Digestion

Research Article

Digestion 2023;104:357–369
DOI: 10.1159/000528282

Received: August 10, 2022
Accepted: October 31, 2022
Published online: May 12, 2023

Relationship between Chemotherapy-Induced Diarrhea and Intestinal Microbiome Composition

Yuka Kawasaki[a] Kazuki Kakimoto[a] Yasuyoshi Tanaka[a] Hikaru Shimizu[a] Koji Nishida[a] Keijiro Numa[a] Naohiko Kinoshita[a] Yoshihiro Tatsumi[a] Kei Nakazawa[a] Ryoji Koshiba[a] Yuki Hirata[a] Kazuhiro Ota[a] Naokuni Sakiyama[a] Tetsuji Terazawa[a] Toshihisa Takeuchi[a] Takako Miyazaki[a] Masahiro Goto[a] Haruka Yokota[b] Yutaka Makizaki[b] Yoshiki Tanaka[b] Shunji Nakajima[b] Hiroshi Ohno[b] Kazuhide Higuchi[a] Shiro Nakamura[a] Hiroki Nishikawa[a]

[a]2nd Department of Internal Medicine, Osaka Medical and Pharmaceutical University, Osaka, Japan; [b]R&D Center, Biofermin Pharmaceutical Co., Ltd., Kobe, Japan

Keywords
Chemotherapy · Intestinal microbiome · Fluoropyrimidines · Diarrhea

Abstract

Background and Aim: Fluoropyrimidines (FPs) are key drugs in many chemotherapy regimens; however, recipients are often prone to diarrhea due to gastrointestinal toxicity. Disruption of the intestinal epithelial barrier function by FPs leads to dysbiosis, which may exacerbate intestinal epithelial cell damage as a secondary effect and trigger diarrhea. However, despite studies on chemotherapy-induced changes in the intestinal microbiome of humans, the relationship between dysbiosis and diarrhea is unclear. In this study, we aimed to investigate the relationship between chemotherapy-induced diarrhea and the intestinal microbiome. ***Methods:*** We conducted a single-center prospective observational study. Twenty-three patients who received chemotherapy, including FPs as first-line chemotherapy for colorectal cancer, were included. Stool samples were collected before the start of chemotherapy and after one cycle of treatment to analyze intestinal microbiome composition and perform PICRUSt predictive metagenomic analysis. ***Results:*** Gastrointestinal toxicity was observed in 7 of 23 patients (30.4%), diarrhea was observed in 4 (17.4%), and nausea and anorexia were observed in 3 (13.0%). In 19 patients treated with oral FPs, the α diversity of the microbial community decreased significantly following chemotherapy only in the diarrheal group. At the phylum level, the diarrheal group showed a significant decrease in the abundance of Firmicutes and a significant increase in the abundance of Bacteroidetes with chemotherapy (p = 0.013 and 0.011, respectively). In the same groups, at the genus level, *Bifidobacterium* abundance was significantly decreased (p = 0.019). In contrast, in the non-diarrheal group, Actinobacteria abundance increased

karger@karger.com
www.karger.com/dig

Correspondence to:
Kazuki Kakimoto, kazuki.kakimoto@ompu.ac.jp

significantly with chemotherapy at the phylum level (p = 0.011). Further, *Bifidobacterium*, *Fusicatenibacter*, and *Dorea* abundance significantly increased at the genus level (p = 0.006, 0.019, and 0.011, respectively). The PICRUSt predictive metagenomic analysis revealed that chemotherapy caused significant differences in membrane transport in KEGG pathway level 2 and in 8 KEGG pathway level 3, including transporters and oxidative phosphorylation in the diarrhea group. ***Conclusion:*** Organic-acid-producing bacteria seem to be involved in diarrhea associated with chemotherapy, including FPs.

Introduction

Chemotherapy is an essential part of cancer treatment; however, adverse events are common [1–3], including gastrointestinal toxicity, such as nausea, vomiting, anorexia, and diarrhea [2]. Grade 3 gastrointestinal toxicity (or higher) makes it difficult to continue chemotherapy, leading to discontinuation of chemotherapy, which is associated with poor cancer prognosis [4, 5]. Severe diarrhea can lead to electrolyte abnormalities, dehydration, renal failure, and circulatory failure [5]. Furthermore, diarrhea combined with neutropenia can lead to sepsis, which can be fatal [2]. Therefore, management of chemotherapy-induced diarrhea is of great clinical importance for the treatment and prognosis of cancer.

Known anticancer agents that can cause diarrhea include 5-fluorouracil (5-FU), irinotecan (CPT-11), pyrimidine fluoride, cytarabine, doxorubicin, and high doses of methotrexate [2]. Fluoropyrimidines (FPs), including 5-FU, are vital in many chemotherapy regimens and are often used to treat gastrointestinal cancers. It mechanistically interferes with DNA and RNA synthesis by inhibiting thymidylate synthesis, thereby exerting antitumor activity [6, 7]; however, it also damages intestinal epithelial cells, symptomatically resulting in diarrhea. In experimental mouse models, 5-FU-induced epithelial cell damage has been reported to result in the release of various proinflammatory cytokines and expression of stress response factors [8]. In contrast, when the barrier function of the intestinal epithelium is compromised by inflammation, the diversity of the intestinal microbiome is reduced, resulting in dysbiosis [9]. Hamouda et al. [10] showed that 5-FU-induced dysbiosis triggered intestinal mucosal injury in an experimental mouse model, indicating that oral administration of broad-spectrum antibiotics or probiotics to alter the intestinal microbiome improves mucosal injury in the small intestine. Therefore, dysbiosis associated with 5-FU may exacerbate intestinal mucosal injury secondarily. However, few studies have analyzed chemotherapy-induced changes in the human intestinal microbiome, and the relationship between chemotherapy-induced dysbiosis and diarrhea remains unclear. In this study, we attempt to elucidate the relationship between diarrhea associated with FPs chemotherapy and the intestinal microbiome.

Methods

Patients and Chemotherapy

We conducted a single-center, prospective, observational study. Patients who received chemotherapy, including FPs as first-line chemotherapy for colorectal cancer at Osaka Medical and Pharmaceutical University Hospital, from December 2018 to March 2020, were included. The exclusion criteria included the use of antibiotics between 3 weeks before the start of chemotherapy and the end of the first course of chemotherapy and initiation or change of oral probiotics between 3 weeks before the start of chemotherapy and the end of the first course of chemotherapy. Blood tests and stool samples were collected before the start of chemotherapy and after the completion of one cycle of treatment. Bacterial flora and fecal calprotectin levels were analyzed in fecal samples. Patients with diarrhea at the end of the first chemotherapy cycle were classified into the diarrhea group, and those without diarrhea were classified into the non-diarrhea group.

The FPs-based drugs are TS-1®, capecitabine, and 5-FU. With regard to the FPs-based regimens, patients were treated with TS-1® + oxaliplatin, capecitabine, capecitabine + oxaliplatin, and folinic acid + 5-FU + oxaliplatin. In addition, bevacizumab and panitumumab were also added as molecular-targeting agents for some patients. TS-1® and capecitabine were administered orally, whereas 5-FU was administered intravenously. Analyses were performed separately for the group that received oral FPs and the group that received only intravenous FPs.

