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Diverse gut microbiota pattern between mild and severe cancer-related fatigue in lung cancer patients treated with first-line chemotherapy: A pilot study

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Abstract

Background: Cancer survivors perceive cancer-related fatigue (CRF) as one of the most common symptoms. However, the potential relationship between CRF and gut microbiota has not been elucidated. Our study aimed to preliminary explore the diverse gut microbiota composition between mild and severe CRF in advanced lung cancer patients undergoing first-line chemotherapy.

Methods: A total of 20 advanced lung patients treated with first-line chemotherapy were enrolled, 10 with mild CRF and 10 with severe CRF. The self-reported Piper Fatigue Scale and stool samples were collected from all eligible patients. The 16 S ribosomal ribonucleic acid gene was performed to analyze the intestinal microbiome.

Results: We identified the significantly diverse gut microbiota composition among patients with mild and severe CRF. The pattern was characterized by the increasing abundance in short-chain fatty acid-producing taxa for mild CRF patients (genus Lachnospiraceae-UCG-008 and family Lachnospiraceae, $p < 0.05$), whereas higher abundance in taxa related to inflammation (family Enterobacteriaceae and genus Escherichia-Shigella, $p < 0.05$) for severe CRF patients. Significantly different Kyoto Encyclopedia of Genes and Genomes pathways between mild and severe CRF patients were evaluated concerning fatty acid metabolism, nucleotide metabolism, brain function, amino acid metabolism, and so on ($p < 0.05$).

Conclusions: Our study observed a plausible association between different levels of CRF and the diverse gut microbiota composition, with increasing proinflammation taxa in severe CRF patients and anti-inflammation taxa growing in mild CRF patients. Further studies are warranted to evaluate whether CRF can be improved by modulating the gut microbiota composition.

KEYWORDS
cancer-related fatigue, chemotherapy, gut microbiota, lung cancer

INTRODUCTION

Cancer-related fatigue (CRF) is perceived by cancer survivors to be one of the most common and distressing side effects of cancer and its treatment with deterioration in all aspects of quality of life may be aggravated during cancer treatment.[1,2] In most studies, the prevalence evaluation of moderate to severe CRF during anticancer treatment ranges from 30% to 60%.[1] CRF is typically more frequently reported in patients receiving chemotherapy (80%–96%) than patients undergoing radiotherapy

Hao Wei, Lingling Xie, Yihan Zhao and Jun He have contributed equally to this study and share first authorship.

TABLE 1 Baseline demographic characteristics of patients

Characteristic	Mild CRF ($N = 10$) Mean ± SD or N (%)	Severe CRF ($N = 10$) Mean ± SD or N (%)	Total ($N = 20$) Mean ± SD or N (%)	p-value
Age (years)				
Mean ± SD	64.20 ± 7.54	61.60 ± 10.09	62.90 ± 8.77	0.522
Median (range)	66.50 (46.00–74.00)	63.00 (46.00–78.00)	66.00 (46.00–78.00)	
Gender				
Male	6 (60.00%)	9 (90.00%)	5 (25.00%)	0.303
Female	4 (40.00%)	1 (10.00%)	15 (75.00%)	
Histology				
Adenocarcinoma	4 (40.00%)	2 (20.00%)	6 (30.00%)	0.553
Squamous	3 (30.00%)	3 (30.00%)	6 (30.00%)	
Small cell lung cancer	3 (30.00%)	5 (50.00%)	8 (40.00%)	
Smoking status				
Nonsmoker	7 (70.00%)	8 (80.00%)	15 (75.00%)	1.000
Smoker	3 (30.00%)	2 (20.00%)	5 (25.00%)	
Alcoholism				
Yes	1 (10.00%)	3 (30.00%)	4 (20.00%)	0.582
No	9 (90.00%)	7 (70.00%)	16 (80.00%)	
Married				
Yes	8 (80.00%)	9 (90.00%)	17 (85.00%)	1.000
No	2 (20.00%)	1 (10.00%)	3 (15.00%)	
BMI (kg/m^2)	21.51 ± 2.27	24.55 ± 4.16	23.03 ± 3.61	0.058
Chemotherapy regimen				
Etoposide + carboplatin	0 (0.00%)	3 (30.00%)	3 (15.00%)	0.121
Etoposide + cisplatinum	3 (30.00%)	2 (20.00%)	5 (25.00%)	
Paclitaxel + cisplatinum	2 (20.00%)	3 (30.00%)	5 (25.00%)	
Paclitaxel + carboplatin	3 (30.00%)	0 (0.00%)	3 (15.00%)	
Pemetrexed + carboplatin	1 (10.00%)	1 (10.00%)	2 (10.00%)	
Pemetrexed + cisplatinum	1 (10.00%)	1 (10.00%)	2 (10.00%)	
ECOG scores				
0	2 (20.00%)	7 (70.00%)	9 (45.00%)	0.070
1	8 (80.00%)	3 (30.00%)	11 (55.00%)	
Piper total scores	1.91 ± 0.60	7.86 ± 0.87	4.88 ± 3.14	0.000

Abbreviations: BMI, body mass index; CRF, cancer-related fatigue; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation.

