ORIGINAL ARTICLE

Characterization of the gut microbiota in frail elderly patients

Ling Zhang[1] · Jianjun Liao[1] · Qiaochao Chen[1] · Miaohong Chen[1] · Yingfei Kuang[1] · Long Chen[1] · Wen He[1]

Abstract

Background The change in the composition of gut microbiota has been reported in the elderly and in the frail individuals; however, studies on gut microbiota in frail elderly are limited.

Aims This study aimed to investigate the gut microbiota of the frail elderly.

Methods From September 2017 to February 2018, 27 elderly patients hospitalized in the Department of Geriatrics of our hospital were enrolled and divided into the frailty group ($n = 15$) and the control group ($n = 12$) based on the cutoff of 0.25 for the frailty index. The fecal samples were collected for 16S rRNA-amplicon sequencing to analyze the composition and richness of gut microbiota. Operational taxonomic unit (OTU) clustering was performed using Usearch software. Intra-sample diversity (alpha-diversity) analysis and inter-sample diversity (beta-diversity) analyses were performed. The community richness was compared between the two groups at family and genus levels.

Results There were 1903 and 1880 OTUs identified in the control and frailty groups, respectively, with 1282 OTUs overlap between the two groups. The alpha diversity of microbiota community was similar between the two groups, whereas the frailty group had larger beta diversity than the control group. The top-10 taxonomy categories and abundances of gut microbiota between the two groups were similar. As for the gut microbiota composition, 4 families and 17 genera were significantly different between the two groups ($p < 0.05$).

Conclusion These results suggested that frailty can affect gut microbiota diversity and compositions in late elderly hospitalized patients.

Keywords Elderly · Frailty · Frailty index · Gut microbiota

Introduction

Population aging is a global issue, and the speed of population aging is much faster than in the past. According to the United Nations, the number of people aged over 60 is estimated at 1.2 billion by 2025 [1]. In China, the population over 60 years old is estimated at 300 million by 2025 [2]. This demographic shift brings major challenges to the health and social systems of all countries. Frailty is an aging-associated syndrome characterized by significant vulnerability to

stressors due to physiological decline across multiple organ systems, elevating the risk of adverse outcomes, such as falls, disability, hospitalization and even death [3]. Frailty is associated with aging, malnutrition, sarcopenia, concurrent multiple diseases, sleep disorders, anxiety and depression [4–6].

Gut microbiota is defined as the community of microbes (including bacteria, archaea, and eukarya) inhabiting the GI tract which has a complicated and mutually beneficial relationship with the host [7]. The number of gut microorganisms is estimated to over 10^{14} [8]. Gut microbiota plays an important role in the metabolism and immune function of the host. Human gut microbiota composition is affected by multiple factors, such as age, race, disease, and medication intake [9–11]. Intestinal microorganisms can hydrolyze complex plant polysaccharides to promote the host's metabolic capacity and produce short-chain fatty acids, which can improve the energy metabolism of colon cells [12]. Some short-chain fatty acids even have anti-inflammatory effects

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[13]. Human gut microbiota composition is relatively stable during adulthood but undergoes the age-related reduction in biodiversity [9, 14–16]. Changes in the composition of the gut microbiota have been reported to be related with several clinical conditions, such as obesity, diabetes, nonalcoholic fatty liver disease, allergic diseases, diabetes, gastrointestinal diseases, autoimmune diseases, and even cancer [17, 18]. Verdi et al. have shown that frailty is associated with gut microbiota in the TwinsUK British twin cohort aging over 40 years [19]. Theou et al. have reported that a prebiotic intervention can reduce frailty levels in non-demented nursing home elder over 65 years, suggesting that gut microbiota has an effect on the frailty levels in the elderly [20]. Although the change in the composition of gut microbiota has been reported in elderly [21] and in frail individuals [22]; however, studies on gut microbiota in frail elderly are still limited [23, 24], especially in late elderly (aged ≥ 75 years). The purpose of this study was to characterize the gut microbiota in late elderly patients with frailty.