16S rRNA Analysis

Analysis of bacterial flora was performed by comprehensive 16S rRNA sequencing using next-generation sequencing. DNA was extracted from feces using the bead-phenol method [11], and sequence analysis of the V3–V4 region of the 16S rRNA gene was carried out using the MiSeq platform, following the method described by Fadrosh et al. [12]. The analysis of sequenced read data obtained from MiSeq was performed using the QIIME pipeline [13], i.e., read merge was performed using Fastq-join and quality filtering (QV ≥25) was performed using USEARCH v6.1. Chimeric reads were then removed from the read data by filtering, and the resulting read data were used for bacterial flora analysis. Five thousand reads per sample were randomly selected to create operational taxonomic units (OTUs), with a homology threshold of 97%, using USEARCH. A homology search was performed on representative sequences of the OTUs using UCLIST, to identify the relative abundance of bacterial flora and the bacteria in each read to the genus level. Comparisons of alpha diversity (using observed OTUs, chao1, abundance-based coverage estimator, and Shannon index) and beta diversity (using UniFrac analysis) were

Table 1. Baseline demographics and clinical characteristics

Patients, *n*	23
Male/female, *n*	19/4
Age, median (IQR), years	62 (52–68)
Tumor location	
Ascending colon/sigmoid colon/rectum	2/4/17
TNM stage	
II/III/IV	3/12/8
Route of FPs administration, *n*	
Including oral FPs/intravenous FPs only	19/4
Chemotherapy regimens, *n*	
SOX/capecitabine/CAPOX/FOLFOX	3/2/14/4
Combination of molecular targeted drugs, *n*	
Bevacizumab/panitumumab	12/1

FPs, fluoropyrimidines; SOX, TS-1® + oxaliplatin; CAPOX, capecitabine + oxaliplatin; FOLFOX, folinic acid + fluorouracil + oxaliplatin.

performed to examine the similarity of each flora [14]. PICRUSt predictive metagenomic analysis was performed based on the 16S rRNA gene analysis.

Evaluation of Diarrhea

Diarrhea was graded according to the Common Terminology Criteria for Adverse Events Version 4.0, as follows: grade 0 (no diarrhea), grade 1 (an increase to <4 bowel movements/day compared to pre-treatment), grade 2 (an increase to 4–6 bowel movements/day compared to pre-treatment or nocturnal bowel movements), grade 3 (increase in defecation frequency to ≥7 times/day or need for intravenous fluids for incontinence or dehydration compared to pre-treatment), and grade 4 (conditions requiring intensive care or circulatory collapse).

Statistics

Statistical analysis was performed using the paired *t* test for the analysis of blood sample data and the Wilcoxon rank-sum test for the analysis of fecal calprotectin. In the analysis of the flora, Welch's *t* test and paired *t* test were used to test the relative proportion of flora composition and alpha diversity, while PERMANOVA was used to test beta diversity. All statistical analyses were performed using the JMP v15.2.1 software (SAS Institute, Cary, NC, USA). Statistical significance was set as $p < 0.05$ (two-sided test).

Results

Patients and Gastrointestinal Toxicity

Twenty-three patients were included in the study, including 19 men and four women (Table 1). Their median age was 62 years. Chemotherapy consisted of TS-1® + oxaliplatin in 3 patients, capecitabine in 2 patients, capecitabine + oxaliplatin in 14 patients, and folinic acid + fluorouracil + oxaliplatin in 4 patients. Bevacizumab was added for 12 patients and panitumumab for 1 patient. FPs were administered orally to 19 patients and intravenously only in 4 patients.

Seven patients (30.4%) developed gastrointestinal toxicity, including diarrhea in 4 patients (17.4%) and nausea and anorexia in 3 patients (13.0%). In patients treated with oral FPs, three developed diarrhea (2 cases of grade 2 and 1 case of grade 1) and three developed nausea and anorexia. In patients treated with intravenous FPs, only 1 case of grade 1 diarrhea was observed.

Analysis in Patients Treated with Oral FPs

Intestinal Microbiome Comparisons before Chemotherapy in the Diarrhea and Non-Diarrhea Groups

In 19 patients treated with oral FPs, the intestinal microbiome before chemotherapy was compared between the diarrheal and non-diarrheal groups. Observed OTUs, chao1, abundance-based coverage estimator, and Shannon indices were used to reflect the alpha diversity of the microbial communities. Principal coordinate analysis was performed to estimate the beta diversity and determine the overall similarity of the microbial community structure among samples. There were no significant differences in alpha and beta diversity (Fig. 1a–f). Furthermore, there were no significant differences in the intestinal microbiome at the phylum level either (Fig. 2a, b). In contrast, at the genus level, the diarrhea group had a significantly lower abundance of *Ruminococcus* and a significantly greater abundance of *Phascolarctobacterium* ($p = 0.041$ and 0.012, respectively; Fig. 2c, d).

Taxonomic Shifts in the Microbiome following Chemotherapy

The intestinal microbiome was compared before and after chemotherapy administration in 19 patients who received oral FPs. There were no significant changes in the alpha and beta diversity with chemotherapy (Fig. 3a–f). On the other hand, at the phylum level, the diarrhea group showed a significant decrease in the abundance of Firmicutes and a significant increase in the abundance of Bacteroidetes ($p = 0.013$ and 0.011, respectively; Fig. 4a, c). At the genus level, *Bifidobacterium* abundance decreased significantly ($p = 0.019$; Fig. 5a, c). In the non-diarrheal group, Actinobacteria abundance increased significantly with chemotherapy at the phylum level ($p = 0.011$; Fig. 4a, b), and *Bifidobacterium, Fusicatenibacter,* and *Dorea* abundance increased significantly at the genus level ($p = 0.006$, 0.019, and 0.011, respectively; Fig. 5a, c).

Blood Test and Fecal Calprotectin in Patients Treated with Oral FPs

We assessed changes in the blood test data and fecal calprotectin levels after chemotherapy. CRP, ALB, HGB,