(60%–93%).[3] By changing a patient's mood, work, social relationships, and daily activities, CRF significantly affects the quality of life during treatment.[1,3] However, despite its prevalence and detrimental impact, the underlying mechanisms of CRF are still not fully understood. Shreds of evidence suggest that the etiology of CRF may involve multifactorial processes. These include elevated levels of proinflammatory cytokines, 5-hydroxytryptophan dysregulation, hypothalamic–pituitary–adrenal axis dysregulation, circadian rhythm disturbances, and increased vagal tone.[4–8] Currently, most studies exploring the mechanism of CRF focus on inflammation, especially proinflammatory cytokines.[1] Although the association between inflammation and CRF has been revealed, further studies on how inflammation leads to CRF are warranted.[9,10]

Gut microbiota plays a vital role in multiple physiological functions of humans, particularly metabolism, inflammation, and immunity.[11] Short-chain fatty acids (SCFA) are produced with specific gut microbiota during the fermentation of indigestible carbohydrates.[12] These SCFAs may have potential anti-inflammatory and immunomodulatory abilities.[12,13] For instance, butyrate, an anti-inflammatory SCFA, may inhibit the production of proinflammatory cytokines.[14,15] Recently, accumulating evidence favors that gut microbiota exerts profound effects on local and systemic immune responses,[11]

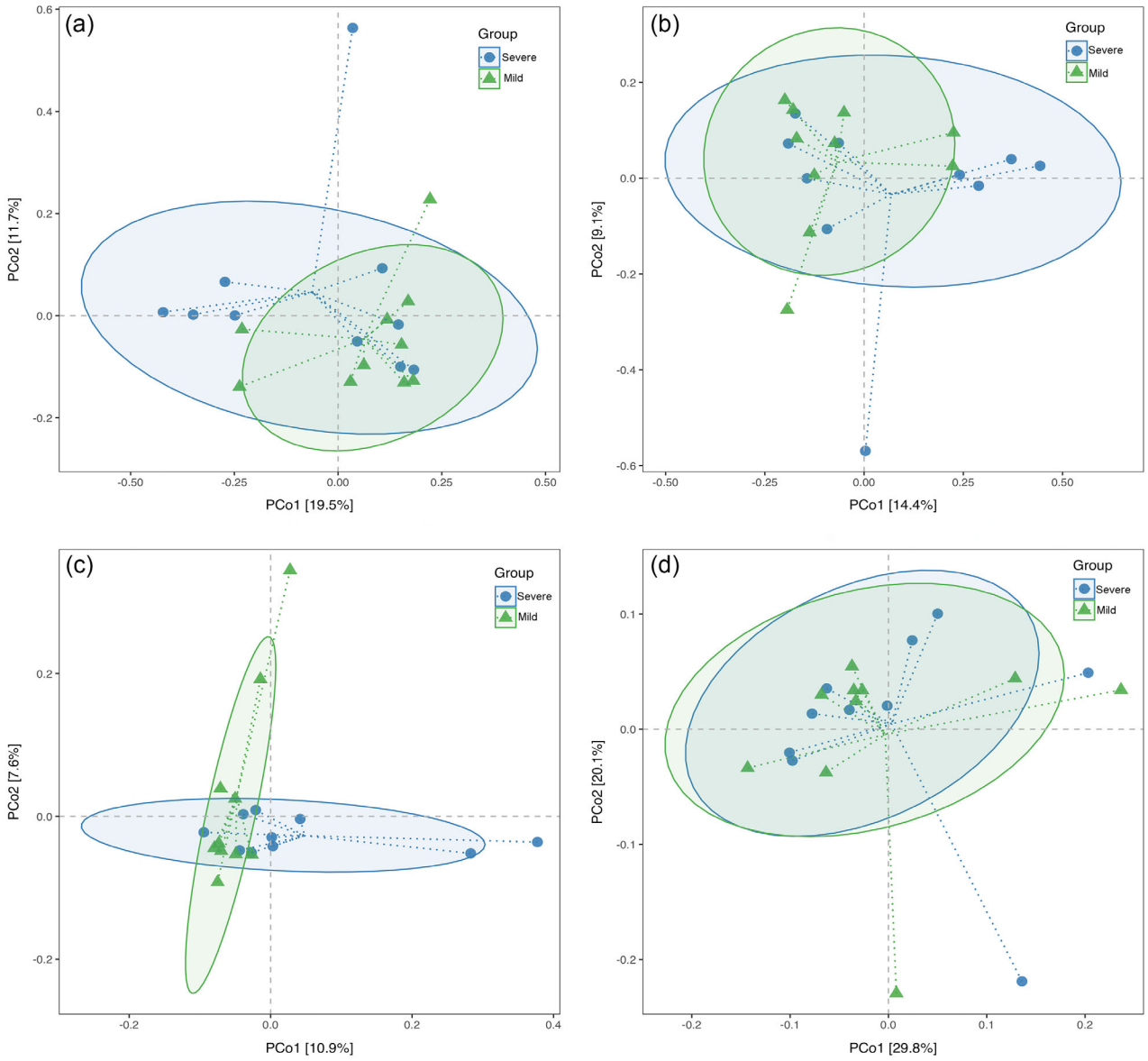

FIGURE 1 Principal coordinate analysis (PCoA) between mild versus severe CRF groups based on (a) Bray-Curtis distance, (b) Jaccard distance, (c) unweighted Unifrac distance, and (d) weighted Unifrac distance. CRF, cancer-related fatigue

representing a fundamental to exploring the possible mechanism of CRF based on the relationship between gut microbiota and inflammation. González-Mercado et al.[16] performed a cross-sectional pilot study that primarily showed fatigued rectal cancer patients owned differentially abundant microbial taxa relative to nonfatigued rectal cancer patients at the end of chemotherapy and radiotherapy. A pilot study conducted by Xiao et al.[10] demonstrates the diverse composition of gut microbiota in head and neck cancer patients who receive radiotherapy with high versus low CRF, which indicates the potential associations between gut microbiota and CRF. However, research exploring the relationship between gut microbiota and CRF is sparse, particularly for lung cancer patients with CRF undergoing chemotherapy.

This pilot study was performed on lung cancer patients with CRF undergoing chemotherapy to primarily identify the diverse composition of gut microbiota between severe and mild CRF.