Methods

Participants and grouping

From September 2017 to February 2018, 27 elderly patients hospitalized in the Department of Geriatrics, First Affiliated Hospital of Sun Yat-sen University were enrolled. Admission reasons, dietary regimen during hospital stay, and pharmacologic treatments of the enrolled patients have been summarized in Table 1. Frail status of the patient was evaluated by Rockwood Clinical Frailty Scale [25]. The exclusion criteria were as follows: acute infectious diseases or end-stage disease; patients receiving antibiotics, prebiotics treatment or other drugs that regulate intestinal flora in the previous month; receiving gastrointestinal surgery within 6 months. This study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of our hospital. Written informed consent was obtained from the patient.

Table 1 Admission reasons, dietary regimen during hospital stay, and pharmacologic treatments of the patients

	Control ($n = 12$)	n	Frailty ($n = 15$)	n
Admission reasons	Dizziness	3	Dizziness	4
	Arrhythmia	1	Bilateral lower limb weakness	2
	Follow-up after cardiac valve replacement	1	Osteoporosis	2
	Follow-up for coronary heart disease	1	Anxiety	1
	Gastrointestinal stromal tumor	1	Arrhythmia	1
	Hyperuricemic nephropathy	1	Follow-up after cerebral infarction	1
	Osteoporosis	1	Follow-up for coronary heart disease	1
	Post-herpetic neuralgia	1	Joint pain	1
	Transient ischemic attack	1	Parkinson disease	1
	Urinary retention	1	Transient ischemic attack	1
Dietary regimen during hospital stay	Low-salt, low-sugar	7	Low-salt, low-sugar	5
	Low-salt	2	Low-salt	4
	Low-salt, low-purine	1	Low-sugar	2
	Low-salt, low-sugar, low-purine	1	Ordinary diet	2
	Ordinary diet	1	Low-purine	1
			Low-salt, low-sugar, low-purine	1
Pharmacologic treatments	Antihypertensive and secondary prevention of coronary heart disease drugs	3	Antihypertensive and secondary prevention of coronary heart disease drugs	4
	Improve circulation, antiplatelet drugs	3	Improve circulation, antiplatelet drugs	4
	Antihypertensive and antiarrhythmic drugs	1	Anti-osteoporosis drugs	2
	Anti-osteoporosis drugs	1	Antihypertensive and antiarrhythmic drugs	1
	Antiplatelet drugs	1	Antihypertensive drugs	1
	Anti-prostatic hyperplasia drug	1	Anti-Parkinson drug	1
	Painkiller	1	Anxiolytic	1
	Secondary prevention of coronary heart disease and uric acid lowering drugs	1	Painkiller	1

Frailty assessment

Frail status of the patient was evaluated by modified Rockwood frailty questionnaire [26], which consisted of 50 health defects items, mainly including: disease history: hypertension, diabetes, stroke, depression, degenerative diseases, family history of cognitive impairment, etc.; self-care ability: ability to dress, bath, toilet independently, etc.; mental health: emotions problems, anxiety or depression, loneliness, etc.; auxiliary examinations: albumin, BMI, etc. Each item represented a health defect issue. The patient having the defect of the item was recorded as "1", otherwise it was recorded as "0". The frailty index was calculated as follows: FI = cumulative health defects/ total defects (50). Patients with frailty index < 0.25 were grouped into the frailty group ($n = 15$), while those with frailty index ≥ 0.25 were grouped into the control group ($n = 12$) [26].

16S rRNA sequencing

To analyze the gut microbiota diversity, fecal samples of the participants were collected for 16S rRNA sequencing. Fresh fecal samples were collected within 3 days after enrollment. After collection, fresh stool samples were stored at − 80 °C refrigerator or liquid nitrogen tanks within 4 h. The genomic bacterial DNA was extracted from fecal samples using the freeze–thaw method. The 16S rDNA was PCR-amplified and then sequenced on the MiSeq system (Illumina, USA). The primer sequence used in the PCR were F: 5′GTGCCA GCMGCCGCGGTAA3′ and R:5′ GGACTACHVGGGTWT CTAAT3′.

Operational taxonomic unit (OTU) clustering

The sequence reads with a similarity greater than 97% were identified and clustered into an OTU using the Usearch software [27]. The Ribosomal Database Project (RDP) classifier Bayesian algorithm was applied to classify the OTUs clusters, and the community composition was analyzed in each taxonomic rank: domain, kingdom, phylum, class, order, family, genus, and species.