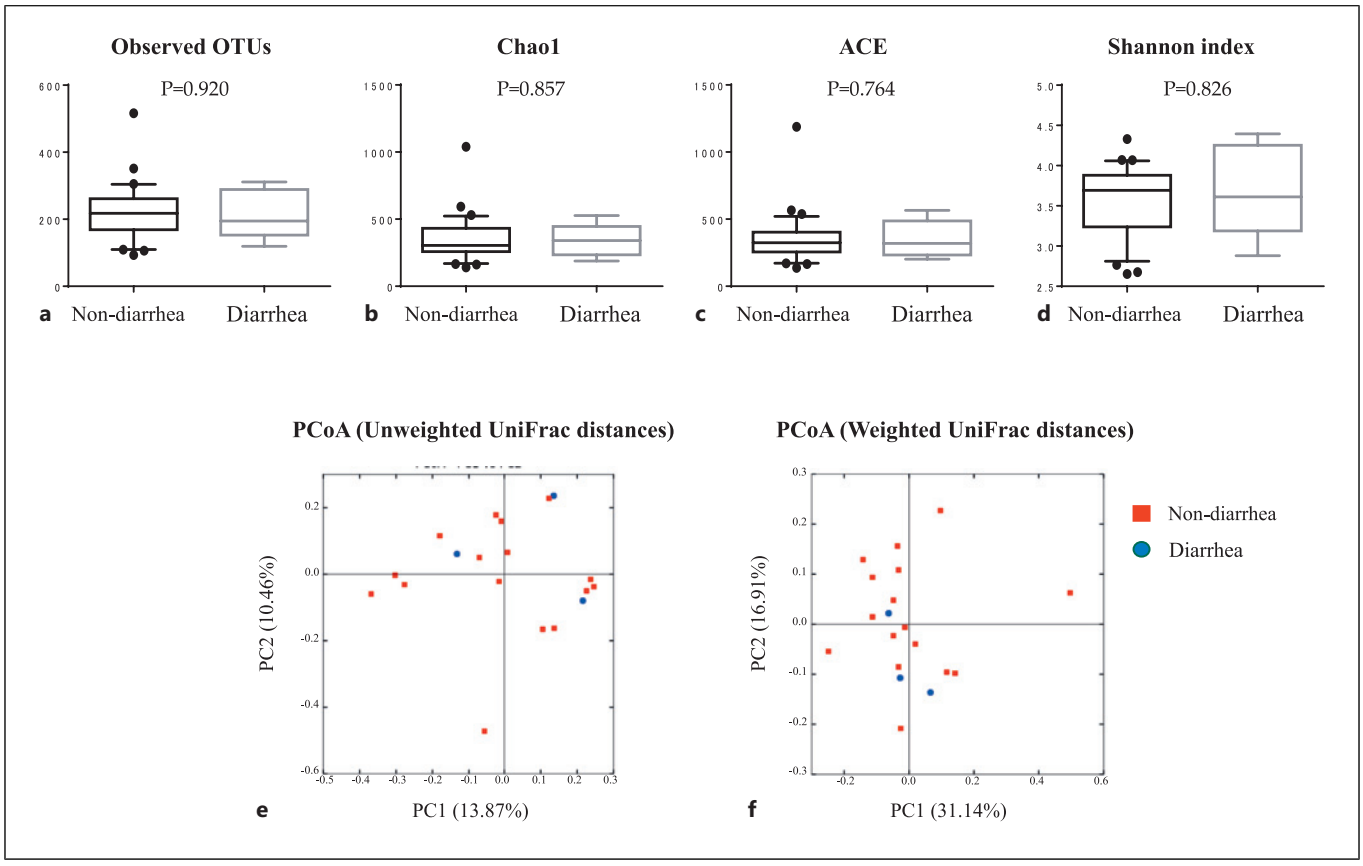

Fig. 1. Comparison of alpha and beta diversity of microbial communities before chemotherapy between the diarrhea and non-diarrhea groups. Alpha diversity indexes include observed OTUs (**a**), Chao1 (**b**), ACE (**c**), Shannon index (**d**). PCoA was generated using an unweighted (**e**) and weighted (**f**) UniFrac distances. ACE, abundance-based coverage estimator; PCoA, principal coordinate analysis.

and fecal calprotectin levels did not change significantly after chemotherapy in either the diarrhea and non-diarrhea groups (Table 2).

Analysis in Patients Treated with Intravenous FPs

Changes in Intestinal Microbiome

The intestinal microbiome was compared before and after chemotherapy administration in 4 patients who received FPs intravenously. There were no significant changes in the alpha and beta diversity after chemotherapy. In addition, there were no significant changes in the microbiome at the phylum or genus level (data not shown).

Functional Shifts in the Microbiome following Chemotherapy in Patients Treated with Oral FPs

Bacterial gene functions were predicted via 16S rRNA gene-based microbial compositions, using the PICRUSt algorithm to make inferences from the KEGG annotation databases. In the diarrhea group, chemotherapy caused significant differences between the 1 KEGG pathway level 2 and 8 KEGG pathway level 3 (Fig. 6, 7b). The microbiome displayed differences in membrane transport in KEGG pathway level 2 and in transporters; oxidative phosphorylation; valine, leucine, and isoleucine biosynthesis; DNA replication; prenyltransferases; tryptophan metabolism; and caprolactam degradation in KEGG pathway level 3.

In the non-diarrhea group, chemotherapy was not associated with significant changes in KEGG pathway level 2 but was associated with significant changes in 6 KEGG pathway level 3 (Fig. 6, 7a). The microbiome showed changes in energy metabolism, nicotinate and nicotinamide metabolism, other transporters, ribosome biogenesis in eukaryotes, and proteasomes in KEGG pathway level 3.

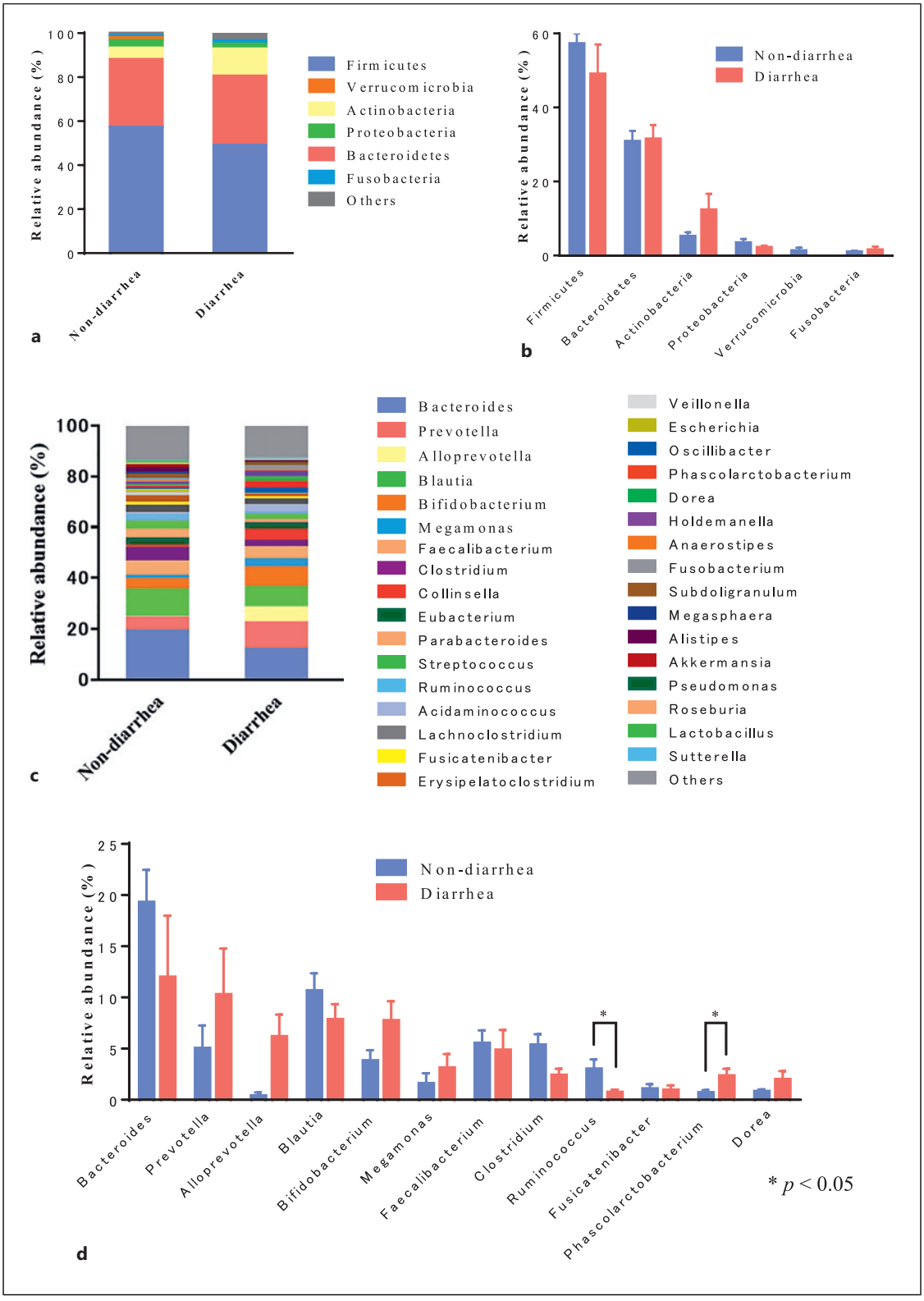

Fig. 2. Comparison of the composition and structure of the microbial community before chemotherapy between the diarrhea and non-diarrhea groups. **a** Stacked bar plot of phyla abundances. **b** Relative abundance of each phylum. **c** Stacked bar plot of genera abundances. **d** Relative abundance of each key genus. Data are expressed as the mean ± SEM. *$p < 0.05$.