METHODS

This pilot study designed with a questionnaire and biosample gathered during chemotherapy treatment primarily evaluated the diverse composition of gut microbiota between severe and mild CRF for lung cancer patients receiving chemotherapy at the West China Hospital, Sichuan University. This study was approved by the Ethics Committee of West China Hospital of Sichuan University. Written informed consent was obtained from all eligible patients.

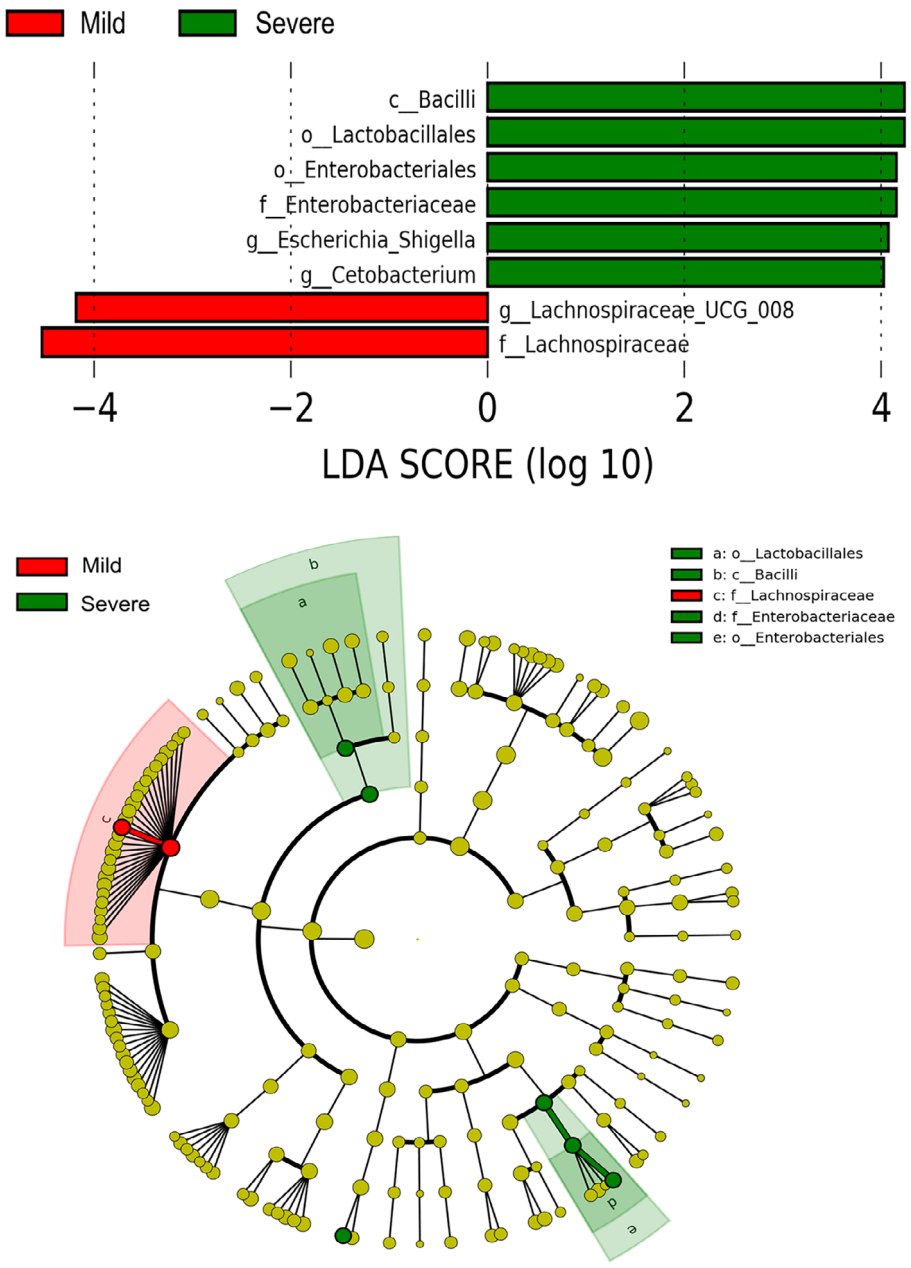

FIGURE 2 LEfSe analysis of taxa abundance between mild and severe CRF patients. LEfSe, linear discriminant analysis effect size; CRF, cancer-related fatigue; LDA, linear discriminant analysis; c, class; o, order; f, family; g, genus

Patients

Patients were enrolled if they met the following inclusion criteria: pathologically diagnosed with lung cancer, presented with mild or severe CRF, aged 18 years or older, stage IV based on the eighth edition of the American Joint Committee on Cancer staging manual, and undergoing first-line chemotherapy. The main exclusion criteria consisted of the following: (1) history of second malignancy; (2) receiving immunotherapy, radiotherapy, or targeted therapy combined with chemotherapy; (3) long-term immunosuppressive medication treatment; and (4) regular usage of antibiotic or probiotic preparations.

Baseline demographic characteristics such as age, gender, chemotherapy regimen, marital status, smoking status, alcohol status, Eastern Cooperative Oncology Group Performance Status (ECOG PS) and body mass index (BMI) were collected.

Cancer-related fatigue evaluation

CRF was evaluated with the Mandarin Chinese version of the Piper Fatigue Scale at baseline. The Mandarin Chinese version of the Piper Fatigue Scale is a self-administered multidimensional assessment tool consisting of 24 items covering four attributes: affective, sensory, behavioral, and cognitive/mood.[17,18] The Mandarin Chinese version of the Piper Fatigue Scale has established excellent reliability; the Cronbach α is 0.96 to 0.97.[18] Patients determined the degree of their CRF from 0 to 10, where 0: not at all, 1–3: mild CRF, 4–6: moderate CRF, 7–10 severe CRF.