Rank-abundance curve

Within a single sample, the number of sequences included in every single OTU was counted, and the OTUs were sorted by the order of richness. Then the percentage of the sequences number in each OTU was plotted on the rank-abundance curve, which can be used to explain the species abundance and uniformity.

Rarefaction curve

Rarefaction curves were created by repeatedly randomly re-sampling the whole population multiple times and then plotting the average number of species found in each sample. It can be used to compare the species richness between samples, and it can also be used to indicate whether the sampling size of the sample is reasonable.

Intra-sample diversity analysis (alpha-diversity)

The single sample diversity analysis (alpha diversity) can reflect the richness and diversity of microbial communities, including a series of statistical analysis indices to estimate the species abundance and diversity of communities. The community richness indexes included Chao1, Ace, and SOBs, while the community diversity indexes were Shannon and Simpson. Sequencing depth was evaluated by coverage.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) while categorical data were expressed as number and percentage (%). Student's independent t test was used to compare the differences between groups. If normality of continuous variables was not assumed, non-parametric analysis Mann–Whitney U test would be used instead. Chi square test and Fisher's exact test (if any expected value lower than 5 was observed) were used for categorical data. Principal co-ordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS) were used to analyze the beta diversity. Hierarchical and K means clustering methods were used to examine the grouping results from diversity index. A p value lower than 0.05 would be recognized as significant. All analyses were performed using IBM SPSS Version 20 (SPSS Statistics V20, IBM Corporation, Somers, New York). Bioinformatics results were analyzed using R software (version 3.5.2).

Results

Patient's clinical characteristics and laboratory index

A total of 27 patients (17 males and 10 females, mean age 81.63 ± 7.90) were enrolled and divided into frailty group (frailty index < 0.25, $n = 15$) and control group (frailty index ≥ 0.25, $n = 12$). Patient's demographic and clinical characteristics were summarized in Table 2. Frailty index

Table 2 Patient's demographic and clinical characteristics

Parameters	Frailty (n = 15)	Control (n = 12)	Total (n = 27)	p
Gender				0.706
Male	10 (66.67)	7 (58.33)	17 (62.96)	
Female	5 (33.33)	5 (41.67)	10 (37.04)	
Age, years	82.00 ± 7.63	81.17 ± 8.54	81.63 ± 7.90	0.922
BMI, kg/m^2	21.07 ± 4.27	23.83 ± 2.48	22.30 ± 3.79	0.059
Frailty index	0.35 ± 0.09	0.14 ± 0.07	0.26 ± 0.13	<0.001
Heathy assessment score	51.87 ± 6.45	56.92 ± 7.66	54.11 ± 7.33	0.083
Complications				
Hypertension	10 (66.67)	10 (83.33)	20 (74.07)	0.408
CAD	7 (46.67)	4 (33.33)	11 (40.74)	0.696
Diabetes	6 (40.00)	8 (66.67)	14 (51.85)	0.252
Hyperuricemia	7 (46.67)	4 (33.33)	11 (40.74)	0.696
COPD	3 (20.00)	2 (16.67)	5 (18.52)	1.000
Stroke	5 (33.33)	1 (8.33)	6 (22.22)	0.182
Dementia	2 (13.33)	0	2 (7.41)	0.487
Parkinson's disease	2 (13.33)	0	2 (7.41)	0.487
Renal disease	0	1 (8.33)	1 (3.70)	0.444
Tumor	9 (60.00)	3 (25.00)	12 (44.44)	0.069
Blood biochemical indexes				
White blood cell, 10^9/L	6.63 ± 2.27	6.15 ± 1.53	6.42 ± 1.95	0.533
Hemoglobin, g/L	122.80 ± 19.74	124.83 ± 16.52	123.70 ± 18.06	0.778
Platelet, 10^9/L	223.20 ± 120.14	176.50 ± 40.15	202.44 ± 94.94	0.210
Albumin, g/L	37.03 ± 3.98	37.74 ± 3.52	37.35 ± 3.72	0.633
Cr, umol/L	81.13 ± 22.30	95.00 ± 50.69	87.30 ± 37.47	0.349
Potassium, mmol/L	13.16 ± 36.20	4.00 ± 0.31	9.09 ± 26.96	0.391
Sodium, mmol/L	130.66 ± 35.23	139.75 ± 2.90	134.70 ± 26.33	0.383
Total cholesterol, mmol/L	4.11 ± 1.22	4.31 ± 0.83	4.20 ± 1.05	0.641
Triglycerides, mmol/L	1.05 ± 0.40	13.16 ± 40.27	6.43 ± 26.90	0.253
Low density lipoproteins, mmol/L	2.42 ± 0.77	2.69 ± 0.69	2.54 ± 0.74	0.345
High density lipoproteins, mmol/L	1.33 ± 0.42	1.12 ± 0.24	1.24 ± 0.36	0.130
Uric acid, μmol/L	354.87 ± 94.37	389.67 ± 116.26	370.33 ± 104.04	0.398
Hemoglobin A1c, %	6.10 ± 0.96	6.51 ± 1.30	6.28 ± 1.12	0.355

BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease

was significantly higher in the frailty group than in the control group (0.35 ± 0.09 vs. 0.14 ± 0.07; $p < 0.001$). However, there was no significant difference in other outcomes, including all demographic and clinical characteristics, and all blood biochemical indexes (all $p > 0.05$, Table 2), These results indicated the comparability between the two groups.

Operational taxonomic unit (OTU) clustering and community richness

To characterize gut microbiota, fecal samples were collected for 16S rRNA-amplicon sequencing. The 16S rRNA sequencing reads were clustered using the Usearch software with a 97% sequence similarity threshold. A total of 2501 OTUs were detected (range 187–794). Figure 1 showed the rank-abundance curve of each sample. As the number of

OTUs increased, the relative abundance decreased. Most samples had an abundance between 200 and 600.

The Venn diagram showed the number of common microbial taxonomies between the frailty and control groups (Fig. 2). There were 1282 OTUs overlapping between the two groups, while 621 and 598 OTUs were exclusively belonging to the control and frailty groups, respectively.

Alpha diversity analyses

Rarefaction curve indicates the results of observed species corresponding to random sampling sequences per sample (Fig. 3). In all samples, a flat trend was observed as the number of sequences increased, indicating that maximum sequencing level had been reached and the sequencing results were reliable.
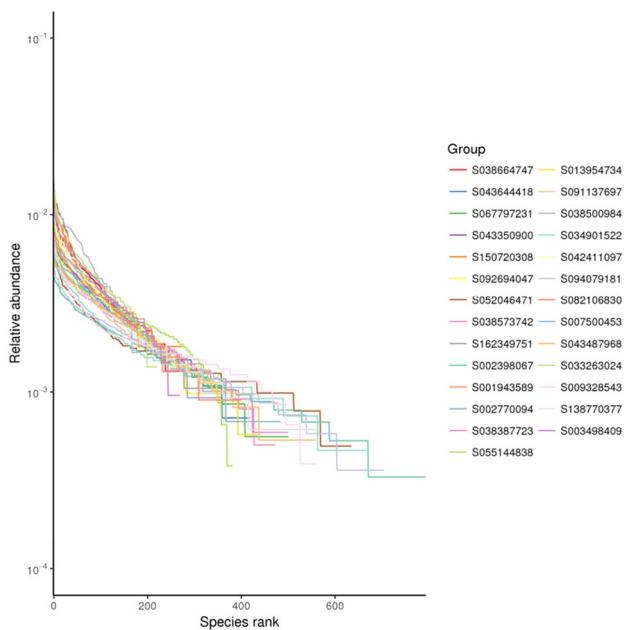
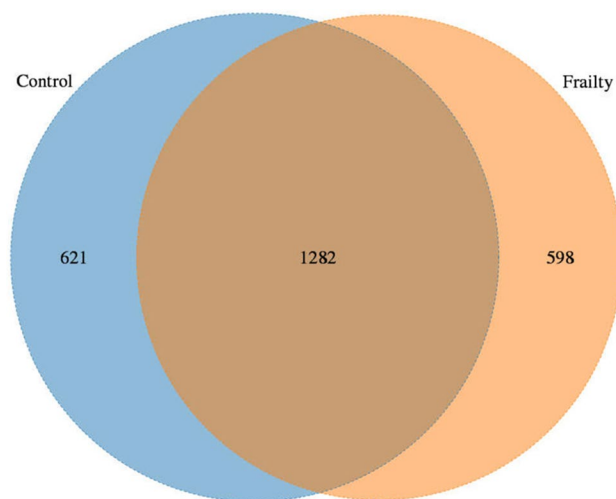**Fig. 1** The rank-abundance curve of per sample