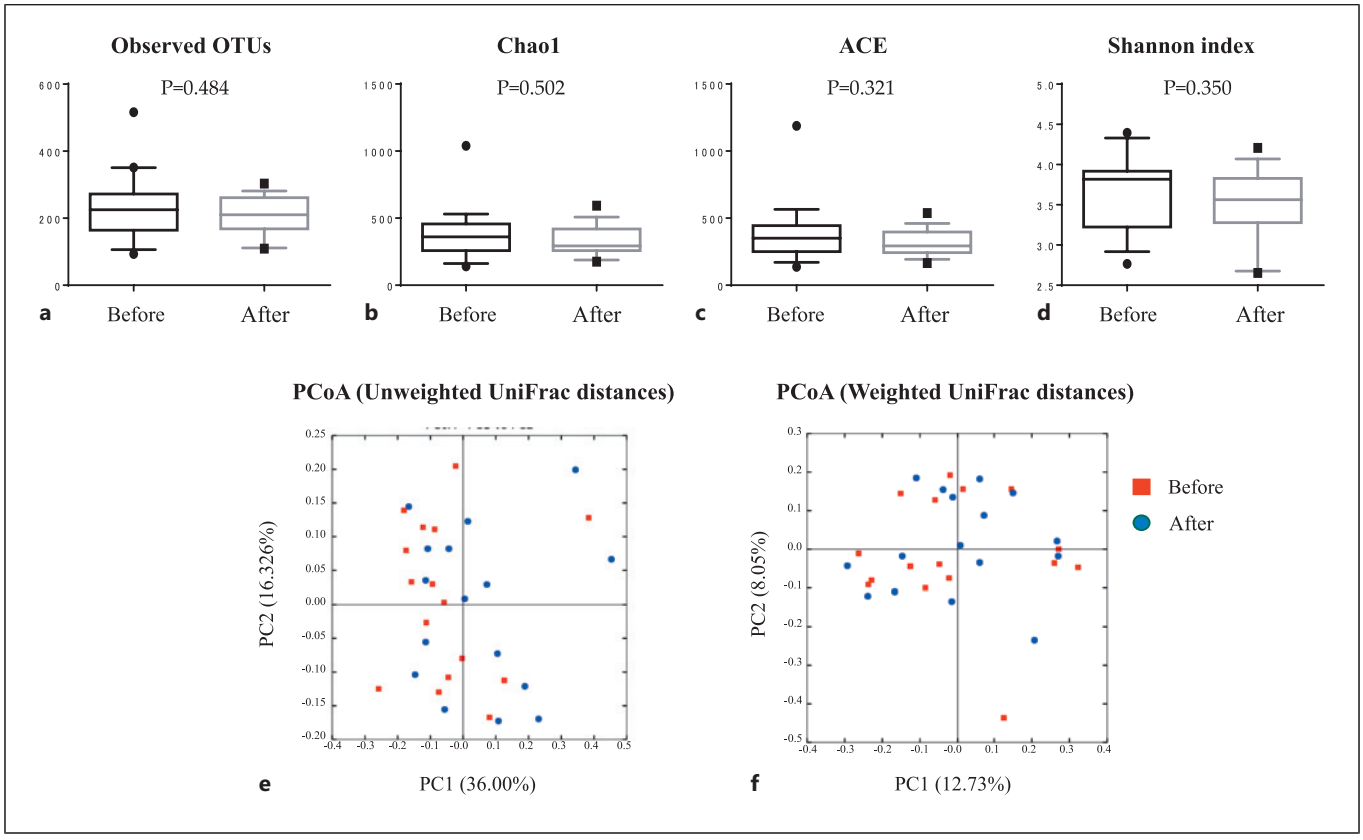

Fig. 3. Comparison of alpha and beta diversity of microbial communities following chemotherapy. Alpha diversity indexes include observed OTUs (**a**), Chao1 (**b**), ACE (**c**), Shannon index (**d**). PCoA generated using an unweighted (**e**) and weighted (**f**) UniFrac distances. ACE, abundance-based coverage estimator; PCoA, principal coordinate analysis.

Discussion

Mechanistically, FPs are toxic to intestinal epithelial cells because it leads to villous atrophy and destruction of the glandular epithelium in the small intestine [15, 16]. In intestinal epithelial cells, NF-κB signaling is activated as an inflammatory response, and inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6, and stress response factors, such as cyclooxygenase-2, are expressed [17, 18]. This induces the apoptosis of epithelial cells, which increases the permeability of the intestinal mucosa, hence reducing barrier function. As a result, intestinal bacteria invade the mucosa, and pathogen-associated molecular patterns and damage-associated molecular patterns are released from the invading bacteria, activating signals via toll-like receptors and inducing the expression of various proinflammatory cytokines and chemokines [17, 18]. In contrast, Hamouda et al. [19] recently reported that dysbiosis, caused by the disruption of the epithelial barrier associated with 5-FU, leads to secondary intestinal mucosal injury. In their study, 5-FU administration in mice decreased the abundance of Firmicutes and increased the abundance of Bacteroidetes and Verrucomicrobia, whereas a marked decrease in the abundance of the intestinal microbiome after administration of broad-spectrum antibiotics improved mucosal injury in the small intestine. This finding suggests that chemotherapy-induced dysbiosis may further aggravate intestinal mucosal injury.

In the present study, we investigated the relationship between the intestinal microbiome and diarrhea associated with chemotherapy, including FPs, in human colorectal cancer. After chemotherapy, the intestinal microbiome showed a decrease in the abundance of Firmicutes and an increase in the abundance of Bacteroidetes at the phylum level and decreased *Bifidobacterium* abundance at the genus level in patients with diarrhea, similar to results observed in mice by Hamouda et al. [19], suggesting that chemotherapy-induced dysbiosis may exacerbate diarrhea in humans, as it does in mice.