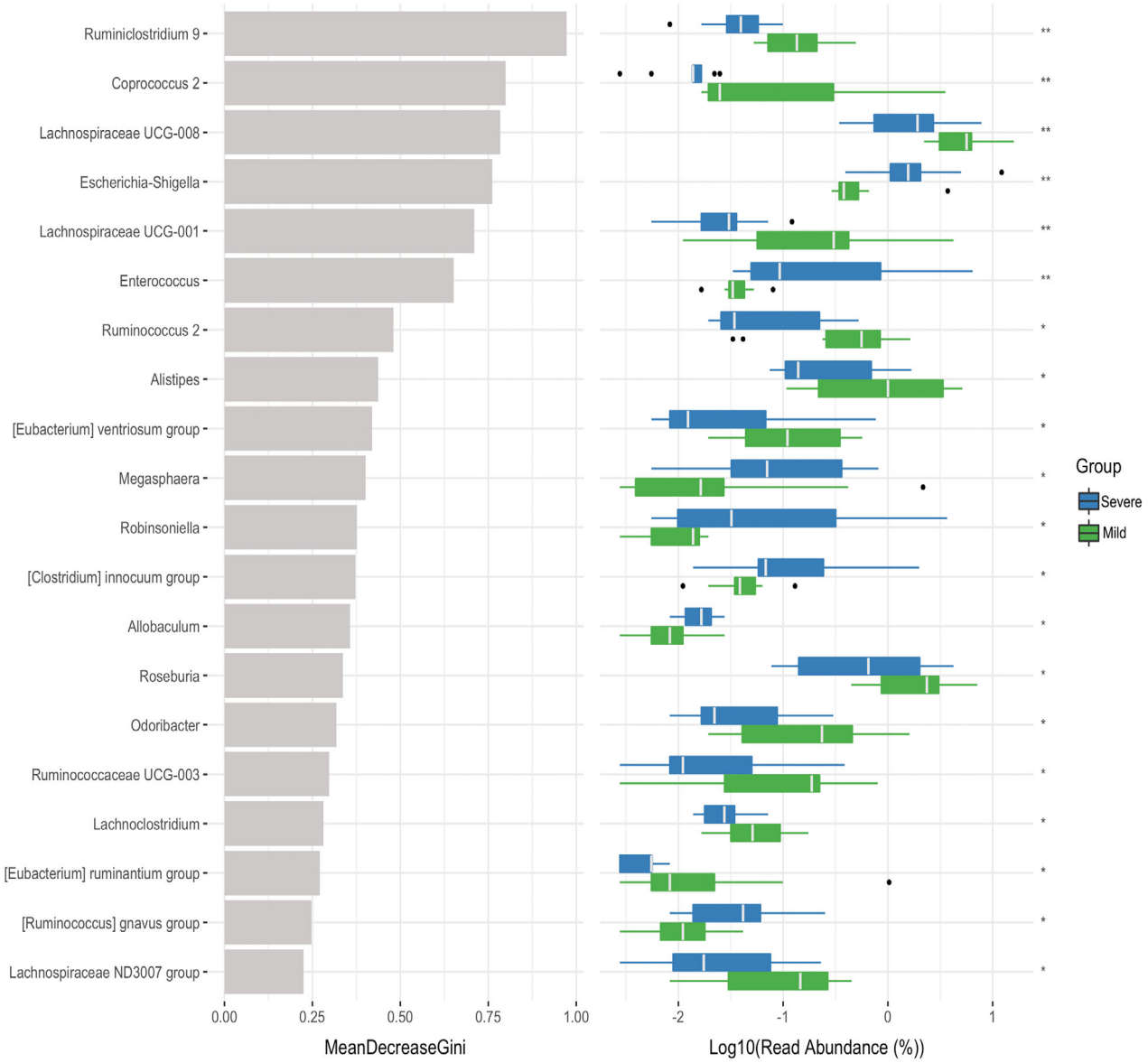

FIGURE 3 Comparison between mild versus severe CRF patients in gut microbiota pattern based on Random Forest analysis. CRF, cancer-related fatigue; **, $p < 0.01$; *, $p < 0.05$

Gut microbiota, bioinformatic analysis, and statistical analyses

The detailed methods are presented in the supplementary materials (Supporting information).

RESULTS

Patients

Twenty lung cancer patients were enrolled in this pilot study. Table 1 describes the baseline demographic characteristics of the participants. Of the 20 eligible patients, mild CRF patients did not significantly differ from severe CRF participants according to age, gender, histology, smoking status, alcohol status, marital status, BMI, chemotherapy regimen, and PS scores ($p > 0.05$), except for the mean fatigue ($p = 0.000$).

Bacterial diversity and composition

There was no significant difference of the common phyla between the severe CRF and mild CRF group, 43.68% versus 47.76% in the phyla Bacteroidetes ($p = 0.496$), 40.70% versus 43.37% in the phyla Firmicutes ($p = 0.364$), 7.84% versus 4.96% in the phyla Proteobacteria ($p = 0.199$), 3.46% versus 1.47% in the phyla Actinobacteria ($p = 0.450$), 3.43% versus 0.76% in the phyla Fusobacteria ($p = 0.226$).