Fig. 2 The Venn diagram of OTUs distribution of the control and frailty groups

Fig. 3 The rarefaction curve of random sequences per sample and their corresponding number of observed species

Beta diversity analyses

For beta diversity analysis, principal co-ordinates analysis (PCoA) and non-metric multidimensional scaling (NMDS) were performed. Although a major overlapping was observed in the PCoA plot, the PC2 axis shows a relatively larger distribution in the frailty group than the control group (Fig. 5a). Likewise, in the NMDS plot, the frailty group had a relatively larger distributed area than the control group. These results suggested that the frailty group had a larger beta diversity than the control group.

Gut microbiota diversity analysis in the phylum and genus level

The diversity of intestinal microbe between the frailty and control groups were analyzed on phylum (Fig. 6a) and genus (Fig. 6b). The top-10 taxonomy categories and abundances of gut microbiota were similar between the two groups.

The top-10 abundant phyla were *Firmicutes*, *Bacteroidetes*, *Proteobacteria, Actinobacteria, Verrucomicrobia*, *Fusobacteria*, *Acidobacteria*, *Chloroflexi*, *Patescibacteria*, and *Epsilonbacteraeota*. The top-10 abundant genera included *Bac Bacteroides*, *Lac Lachnospiraceae UCG-008*, *Lac Blautia*, *Rum Faecalibacterium*, *Lac Lachnoclostridium*, *Str Streptococcus*, *Lac Roseburia*, *Tan Parabacteroides*, *Rik Alistipes*, and *Bif Bifidobacterium.*

The community richness at family and genus levels were compared between the frailty and control groups. As shown in Table 3, there were 4 and 17 significant the gut microbiota composition at family and genus levels, respectively ($p < 0.05$), suggesting that microbial diversity and distribution are different between the frailty and control groups.