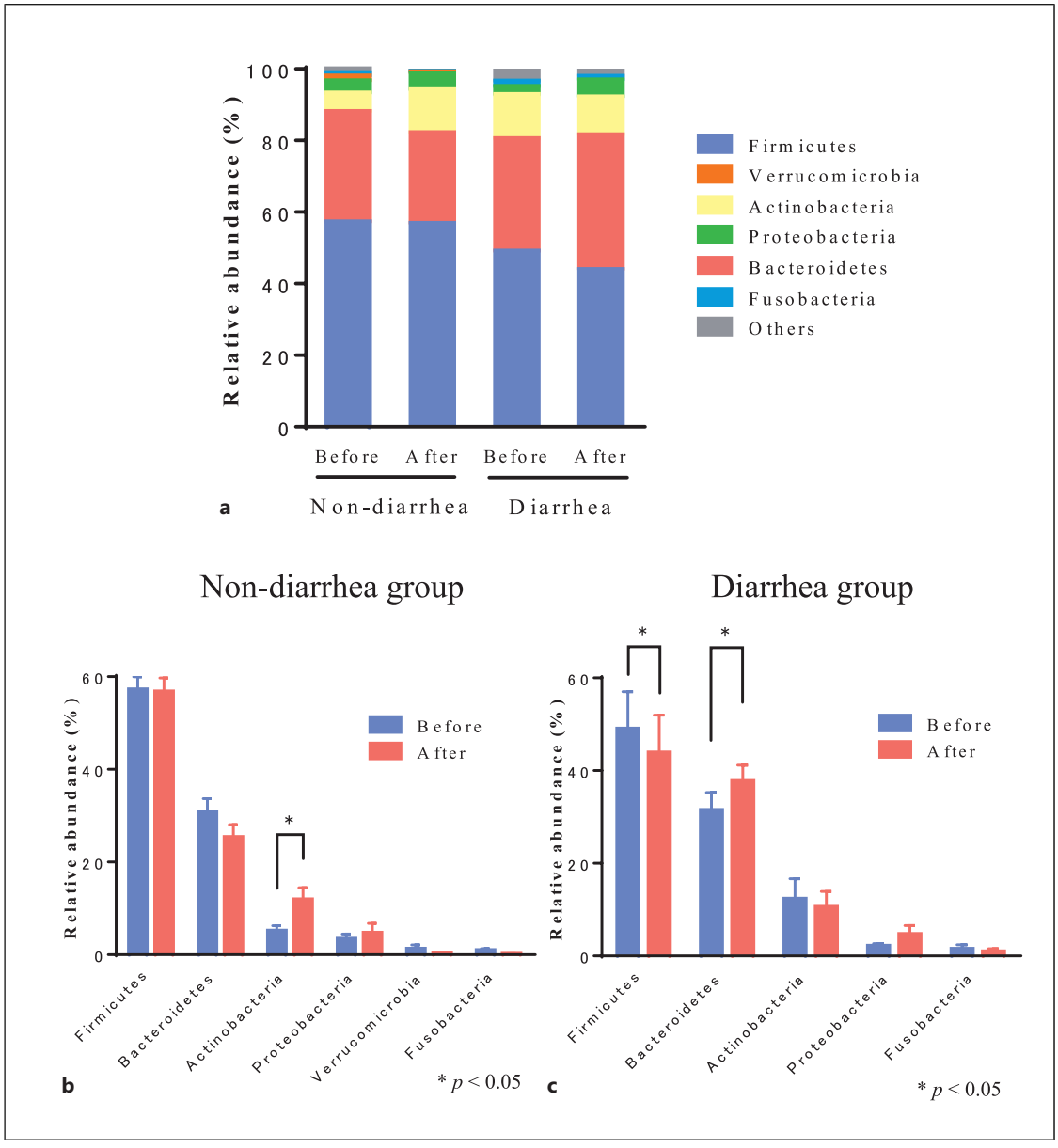

Fig. 4. Comparison of the composition and structure of the microbial community following chemotherapy at the phylum level in the diarrhea and non-diarrhea groups. **a** Stacked bar plot of phyla abundances. **b**, **c** Relative abundance of each key phylum. Data are expressed as the mean ± SEM. *$p < 0.05$.

Interestingly, the abundance of *Bifidobacterium* decreased in the diarrhea group but increased in the non-diarrhea group. *Bifidobacterium* produces short-chain fatty acids, acetic acid, and lactic acid, resulting in an increase in butyric acid bacteria [20, 21]. Butyrate induces regulatory T cells and has anti-inflammatory properties [22]. The genus *Bifidobacterium* inhibits 5-FU-induced intestinal mucosal injury in mouse models [23–25], and FPs-associated diarrhea may have been attributable to that the alteration in the proportion of the genus *Bifidobacterium*. In the non-diarrhea group, *Fusicatenibacter* and *Dorea* abundance also increased significantly after chemotherapy. *Fusicatenibacter* is an organic-acid-producing bacterium that has been reported to be less common in patients with ulcerative colitis [26]. Furthermore, in the post-chemotherapy flora, the genera *Blautia* and *Veillonella*, which are members of the phylum Firmicutes, were less abundant in the diarrhea

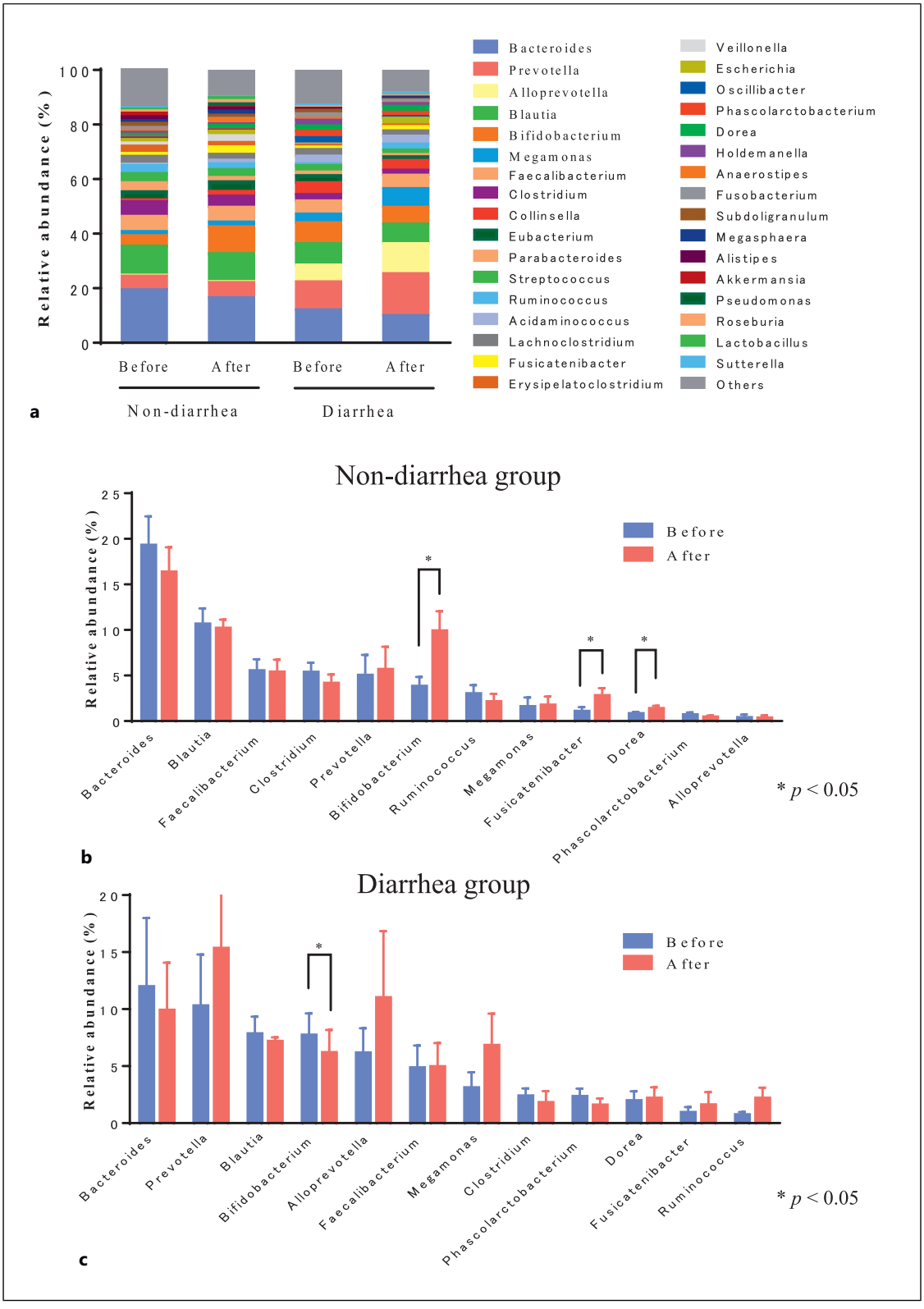

Fig. 5. Comparison of the composition and structure of the microbial community following chemotherapy at the genus level in the diarrhea and non-diarrhea groups. **a** Stacked bar plot of genera abundances. **b**, **c** Relative abundance of each key genus. Data are expressed as the mean ± SEM. *$p < 0.05$.

