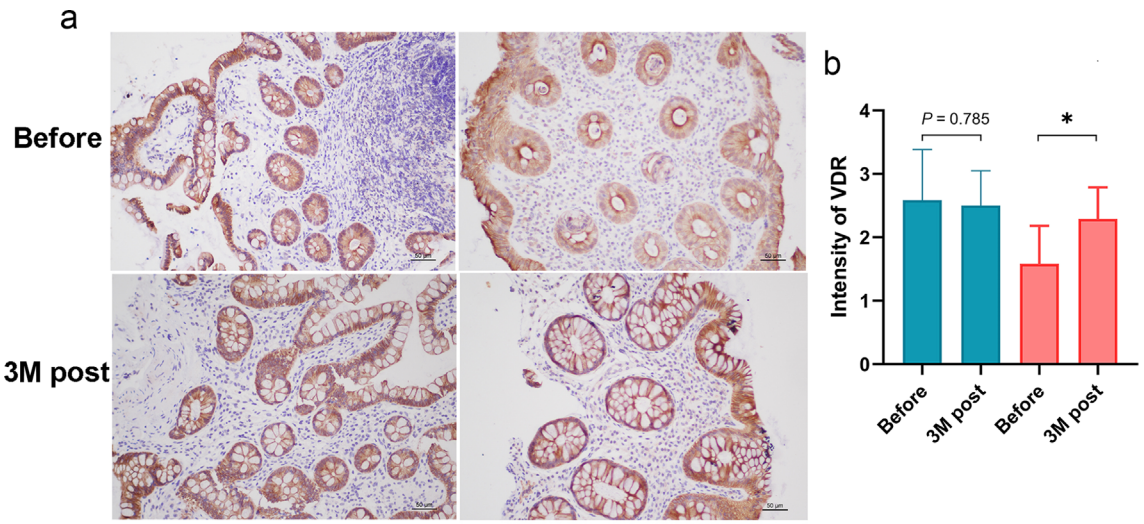

Figure 4 VDR expression in the intestinal mucosa after WMT. (a) IHC scoring for VDR in the intestinal mucosa at 200× magnification, scale bars = 50 μm. VDR staining of the terminal ileum and sigmoid lesion mucosa before and 3 months after WMT. (b) The intensity of VDR score at baseline and 3 months after treatment in the sigmoid lesion and terminal ileum, $*P < 0.05$, paired-samples *t*-tests. VDR, vitamin D receptor; WMT, washed microbiota transplantation. ■, Terminal ileum; ■, Sigmoid lesion.

significantly increased after WMT compared with that before treatment ($P = 0.011$) (Fig. 2c). In contrast, we had no obvious founds in the non-WMT group ($P = 0.903$) (Fig. 2d). The results indicated that WMT might increase VD levels in patients with UC.

Associations between gut microbiota and serum 25(OH)D level. To explore the relationship between the change of serum 25(OH)D level and the composition of gut microbiota in UC, metagenomic sequencing was conducted to evaluate the microbial composition in patients at baseline and 3 months after the treatment ($n = 16$, eight with WMT and eight without WMT). In the WMT group, α-diversity measured by Chao 1 index at the species level was significantly higher after WMT than baseline ($P = 0.01$), while the Simpson index showed no difference. Subgroup analysis showed a significant increase of Chao1 index in the IVD group ($P = 0.029$), but no noticeable change in the NIVD group (Fig. 3a). In the non-WMT group, the α-diversity (Chao1 index and Shannon index) showed no significant change after treatment compared with the baseline level (Fig. 3b). However, both WMT and non-WMT group had a striking differences impact on β-diversity analysis at baseline and 3 months (Fig. 3c,d).

Next, we assessed the relative differential abundance using LEfSe at the species level. Compared to those at baseline, the IVD patients showed a significant increase in the abundances of *Erysipelotrichaceae_bacterium*, *Gordonibacter_pamelaeae*, *Prevotella_copri*, *Parvimonas_unclassified*, *Adlercreutzia_equolifaciens*, and *Ruminococcus_obeum* after WMT. Meanwhile, the abundance of *Escherichia_unclassified* significantly decreased. In NIVD patients, the LDA diagram showed higher abundances of *Eubacterium_biforme* and *Barnesiella_intestinihominis* and a lower abundance of *Dorea_unclassified* after WMT (Fig. 3e). However, in the non-WMT group, there was no significant difference between the baseline level and that at 3 months after the treatment at the species level.

In total, 94 pathways differed before and after treatment in IVD patients, while in the NIVD group, only four pathways differed. Ten metabolic pathways with top absolute values were selected based on the LDA score, including energy metabolism, lipids metabolism, vitamin biosynthesis, glycan biosynthesis and metabolism, nucleotide biosynthesis, amino acid biosynthesis, and xenobiotics biodegradation (Fig. 3f). The branched-chain amino acids (BCAAs) biosynthesis pathway, including L-isoleucine and L-valine biosynthesis, was significantly altered after treatment in the IVD group, compared with the baseline. A principal coordinate analysis (PCoA) revealed a significant difference in gut microbiota communities among the four groups undergoing WMT ($P = 0.002$) (Fig. 3g).