For alpha diversity analysis, we calculated the Shannon index and Simpson index. The Shannon index of frailty and control groups were 4.39 ± 1.34 and 4.63 ± 0.65, respectively ($p = 0.722$, Fig. 4a); while the Simpson index of frailty and control groups were 0.84 ± 0.13 and 0.89 ± 0.04, respectively ($p = 0.887$, Fig. 4b). These results suggested that there was no statistical difference in alpha diversity of microbiota community between the two groups.
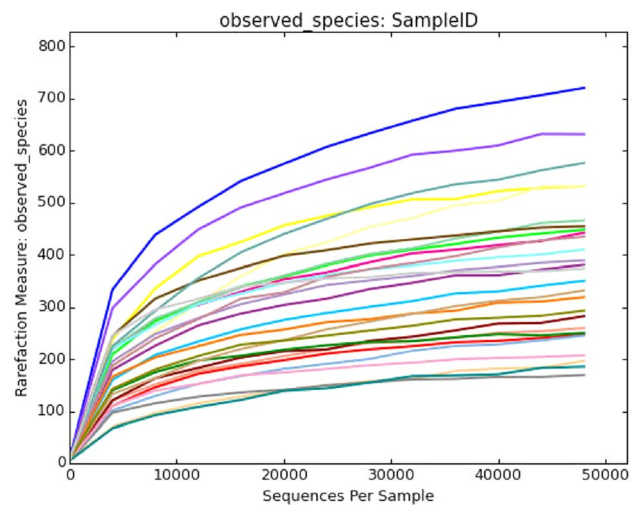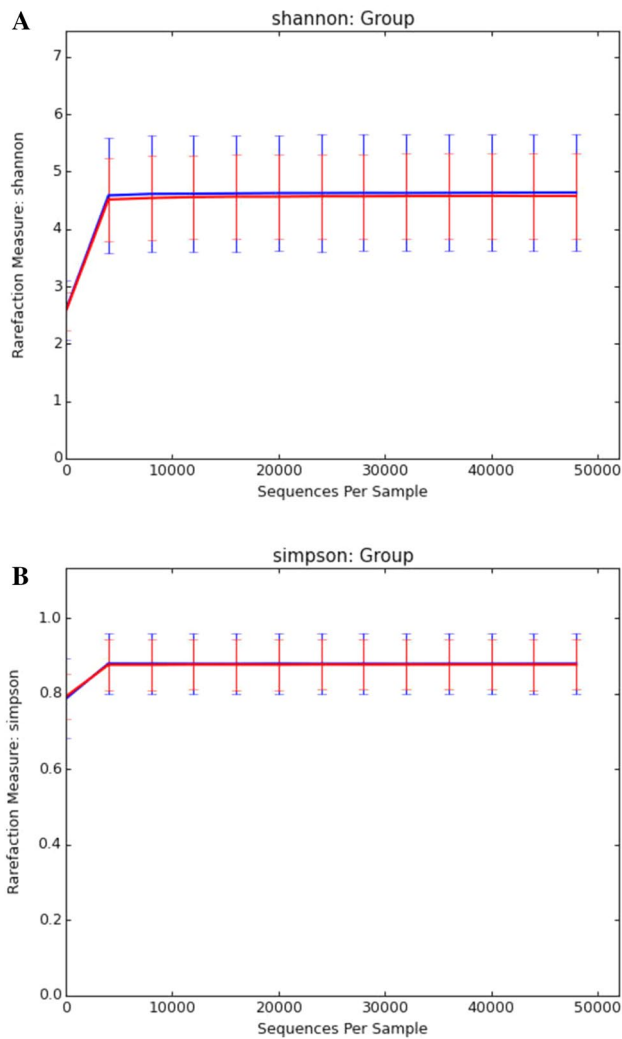
A

B

Fig. 4 The Shannon index (**a**) and Simpson index (**b**) of the frailty group (red line) and control group (blue line)

A

B

Fig. 5 The distributed results of PCoA (**a**) and NMDS (**b**)

Clustering analyses

Hierarchical clustering and K-means clustering methods were used to investigate the grouping by microbial diversity indexes, including Shannon index, Simpson index, chao1, ACE, and goods coverage. As indicated in Fig. 7, the hierarchical clustering correctness rates of the frailty and control groups were 60% and 72.7%, respectively.

The *K* means clustering correctness rates of the frailty and control groups were 55.6% and 75%, respectively. These results suggested that the frailty and control groups indeed possessed different gut microbiota diversity, and the diversity indexes could be used to discriminate the frailty and non-frailty elderly.
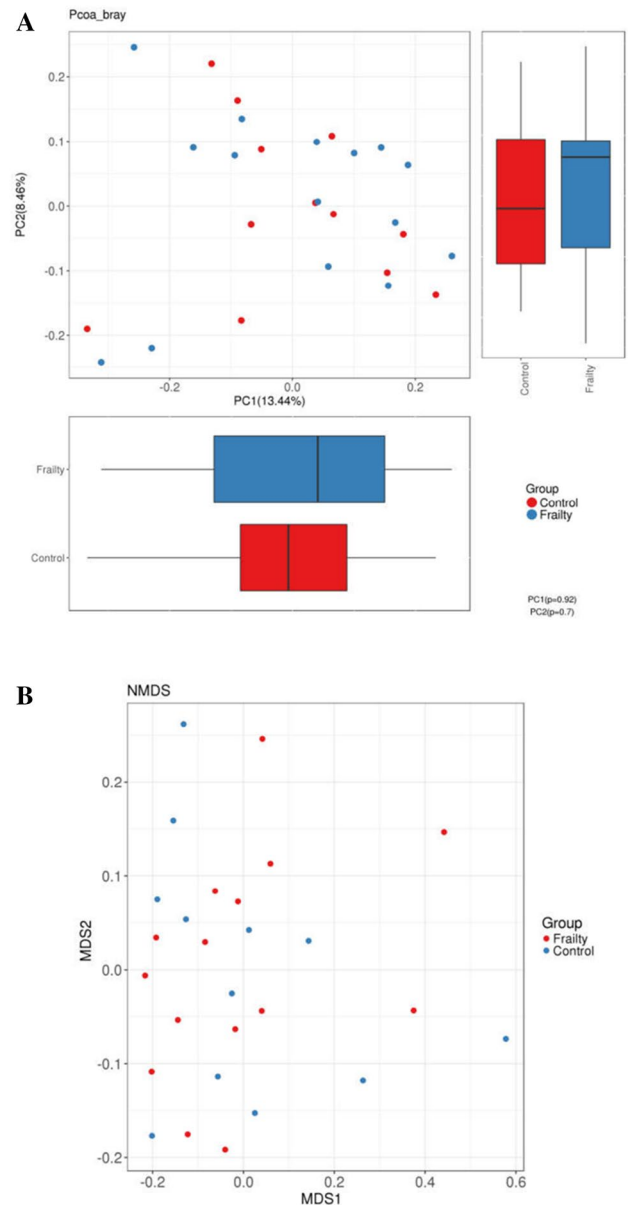
Discussion