Table 2. Changes in blood tests and fecal calprotectin following chemotherapy

	Before chemotherapy	After chemotherapy	*p* value
Non-diarrhea group			
Fecal calprotectin, μg/g	184 (93.3–731)	263 (99.6–1,267.8)	0.562
CRP, mg/L	0.11 (0.05–0.24)	0.05 (0.03–0.16)	0.39
ALB, g/L	4.1 (3.7–4.3)	4.1 (3.9–4.2)	0.338
HGB, mg/dL	12.4 (11.8–13.4)	12.9 (12.0–14.1)	0.148
Diarrhea group			
Fecal calprotectin, μg/g	1,151 (457.0–2,755)	1,210 (638.5–1,430)	0.748
CRP, mg/L	0.09 (0.05–0.14)	0.03 (0.02–0.05)	0.092
ALB, g/L	4.2 (4.0–4.4)	4.2 (4.0–4.3)	0.69
HGB, mg/dL	13.6 (12.6–14.3)	12.7 (11.9–13.2)	0.755

CRP, C-reactive protein; ALB, albumin; HGB, hemoglobin.

group than in the non-diarrhea group. Both *Blautia* and *Veillonella* are organic-acid-producing bacteria, suggesting that organic acids may be associated with chemotherapy-induced diarrhea.

There have been few reports on chemotherapy-induced changes in the human intestinal microbiome. Montassier et al. [27] performed 16S rRNA gene analysis of feces after chemotherapy in 28 patients with non-Hodgkin's lymphoma and reported that dysbiosis occurring after chemotherapy was associated with gastrointestinal mucositis. In addition, van Vliet et al. [28] reported that in pediatric patients treated with chemotherapy for acute myeloid leukemia, chemotherapy was associated with a decreased abundance of anaerobic bacteria and increased abundance of pathogenic aerobic enterococci in the intestinal microbiome, thereby increasing the risk of Gram-positive aerobic infection in immunocompromized individuals. Although there have been few reports on the intestinal microbiome during chemotherapy for hematologic cancers, to the best of our knowledge, this is the first report on changes in the intestinal microbiome during chemotherapy for solid tumors and on the relationship between chemotherapy-induced diarrhea and the intestinal microbiome in humans. In contrast, Osterlund et al. [29] reported that diarrhea was suppressed when probiotics were administered to colorectal cancer patients receiving 5-FU. Probiotics improve dysbiosis-related secondary inflammatory responses to 5-FU-induced intestinal mucositis in mice [19]. Although further prospective studies are needed, a therapeutic strategy involving prophylactic administration of probiotics for chemotherapy-induced diarrhea warrants consideration.

Chemotherapy-induced diarrhea is more likely to occur with oral than with intravenous administration of drugs [30]. In this study, most gastrointestinal toxicities occurred in patients who received oral FPs, and patients who received intravenous FPs did not show significant changes in the intestinal microbiome, possibly due to the small number of patients. Therefore, to clearly analyze the impact of FPs on the intestinal microbiome, the analysis focused primarily on patients who received oral FPs.

To investigate whether individual differences in the original intestinal microbiome predispose patients to chemotherapy-induced diarrhea, the intestinal microbiome before chemotherapy was compared between those with and without diarrhea. The results showed that there was no difference in α and β diversity between the diarrhea and non-diarrheal groups, as well as at the phylum level. At the genus level, there was a significantly lower abundance of *Ruminococcus* spp. and a significantly greater abundance of *Phascolarctobacterium* spp. in the diarrhea group, but their proportions were less than 5%. Therefore, the results of this study suggest that the original individual intestinal microbiome has little influence on the occurrence of diarrhea and that diarrhea may largely be due to chemotherapy-induced changes in the intestinal microbiome.

PICRUSt analysis, a computational approach to predict the functional composition of a metagenome, revealed a significant reduction in membrane transport and an increase in oxidative phosphorylation in the diarrhea group. Decreased membrane transport may have led to decreased water absorption capacity in the intestinal tract, causing diarrhea. Furthermore, an increase in oxidative phosphorylation indicates an increased generation of reactive oxygen species and potential oxidative stress on the intestinal microbiome. This may result in dysbiosis and ultimately contribute to the development of diarrhea.

Blood tests and fecal calprotectin tests were performed before and after chemotherapy, and no inflammatory response or elevated fecal calprotectin levels were observed in the diarrhea group, suggesting that even in patients with diarrhea, inflammation of the intestinal