TABLE 2 Relative abundance between severe and mild CRF in level 2 KEGG pathways

KEGG pathways	Severe CRF Mean (SD)	Mild CRF Mean (SD)	*p*-value
Nucleotide metabolism	6.91×10^{-2} (3.17×10^{-3})	7.10×10^{-2} (2.19×10^{-3})	0.009
Amino acid metabolism	1.12×10^{-1} (8.06×10^{-3})	1.16×10^{-1} (7.68×10^{-3})	0.190
Xenobiotics biodegradation and metabolism	2.49×10^{-2} (2.05×10^{-3})	2.50×10^{-2} (1.41×10^{-3})	1.000
Metabolism of other amino acids	2.25×10^{-2} (6.77×10^{-4})	2.22×10^{-2} (9.67×10^{-4})	0.353
Carbohydrate metabolism	1.44×10^{-1} (6.98×10^{-3})	1.42×10^{-1} (5.64×10^{-3})	0.353
Biosynthesis of other secondary metabolites	8.33×10^{-3} (3.72×10^{-4})	8.13×10^{-3} (3.75×10^{-4})	0.165
Glycan biosynthesis and metabolism	3.30×10^{-2} (6.85×10^{-3})	3.18×10^{-2} (5.82×10^{-3})	0.436
Lipid metabolism	2.94×10^{-2} (1.29×10^{-3})	2.83×10^{-2} (1.50×10^{-3})	0.089
Energy metabolism	6.78×10^{-2} (3.23×10^{-3})	6.70×10^{-2} (5.31×10^{-3})	0.912
Metabolism of cofactors and vitamins	6.74×10^{-2} (4.02×10^{-3})	6.73×10^{-2} (4.01×10^{-3})	0.971
Metabolism of terpenoids and polyketides	1.89×10^{-2} (1.60×10^{-3})	1.80×10^{-2} (1.37×10^{-3})	0.019
Translation	6.05×10^{-2} (4.74×10^{-3})	6.16×10^{-2} (4.04×10^{-3})	0.353
Membrane transport	1.14×10^{-1} (1.52×10^{-2})	1.18×10^{-1} (1.38×10^{-2})	0.481
Signal transduction	6.88×10^{-2} (8.46×10^{-3})	6.71×10^{-2} (6.60×10^{-3})	0.353
Cell motility	2.22×10^{-2} (5.18×10^{-3})	2.13×10^{-2} (4.69×10^{-3})	0.529
Folding, sorting and degradation	2.64×10^{-2} (2.17×10^{-3})	2.61×10^{-2} (1.49×10^{-3})	0.853
Transcription	2.77×10^{-3} (2.19×10^{-4})	2.72×10^{-3} (1.99×10^{-4})	0.796
Replication and repair	5.73×10^{-2} (4.53×10^{-3})	5.75×10^{-2} (3.54×10^{-3})	0.631
Cell growth and death	1.74×10^{-2} (5.76×10^{-4})	1.72×10^{-2} (9.79×10^{-4})	0.971
Transport and catabolism	2.38×10^{-3} (5.28×10^{-4})	2.14×10^{-3} (4.58×10^{-4})	0.247
Circulatory system	3.97×10^{-5} (1.51×10^{-5})	3.60×10^{-5} (1.26×10^{-5})	0.165
Cell communication	1.34×10^{-5} (5.02×10^{-6})	1.54×10^{-5} (2.64×10^{-6})	0.393
Signaling molecules and interaction	1.34×10^{-5} (5.03×10^{-6})	1.54×10^{-5} (2.64×10^{-6})	0.393
Immune system	9.60×10^{-4} (2.92×10^{-4})	9.81×10^{-4} (2.71×10^{-4})	0.853
Environmental adaptation	2.42×10^{-3} (1.33×10^{-4})	2.33×10^{-3} (1.23×10^{-4})	0.105
Nervous system	1.13×10^{-3} (6.51×10^{-5})	1.07×10^{-3} (1.23×10^{-4})	0.247
Sensory system	6.09×10^{-11} (1.92×10^{-10})	2.57×10^{-12} (8.11×10^{-12})	1.000
Endocrine system	3.65×10^{-3} (4.46×10^{-4})	3.51×10^{-3} (3.92×10^{-4})	0.280
Endocrine and metabolic diseases	6.51×10^{-4} (3.89×10^{-5})	6.46×10^{-4} (2.67×10^{-5})	0.971
Excretory system	1.95×10^{-4} (4.60×10^{-5})	1.79×10^{-4} (4.58×10^{-5})	0.218
Digestive system	2.35×10^{-3} (1.60×10^{-3})	2.16×10^{-3} (1.25×10^{-3})	0.579
Neurodegenerative diseases	1.19×10^{-3} (2.15×10^{-4})	1.24×10^{-3} (2.08×10^{-4})	0.579
Substance dependence	5.36×10^{-5} (1.65×10^{-5})	4.23×10^{-5} (9.77×10^{-6})	0.063
Infectious diseases	1.68×10^{-2} (9.43×10^{-4})	1.62×10^{-2} (8.45×10^{-4})	0.247
Cancers	9.76×10^{-4} (5.77×10^{-5})	9.86×10^{-4} (8.11×10^{-5})	0.684
Immune diseases	4.92×10^{-4} (1.00×10^{-4})	4.68×10^{-4} (8.73×10^{-5})	0.579
Cardiovascular diseases	6.29×10^{-6} (3.99×10^{-6})	3.48×10^{-6} (2.89×10^{-6})	0.019

Abbreviations: CRF, cancer-related fatigue; KEGG, Kyoto Encyclopedia of Genes and Genomes; SD, standard deviation.

No significant difference was observed based on alpha-diversity between severe and mild CRF in terms of Chao1 (860.02 ± 124.73 vs. 893.16 ± 121.07, $p = 0.554$), Simpson (0.94 ± 0.02 vs. 0.95 ± 0.03, $p = 0.244$), Shannon-Wiener index (3.78 ± 0.27 vs. 4.07 ± 0.41, $p = 0.079$), and PD (56.26 ± 6.62 vs. 55.91 ± 7.33, $p = 0.912$).