In this study, we investigated the gut microbiota of the frail elderly. The results showed that there were 1903 and 1880 OTUs identified in the control and frailty groups, respectively, with 1282 OTUs overlap between the two groups. The alpha diversity of microbiota community was similar between the two groups, whereas the frailty group had larger beta diversity than the control group. The top-10 taxonomy categories and the abundances of gut microbiota between two groups were mainly identical. As for the gut microbiota composition, however, 4 families and 17 genera were significantly different between the two groups. Taken together,
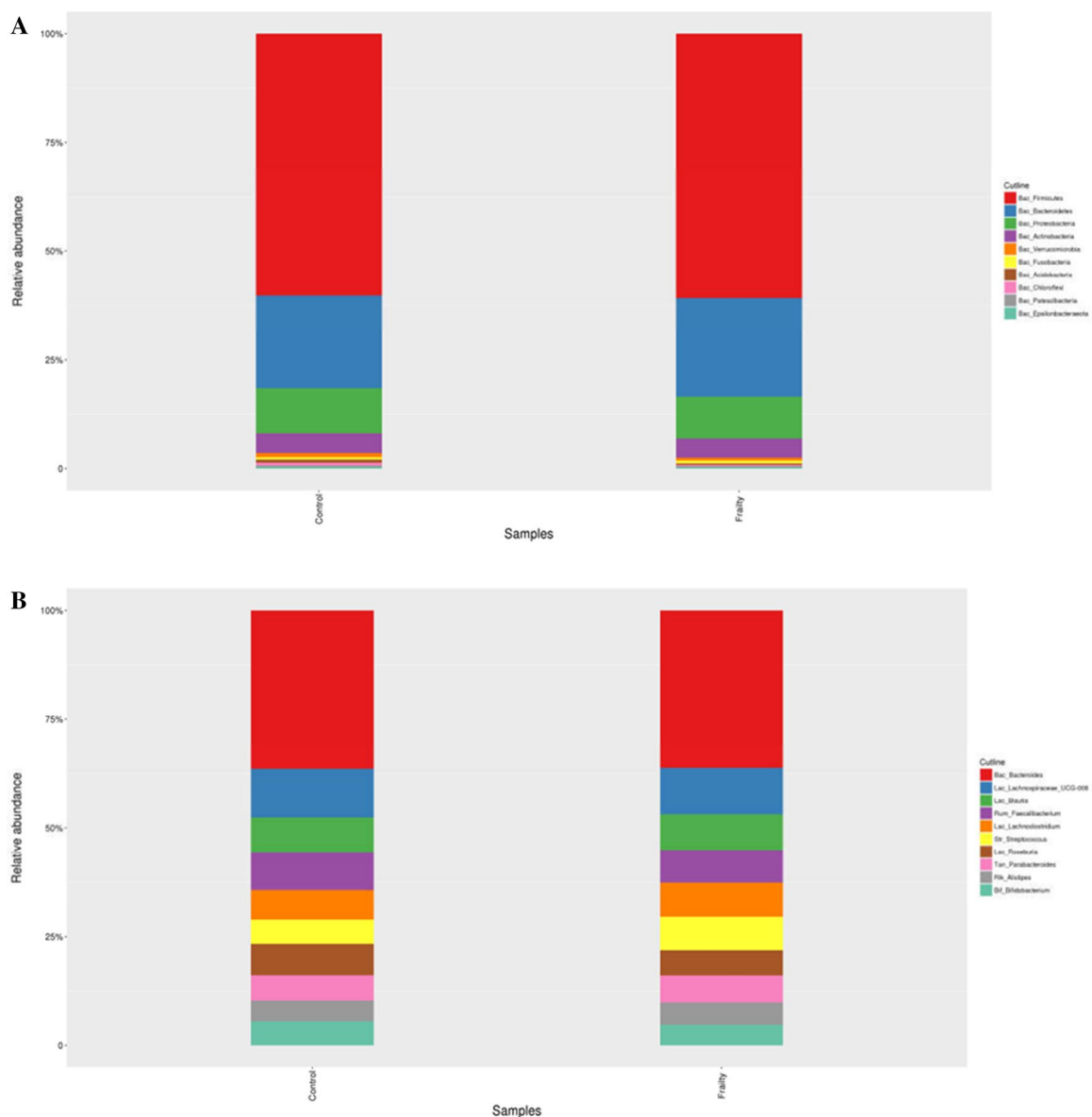
Fig. 6 The percentages of gut microbiota diversity at phylum (**a**) and genus (**b**) levels