VDR expression after WMT. To address the effects of WMT on intestinal epithelial VDR, we further assessed the VDR expression in the terminal ileum and sigmoid colon lesion mucosa at baseline and 3 months after WMT. The mucosa samples from 12 patients who underwent colonoscopy showed that the expression of VDR was increased at sigmoid colon lesions after treatment, compared with that at baseline ($P = 0.026$). However, VDR expression at the terminal ileum showed no significant difference after WMT (Fig. 4).

Discussion

In Part I, 75.2% of patients with UC were deficient and had significantly lower VD levels than HC, and this interesting clinical phenomenon stimulated our interest in further exploring the relationship between gut microbiota and VD levels in patients with UC. Based on the clinical findings, we designed a non-RCT and found that WMT significantly elevated VD levels in patients with UC.

VD is a steroid hormone synthesized in response to sufficient sunlight exposure. A previous study reported that people with more sun exposure had higher 25(OH)D levels than those with less sun exposure.[7] However, this result was not observed in the multiple factor analysis of this study. The univariate analysis in our study showed that UV radiation brought no statistical difference in VD status, which may be explained by patients with UC having difficulty converting sunlight exposure into vitamin D levels. It also indicates that sufficient sunlight exposure is not critical for VD normality in patients with UC. In this study, women had a higher risk of VD deficiency than men. This result is similar to a previous cross-sectional study that noted a higher risk of VD deficiency in pregnant women with IBD,[25] but the mechanism for this remains to be explored.

As indicated by α-diversity, the evenness of the WMT group increased after treatment, while no difference was found in the non-WMT group, which further implicated that VD level is closely related to heterogeneous characteristics of gut microbiota in patients with UC. In addition, the abundance of certain bacteria (e.g. *Ruminococcus_obeum*, *Dorea*, and *Adlercreutzia_equolifaciens*) at the species level significantly correlated with VD, whereas *Escherichia* showed an opposite trend: it was highly enriched in a condition of lower baseline VD level. Furthermore, increasing evidence shows that gut bacteria produce short-chain fatty acid (SCFA), which exerts immunomodulatory and anti-inflammatory impacts in UC.[26] *Ruminococcus_obeum* and *Dorea*, which produce acetate and butyrate, significantly increased their relative abundances in IVD.[27] Sun *et al*. reported that butyrate upregulated the VDR signaling expression and suppressed inflammation in a colitis model.[28] Other studies confirmed that the abundances of *Ruminococcus_obeum* and *Dorea* were increased after FMT treatment, making them potential predictors for the FMT's efficacy in treating UC.[12,29] *Adlercreutzia_equolifaciens* were generally regarded as the species that can produce equol, an active metabolite of isoflavone.[30] Recent researches have reported that isoflavones could inhibit the activity of VD hydroxylases CYP24, a substance that can convert 25(OH)D into an active form, $1,25(OH)_2D$.[19,31,32] Gubatan stated that VD can exert an anti-inflammatory effect by inducing the production of cathelicidin and reducing the abundance of *Escherichia*.[33] The above information further suggests that *Adlercreutzia_equolifaciens* has a positive correlation, but *Escherichia* negatively correlated with 25(OH)D level. These findings might explain the anti-inflammatory mechanism of gut microbiota: It can inhibit intestinal inflammation in IBD patients by increasing the level of VD and the expression of VDR.

Our metabolic pathway analysis showed that the biosynthesis of BCAA increased in the IVD group treated with WMT. BCAAs can enhance intestinal development, intestinal amino acid transportation, mucin production, and innate and adaptive immune responses.[34] In addition, Low *et al*. found that KEGG pathway in colonic biopsies from UC with disease duration more than 20 years showed leucine and isoleucine degradation,[35] suggesting the necessity of understanding the potential role of BCAAs metabolism in IBD.

VD exerts an anti-inflammatory effect mainly by acting on VDR, and the inhibition of VDR can lead to intestinal colitis.[36] Several studies have shown that VD and VDR play a regulatory role in UC.[37] Probiotics increased VD/VDR expression in mouse and human intestinal epithelial cells.[38,39] In line with these findings, our study observed a significant increase in VDR expression at the sigmoid colon following WMT, which may play an important role in the anti-inflammatory effect in patients with UC.

There are several limitations in this study, such as 1,25(OH)2D, as the active form of VD was not measured to meet the limitations of the experiment condition. The current form of the study is still superficial and just indicates the association between VD levels and gut microbes. The part of clinical discovery collected information on a relatively larger number of subjects. However, the subsequent non-RCT had a small sample size. Studies with enlarged sample sizes and randomized trials should be performed in the future to explore the relationship between VD metabolism and gut microbiota.

In conclusion, this study indicated the correlation between serum VD levels and the activity of UC, which was related to the abundance of certain gut microbiota. The increase in serum VD level and VDR expression after WMT might be the mechanism that inhibits the inflammatory response of WMT, which further provides evidence for linking the anti-inflammatory effect of WMT to UC treatment and VD metabolism.

Acknowledgments

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Ethical approval

This study was approved by the institution's ethics committee of the Second Affiliated Hospital of Nanjing Medical University.

Informed consent

All subjects provided written informed consent in accordance with *Declaration of Helsinki*.

Data availability statement. All metagenomic sequences obtained during the current study are available in the [NCBI] repository [https://dataview.ncbi.nlm.nih.gov/object/PRJNA929645]. The other data used and/or analyzed in this study are available from the corresponding author upon reasonable request.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. The characteristics between healthy controls and patients with UC.
Table S2. The clinical characteristics of the collected population.
Table S3. Univariate analysis of vitamin D status.