The beta-diversity was performed with PERMANOVA (weighted Unifrac: $p = 0.519$; unweighted UniFrac: $p = 0.066$; Bray-Curtis: $p = 0.152$; Jaccard: $p = 0.162$), and ANOSIM (weighted Unifrac: $p = 0.659$; unweighted UniFrac: $p = 0.297$; Bray-Curtis: $p = 0.224$; Jaccard: $p = 0.221$) showed no significant difference between severe and mild CRF. Figure 1 showed the results from principal coordinate analysis (PCoA) between severe and mild CRF.

Figure 2 showed that class Bacilli were more abundant in patients with severe CRF, which was further reflected in more abundance in order Lactobacillales, order Enterobacteriales, family Enterobacteriaceae, genus

Escherichia-Shigella, and genus Cetobacterium. Moreover, LDA bar graphs and the cladogram illustrated that Bacilli and subsequent levels were more abundant in patients with severe CRF compared to mild CRF (Figure 2). Results from LEfSe analysis also demonstrated that patients with mild CRF possessed more abundant in genus Lachnospiraceae-UCG-008 and family Lachnospiraceae.

Based on the results of machine learning, a significantly higher abundancy was observed in the mild CRF group relative to the severe CRF group on Ruminiclostridium 9 (0.17 ± 0.14 versus 0.05 ± 0.03, $p = 0.002$), Coprococcus 2 (0.58 ± 1.14 vs. 0.01 ± 0.01, $p = 0.003$), Lachnospiraceae UCG-008 (6.01 ± 4.02 vs. 2.38 ± 2.25, $p = 0.008$), Lachnospiraceae UCG-001 (0.66 ± 1.27 vs. 0.03 ± 0.04, $p = 0.006$), Ruminococcus 2 (0.60 ± 0.49 vs. 0.15 ± 0.20, $p = 0.010$), Alistipes (1.94 ± 1.92 vs. 0.51 ± 0.65, $p = 0.015$), Eubacterium ventriosum group (0.20 ± 0.20 vs. 0.10 ± 0.23, $p = 0.028$), Megasphaera (0.27 ± 0.68 vs. 0.25 ± 0.32, $p = 0.049$), Roseburia (2.81 ± 2.37 vs. 1.27 ± 1.43, $p = 0.049$), Odoribacter (0.40 ± 0.49 vs. 0.07 ± 0.09, $p = 0.019$), Ruminococcaceae UCG-003 (0.23 ± 0.26 vs. 0.07 ± 0.12, $p = 0.041$), Lachnoclostridium (0.07 ± 0.06 vs. 0.03 ± 0.02, $p = 0.048$), Eubacterium ruminantium group (0.12 ± 0.32 vs. 0.00 ± 0.00, $p = 0.015$), and Lachnospiraceae ND3007 group (0.18 ± 0.16 vs. 0.06 ± 0.08, $p = 0.038$). In addition, more abundance of Escherichia-Shigella (2.74 ± 3.56 vs. 0.74 ± 1.05, $p = 0.005$), Enterococcus (1.12 ± 2.05 vs. 0.04 ± 0.02, $p = 0.004$), Robinsoniella (0.52 ± 1.14 vs. 0.01 ± 0.01, $p = 0.028$), Clostridium innocuum group (0.31 ± 0.60 vs. 0.05 ± 0.03, $p = 0.025$), Allobaculum (0.02 ± 0.01 vs. 0.01 ± 0.01, $p = 0.022$), and Ruminococcus gnavus group (0.05 ± 0.07 vs. 0.01 ± 0.01, $p = 0.028$) in the severe CRF group compared to the mild CRF group (Figure 3).

Gut microbiota functional gene analysis

There was no significant difference in the level 1 KEGG pathways between the severe CRF and mild CRF groups, $5.98 \times 10^{-1} \pm 1.33 \times 10^{-2}$ versus $5.97 \times 10^{-1} \pm 1.09 \times 10^{-2}$ in metabolism ($p = 1.000$), $1.47 \times 10^{-1} \pm 1.15 \times 10^{-2}$ versus $1.48 \times 10^{-1} \pm 8.92 \times 10^{-3}$ in genetic information processing ($p = 0.579$), $1.83 \times 10^{-1} \pm 2.31 \times 10^{-2}$ versus $1.85 \times 10^{-1} \pm 1.72 \times 10^{-2}$ in environmental information processing ($p = 0.971$), $4.20 \times 10^{-2} \pm 4.66 \times 10^{-3}$ versus $4.07 \times 10^{-2} \pm 4.80 \times 10^{-3}$ in cellular processes ($p = 0.481$), $1.08 \times 10^{-2} \pm 2.42 \times 10^{-3}$ versus $1.03 \times 10^{-2} \pm 2.03 \times 10^{-3}$ in organismal systems ($p = 0.393$), and $2.02 \times 10^{-2} \pm 1.18 \times 10^{-3}$ versus $1.96 \times 10^{-2} \pm 1.08 \times 10^{-3}$ in human diseases ($p = 0.393$). Results from Table 2 showed the difference in level 2 KEGG pathways between severe and mild CRF. A total of 271 different level 3 KEGG pathways were analyzed in our data. Thirty-six level 3 KEGG pathways were significantly different between mild and severe CRF ($p < 0.05$) (Table 3). Results from the machine learning further demonstrated that the severe CRF group significantly differs from mild CRF according to 36 unique functional pathways (Figure 4). These pathways concerned fatty acid metabolism, nucleotide metabolism, brain function, amino acid metabolism, and so on.

DISCUSSION

This pilot study primarily assessed the diverse composition of gut microbiota for lung cancer patients undergoing first-line chemotherapy with severe and mild CRF. Similar to the previous research, alpha and beta diversity did not differ between mild and severe CRF despite different fatigue evaluation strategies and types of cancer.[10] Nevertheless, our preliminary study found a diverse gut microbiota pattern among patients with mild CRF compared to those with severe CRF during chemotherapy.