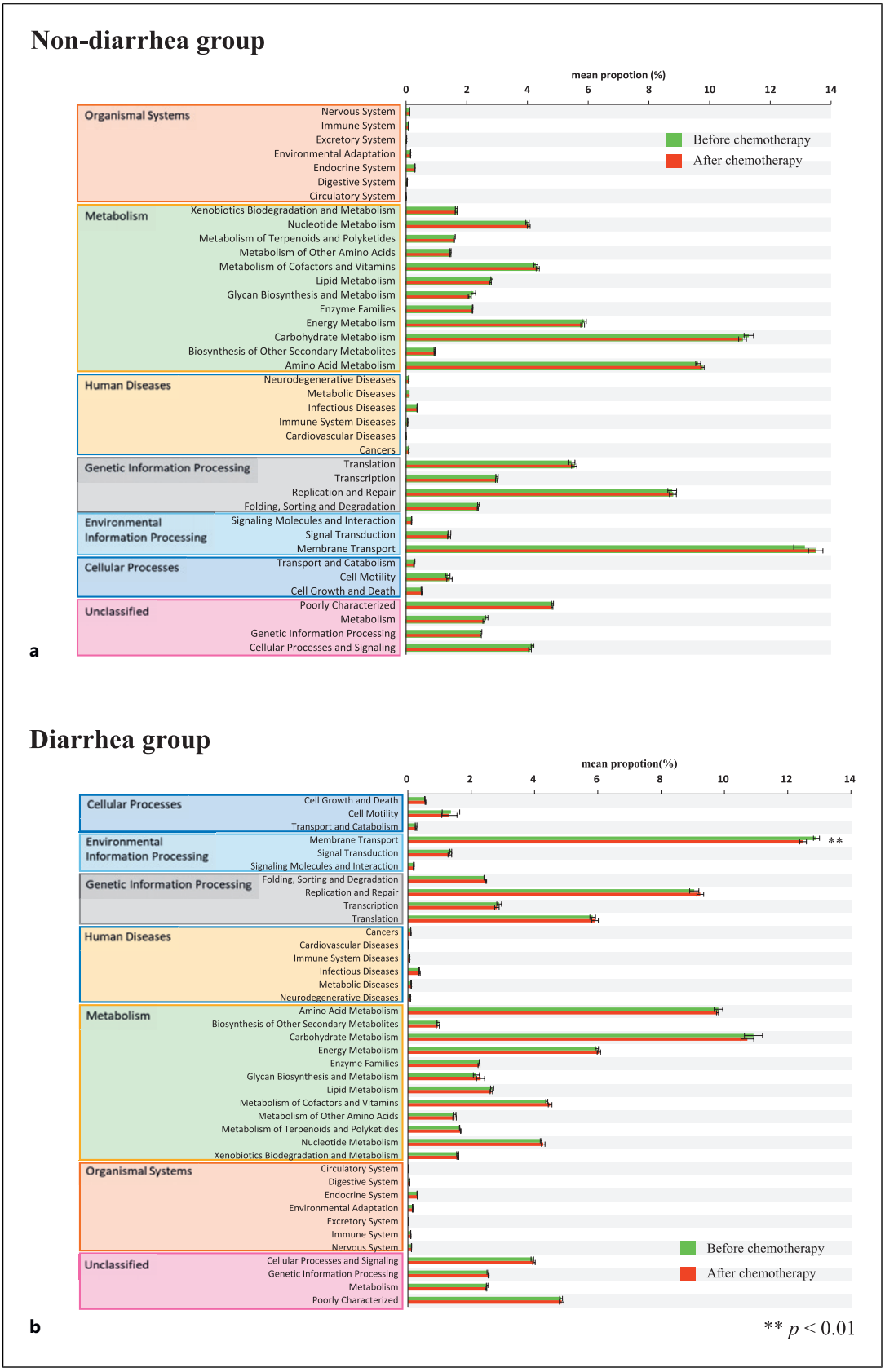

Fig. 6. PICRUSt predictions of the functional composition of intestinal microbiome at KEGG pathway level 2 in the non-diarrhea group (**a**) and diarrhea group (**b**). Data are expressed as the mean ± SEM. $^{**}p < 0.01$.

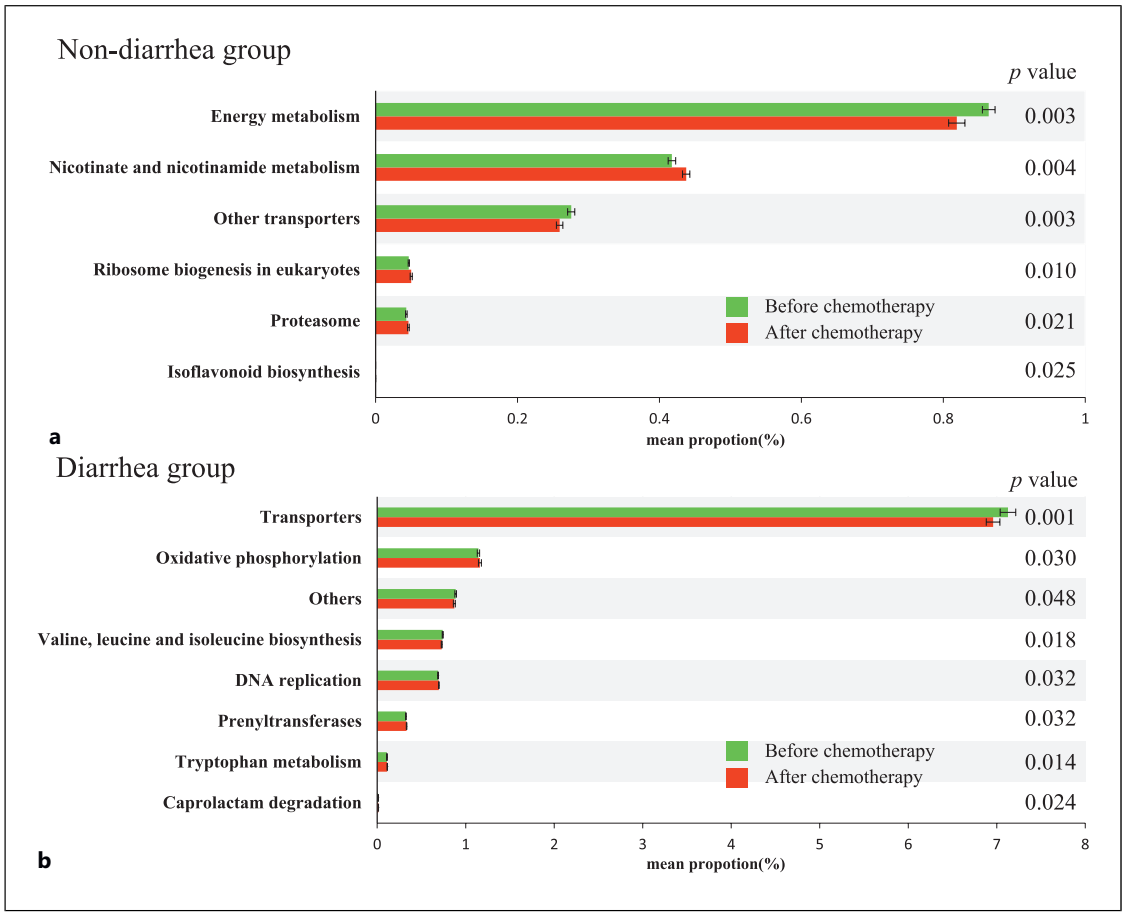

Fig. 7. PICRUSt predictions of the functional composition of intestinal microbiome at KEGG pathway level 3 in the non-diarrhea group (**a**) and diarrhea group (**b**). Data are expressed as the mean ± SEM.

tract may be minimal or inflammation may occur mainly in the small intestine since the fecal calprotectin levels indicate inflammation mainly in the large intestine.

This study has a few limitations. First, the patients in this study were not treated with FPs alone but with chemotherapy that included FPs. Therefore, it is possible that drugs other than FPs may have influenced the intestinal microbiome; however, this analysis could not be performed because of the variety of drugs used. Few studies have evaluated the effects of anticancer agents other than FPs on the intestinal microbiota. Further studies are needed on the effects of each type of anticancer drug. Second, because the observation period was limited to the end of a single course of chemotherapy, we could not examine changes in gastrointestinal toxicity or intestinal microbiome when repeated chemotherapy was administered. Third, because the study was limited to patients with colorectal cancer, it is unclear whether similar changes occur in the intestinal microbiome in patients with other types of solid and nonsolid tumors. Finally, the number of patients with diarrhea was relatively small.

In conclusion, changes in the intestinal microbiome were associated with diarrhea in patients undergoing chemotherapy, including FPs. In particular, organic-acid-producing bacteria may be involved. In the future, it is expected that a therapeutic strategy involving prophylactic administration of probiotics will be examined for efficacy in preventing chemotherapy-induced diarrhea.

Statement of Ethics

This study was performed according to the principles of the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of Osaka Medical and Pharmaceutical University (No. 1533). Written informed consent was obtained from each patient included in this study.

Conflict of Interest Statement

Shiro Nakamura reports receiving speaking fees from AbbVie GK, EA Pharma Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Mochida Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., and Janssen Pharmaceutical K.K. Dr. Shinya Fukunishi is an associate editor of *Digestion*.

Funding Sources

This research was partially supported by Biofermin Pharmaceutical Co., Ltd. (Kobe, Hyogo).