Patients with mild CRF disposition possessed a higher relative abundance of bacterial taxa associated with SCFA production. Among SCFAs, butyrate is associated with keeping intestinal epithelial integrity, and Firmicutes families belonging to Lachnospiraceae and Ruminococcaceae are the key taxa of butyrate-producing bacteria.[19,20] In addition, butyrate can induce the differentiation of T regulatory cells and control intestinal inflammation, which can reduce the risk of inflammatory bowel disease or colorectal cancer.[21–23] A previous study found the absence of SCFA-producing bacterial taxa has the potential relationship with a proinflammatory state and even fatigue in cancer patients.[9,10] Our study found mild CRF patients have a higher abundance of Lachnospiraceae and Ruminococcaceae, which indicated that fatigue state relieving might be associated with increased SCFA production. Interestingly, human gut microbiota capable of SCFA production was positively associated with anti–PD-1/PD-L1 response.[24] Immune-checkpoint inhibitor treatment has become the standard treatment strategy for lung cancer patients. We suggest that patients with mild CRF might benefit from immune-checkpoint inhibitor treatment attributed to specific gut microbiota patterns. However, our study did not enroll patients treated with immunotherapy, and our further extensive sample research will continue to resolve this issue.

Patients with severe CRF changes in the stool microbial composition were characterized by a lower abundance of gut microbiota capable of SCFA production and have a relative overgrowth of potentially pathogenic taxa. Endotoxins derived from the family Enterobacteriaceae seemed to be a key trigger for systemic inflammation.[25] Specific gut microbiota can drive neuroinflammation and even influence brain function and behavior in rodents and humans.[26] Increased Enterobacteriaceae, a peculiar gut microbiota composition of Parkinsonian patients, has been implicated in disease severity.[27] Patients with cognitive impairment and brain amyloidosis have a higher abundance of Escherichia/Shigella, which are related to a proinflammatory status.[26] These studies suggest a higher abundance of proinflammatory gut

TABLE 3 A significant difference in relative abundance between severe and mild CRF in level 3 KEGG pathways

KEGG pathways	Severe CRF Mean (SD)	Mild CRF Mean (SD)	*p*-value
Biosynthesis of siderophore group nonribosomal peptides	6.05×10^{-4} (9.29×10^{-5})	4.78×10^{-4} (6.08×10^{-5})	0.002
Retrograde endocannabinoid signaling	6.08×10^{-5} (2.12×10^{-5})	4.07×10^{-5} (7.78×10^{-6})	0.005
Amyotrophic lateral sclerosis	2.70×10^{-5} (7.65×10^{-6})	1.66×10^{-5} (3.50×10^{-6})	0.005
Fat digestion and absorption	1.72×10^{-7} (1.10×10^{-7})	5.96×10^{-8} (2.71×10^{-8})	0.008
Homologous recombination	1.63×10^{-2} (1.22×10^{-3})	1.70×10^{-2} (7.20×10^{-4})	0.010
Endocytosis	1.57×10^{-6} (7.21×10^{-7})	8.70×10^{-7} (2.75×10^{-7})	0.010
GnRH signaling pathway	1.57×10^{-6} (7.21×10^{-7})	8.70×10^{-7} (2.75×10^{-7})	0.010
Pyrimidine metabolism	3.05×10^{-2} (1.89×10^{-3})	3.26×10^{-2} (1.53×10^{-3})	0.013
Clavulanic acid biosynthesis	8.00×10^{-7} (5.50×10^{-7})	3.60×10^{-7} (1.34×10^{-7})	0.016
Polycyclic aromatic hydrocarbon degradation	2.71×10^{-3} (2.72×10^{-4})	3.01×10^{-3} (3.07×10^{-4})	0.016
Vibrio cholerae infection	9.19×10^{-6} (5.29×10^{-6})	4.42×10^{-6} (2.53×10^{-6})	0.016
Vibrio cholerae pathogenic cycle	1.57×10^{-3} (3.36×10^{-4})	1.35×10^{-3} (3.43×10^{-4})	0.016
Tryptophan metabolism	5.19×10^{-4} (6.33×10^{-5})	4.50×10^{-4} (2.45×10^{-5})	0.019
Toluene degradation	5.26×10^{-4} (6.84×10^{-5})	4.78×10^{-4} (5.45×10^{-5})	0.019
Meiosis-yeast	4.62×10^{-4} (1.02×10^{-4})	3.71×10^{-4} (9.81×10^{-5})	0.019
Salmonella infection	1.61×10^{-3} (1.30×10^{-4})	1.40×10^{-3} (2.20×10^{-4})	0.019
D-Arginine and D-ornithine metabolism	1.79×10^{-5} (4.27×10^{-6})	1.44×10^{-5} (2.95×10^{-6})	0.023
Pantothenate and CoA biosynthesis	8.07×10^{-3} (6.05×10^{-4})	8.68×10^{-3} (7.00×10^{-4})	0.023
Tropane, piperidine and pyridine alkaloid biosynthesis	1.03×10^{-3} (9.74×10^{-5})	9.77×10^{-4} (8.09×10^{-5})	0.023
Shigellosis	1.00×10^{-5} (2.05×10^{-5})	9.52×10^{-7} (1.13×10^{-6})	0.023
Pertussis	7.92×10^{-4} (2.06×10^{-4})	6.29×10^{-4} (1.26×10^{-4})	0.023
Hypertrophic cardiomyopathy	5.80×10^{-6} (4.16×10^{-6})	3.14×10^{-6} (2.95×10^{-6})	0.023
Alcoholism	1.13×10^{-5} (4.68×10^{-6})	7.23×10^{-6} (2.06×10^{-6})	0.028
Glyoxylate and dicarboxylate metabolism	7.18×10^{-3} (3.79×10^{-4})	6.75×10^{-3} (5.15×10^{-4})	0.034
Propanoate metabolism	3.76×10^{-3} (1.49×10^{-4})	3.61×10^{-3} (1.20×10^{-4})	0.034
Biosynthesis of unsaturated fatty acids	8.61×10^{-4} (8.12×10^{-5})	7.92×10^{-4} (4.46×10^{-5})	0.034
Fatty acid metabolism	1.63×10^{-3} (1.05×10^{-4})	1.52×10^{-3} (1.32×10^{-4})	0.041
Valine, leucine and isoleucine biosynthesis	5.22×10^{-3} (8.19×10^{-4})	5.59×10^{-3} (7.03×10^{-4})	0.041
Porphyrin and chlorophyll metabolism	1.59×10^{-2} (1.68×10^{-3})	1.42×10^{-2} (1.53×10^{-3})	0.041
Sesquiterpenoid and triterpenoid biosynthesis	8.95×10^{-6} (8.31×10^{-6})	2.50×10^{-6} (1.56×10^{-6})	0.041
p53 signaling pathway	1.14×10^{-6} (4.88×10^{-7})	7.72×10^{-7} (1.66×10^{-7})	0.041
Cholinergic synapse	6.88×10^{-6} (2.16×10^{-6})	5.09×10^{-6} (1.63×10^{-6})	0.041
Dopaminergic synapse	1.22×10^{-5} (3.01×10^{-6})	9.51×10^{-6} (1.64×10^{-6})	0.041
Pyruvate metabolism	1.10×10^{-2} (1.87×10^{-4})	1.08×10^{-2} (1.61×10^{-4})	0.049
Complement and coagulation cascades	2.71×10^{-6} (2.67×10^{-6})	7.42×10^{-7} (5.69×10^{-7})	0.049
Renal cell carcinoma	1.03×10^{-4} (5.20×10^{-5})	1.38×10^{-4} (5.66×10^{-5})	0.049