Author Contributions

Yuka Kawasaki collected data and wrote the initial draft of the manuscript. Kazuki Kakimoto designed the study, interpreted the data, and drafted the manuscript. Yasuyoshi Tanaka, Hikaru Shimizu, Koji Nishida, Keijiro Numa, Naohiko Kinoshita, Yoshihiro Tatsumi, Kei Nakazawa, Ryoji Koshiba, Yuki Hirata, Kazuhiro Ota, Naokuni Sakiyama, Tetsuji Terazawa, Toshihisa Takeuchi, Takako Miyazaki, Masahiro Goto, Haruka Yokota, Yutaka Makizaki, Yoshiki Tanaka, Shunji Nakajima, Hiroshi Ohno, Kazuhide Higuchi, Shiro Nakamura, and Hiroki Nishikawa contributed to data collection and interpretation and critically reviewed the manuscript. All the authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability Statement

All data generated during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Anilkumar TV, Sarraf CE, Hunt T, Alison MR. The nature of cytotoxic drug-induced cell death in murine intestinal crypts. Br J Cancer. 1992;65(4):552–8.
2 Benson AB, Ajani JA, Catalano RB, Engelking C, Kornblau SM, Martenson JA Jr, et al. Recommended guidelines for the treatment of cancer treatment induced diarrhea. J Clin Oncol. 2004;22(14):2918–26.
3 Wadler S, Benson AB 3rd, Engelking C, Catalano R, Field M, Kornblau SM, et al. Recommended guidelines for the treatment of chemotherapy induced diarrhea. J Clin Oncol. 1998;16(9):3169–78.
4 Blijlevens NM, Donnelly JP, De Pauw BE. Mucosal barrier injury biology, pathology, clinical counterparts and consequences of intensive treatment for haematological malignancy: an overview. Bone Marrow Transplant. 2000;25(12):1269–78.
5 Sonis ST, Oster G, Fuchs H, Bellm L, Bradford WZ, Edelsberg J, et al. Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. J Clin Oncol. 2001;19(8):2201–5.
6 Jones JA, Avritscher EBC, Cooksley CD, Michelet M, Bekele BN, Elting LS. Epidemiology of treatment-associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer. Support Care Cancer. 2006;14(6):505–15.
7 van Vliet MJ, Harmsen HJM, de Bont ESJM, Tissing WJE. The role of intestinal microbiota in the development and severity of chemotherapy induced mucositis. PLoS Pathog. 2010;6(5):e1000879–7.
8 Logan RM, Stringer AM, Bowen JM, Yeoh ASJ, Gibson RJ, Sonis ST, et al. The role of pro-inflammatory cytokines in cancer treatment-induced alimentary tract mucositis: pathobiology, animal models and cytotoxic drugs. Cancer Treat Rev. 2007;733(5):448–60.
9 Ichikawa M, Sujino T, Kanai T. The relationship between gut microbiome, immune system, and cancer. Gan To Kagaku Ryoho. 2019;46(12):1807–13.
10 Hamouda N, Sano T, Oikawa Y, Ozaki T, Shimakawa M, Matsumoto K, et al. Apoptosis, dysbiosis and expression of inflammatory cytokines are sequential events in the development of 5-fluorouracil-induced intestinal mucositis in mice. Basic Clin Pharmacol Toxicol. 2017;121(3):159–68.
11 Matsuki T. Procedure of DNA extraction from fecal sample for the analysis of intestinal microflora. J Int Micro. 2006;20(3):259–62.
12 Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the illumina MiSeq platform. Microbiome. 2014;2(1):6–7.
13 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335–6.
14 Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71(12):8228–35.
15 Wang Q, Wang XD, Jeppsson B, Andersson R, Karlsson B, Westrom B. Influence of colostomy on in vivo and in vitro permeability of the rat colon. Dis Colon Rectum. 1996;39(6):663–70.
16 Ota K, Takeuchi T, Kojima Y, Harada S, Ozaki H, Sugawara N, et al. Fluoropyrimidine-induced intestinal mucosal injury is associated with the severity of chemotherapy-related diarrhea. Scand J Gastroenterol. 2019;54(2):227–32.
17 Yasuda M, Kato S, Yamanaka N, Iimori M, Matsumoto K, Utsumi D, et al. 5-HT(3) receptor antagonists ameliorate 5-fluorouracil-induced intestinal mucositis by suppression of apoptosis in murine intestinal crypt cells. Br J Pharmacol. 2013;168(6):1388–400.
18 Yasuda M, Kato S, Yamanaka N, Iimori M, Utsumi D, Kitahara Y, et al. Potential role of the NADPH oxidase NOX1 in the pathogenesis of 5-fluorouracil-induced intestinal mucositis in mice. Am J Physiol Gastrointest Liver Physiol. 2012;302(10):G1133–42.
19 Kato S, Hamouda N, Kano Y, Oikawa Y, Tanaka Y, Matsumoto K, et al. Probiotic Bifidobacterium bifidum G9-1attenuates 5-fluorouracil-inducedintestinal mucositis in mice via suppression of dysbiosis-related secondary inflammatory responses. Clin Exp Pharmacol Physiol. 2017;44(10):1017–25.
20 Makizaki Y, Maeda A, Oikawa Y, Tamura S, Tanaka Y, Nakajima S, et al. Alleviation of low-fiber diet-induced constipation by probiotic Bifidobacterium bifidum G9-1 is based on correction of gut microbiota dysbiosis. Biosci Microbiota Food Health. 2019;38(2):49–53.
21 Makizaki Y, Uemoto T, Yokota H, Yamamoto M, Tanaka Y, Ohno H. Improvement of loperamide-induced slow transit constipation by Bifidobacterium bifidum G9-1 is mediated by the correction of butyrate production and neurotransmitter profile due to improvement in dysbiosis. PLoS One. 2021;16(3):e0248584.

22 Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504(7480):446–50.
23 Whitford EJ, Cummins AG, Butler RN, Prisciandaro LD, Fauser JK, Yazbeck R, et al. Effects of Streptococcus thermophilus TH-4 on intestinal mucositis induced by the chemotherapeutic agent 5-Fluorouracil (5-FU). Cancer Biol Ther. 2009;8(6):505–11.
24 Yeung CY, Chan WT, Jiang CB, Cheng ML, Liu CY, Chang SW, et al. Amelioration of chemotherapy induced intestinal mucositis by orally administered probiotics in a mouse model. PLoS One. 2015;10(9):e0138746.
25 Justino PFC, Melo LFM, Nogueira AF, Morais CM, Mendes WO, Franco AX, et al. Regulatory role of Lactobacillus acidophilus on inflammation and gastric dysmotility in intestinal mucositis induced by 5-fluorouracil in mice. Cancer Chemother Pharmacol. 2015;75(3):559–67.
26 Sasaki K, Inoue J, Sasaki D, Hoshi N, Shirai T, Fukuda I, et al. Construction of a model culture system of human colonic microbiota to detect decreased lachnospiraceae abundance and butyrogenesis in the feces of ulcerative colitis patients. Biotechnol J. 2019;14(5):e1800555.
27 Montassier E, Gastinne T, Vangay P, Al-Ghalith GA, Bruley des Varannes S, Massart S, et al. Chemotherapy-driven dysbiosis in the intestinal microbiome. Aliment Pharmacol Ther. 2015;42(5):515–28.
28 van Vliet MJ, Tissing WJE, Dun CAJ, Meessen NEL, Kamps WA, de Bont ESJM, et al. Chemotherapy treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. Clin Infect Dis. 2009;49(2):262–70.
29 Osterlund P, Ruotsalainen T, Korpela R, Saxelin M, Ollus A, Valta P, et al. Lactobacillus supplementation for diarrhoea related to chemotherapy of colorectal cancer: a randomised study. Br J Cancer. 2007;97(8):1028–34.
30 Sakumura M, Ando T, Hosokawa A, Nakajima T, Motoo I, Mihara H, et al. Small intestinal mucosal injury and its risk factors in patients with gastrointestinal cancer who developed complicated fluoropyrimidine-induced diarrhea. BMC Gastroenterol. 2020;20(1):355.