Abbreviations: CRF, cancer-related fatigue; GnRH, gonadotropin-releasing hormone; KEGG, Kyoto Encyclopedia of Genes and Genomes; SD, standard deviation.

microbiota associated with neuroinflammation status could promote CRF in lung cancer patients.

We also explored the difference in functional pathways between mild and severe CRF groups and identified a range of pathways in connection with fatty acid metabolism, nucleotide metabolism, brain function, amino acid metabolism, and so on. Retrograde endocannabinoid signaling plays a crucial role in certain synapse activities in many brain regions, contributing to various brain functions concerning learning and memory.[28] Apart from retrograde endocannabinoid signaling, the tryptophan metabolism pathway involved in brain functions and neurotransmitter metabolism[29] was abundant differently between mild and severe CRF patients. Valine, leucine and isoleucine biosynthesis have important mediation effects on regulating metabolism of glucose, lipid, and immunity.[30] Furthermore, pyruvate metabolism is a keystone pathway for numerous human metabolism,

FIGURE 4 Comparison between mild versus severe CRF patients in KEGG pathways based on Random Forest analysis. CRF, cancer-related fatigue; KEGG, Kyoto Encyclopedia of Genes and Genomes; **, $p < 0.01$; *, $p < 0.05$

including fatty acid synthesis and oxidation.[31] Based on this scenario, we speculate that the fatty acid metabolism pathway, amino acid metabolism pathway, and glucose metabolism pathway can directly or indirectly affect SCFA production, affecting the inflammatory state and the brain-gut axis. Our functional studies also reflect that gut microbiota may attenuate or induce CRF by altering inflammatory status and neurological function, but further studies are needed to confirm this.

There are some limitations in our study. First, our preliminary study was small and probably underpowered, and our results should therefore be interpreted cautiously. Nevertheless, we observed promising differences in specific taxa abundances between the mild and severe CRF groups, and our study lays the groundwork for further validation in a more extensive sample study. In addition, ample current evidence shows intervention with prebiotics and probiotics can contribute to SCFA production,[32] indicating that extra probiotics may be valuable for managing CRF. Our study suggests that the composition of gut microbiota is different in patients with various severity of fatigue, which provides a theoretical basis for future evaluation of whether CRF can be improved by intervening in the composition of gut microbiota. Second, our study only included patients with advanced lung cancer who received first-line chemotherapy, ignoring patients with advanced lung cancer who received combination therapy or immunotherapy alone. Due to the small sample size of our study, to reduce the influence of confounding factors, patients who received combination therapy were excluded, and we will further explore such patients in future studies. Third, due to the preliminary exploratory nature, our study did not include patients with moderate CRF and non-CRF but instead

included the two groups with the most significant differences in the impact on patients' quality of life: cancer patients with mild and severe CRF. In future studies, patients with moderate CRF and non-CRF will be included to further explore the relationship between CRF and gut microbiota composition.

In conclusion, our study observed a plausible association between different levels of CRF and the diverse gut microbiota composition, with increasing proinflammatory taxa in severe CRF patients and anti-inflammation taxa growing in mild CRF patients. Further studies are warranted to evaluate whether CRF can be improved by modulating the gut microbiota composition.

AUTHOR CONTRIBUTIONS

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Yu Sun, Jiang Zhu, and Mei Li. Acquisition of data: Hao Wei, Lingling Xie, Yihan Zhao, and Jun He. Analysis and interpretation of the data: Hao Wei, Lingling Xie, and Yihan Zhan. Drafting of the manuscript: Hao Wei. Critical revision of the manuscript for important intellectual content: Yu Sun and Mei Li. Statistical analysis: Hao Wei, Lingling Xie, Yihan Zhao, and Jun He. Obtained funding: Yu Sun. Administrative, technical and material support: Jiang Zhu. Study supervision: Yu Sun and Mei Li.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

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

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