these results suggested that frailty can affect gut microbiota diversity and compositions in late elderly hospitalized patients.

The gut microbiota composition and function are stable in adulthood up to 65–70 years, and then the inter-individual variability would be increased with the decline of biodiversity and a tendency to dysbiosis, leading to significant disturbance in host physiology [28]. Animal studies have demonstrated that dysbiosis of gut microbiota can elevate intestinal permeability [29], which promote endotoxin and other microbial products [e.g., lipopolysaccharide (LPS)] entering the circulation. Circulating LPS induces skeletal muscle inflammation and insulin resistance [30], which impairs both the metabolic and contractile characteristics

of skeletal muscle. The relevant systemic inflammation has been demonstrated in the pathophysiology of sarcopenia patients [31]. These findings suggest the possibility of a "gut–muscle axis", namely gut microbiota act as the mediator of the effects of nutrition on muscle cells. This phenomenon leads to a decrease in muscle strength and quality, further enhances insulin resistance, and ultimately leads to sarcopenia and physical frailty [32, 33].

Accumulating evidence has revealed the correlation between gut microbiota and frailty [34–36], as well as the composition of gut microbiota in the elderly [37–39], but little attention has been paid to the gut microbiota in frail elderly. In a small pilot study, van Tongeren et al. report that the abundance of *Bacteroides*/*Prevotella* and the

**Table 3** Significant results of community richness differences between groups

Bacteria species	Frailty (n = 15)	Control (n = 12)	p
Family level			
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Family_XI	0.000268	0.001240	0.022
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae	0.003135	0.001083	0.042
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mollicutes_RF39;f__KF843164_f	0.000889	0.000311	0.046
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Betaproteobacteriales;f__A21b	0.000000	0.000223	0.046
Genus level			
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Acetanaerobacterium	0.000529	0.000000	0.010
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Family_XI;g__Gemella	0.000268	0.001240	0.022
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnoanaerobaculum	0.000135	0.000835	0.023
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Eubacterium]_ruminantium_group	0.001107	0.002764	0.024
k__Bacteria;p__Firmicutes;c__Erysipelotrichia;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Catenibacterium	0.000698	0.000000	0.025
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Betaproteobacteriales;f__Rhodocyclaceae;g__Azospira	0.000000	0.000399	0.029
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]_torques_group	0.006146	0.003805	0.030
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__DQ801572_g	0.000627	0.000030	0.031
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Tyzzerella	0.000822	0.001825	0.031
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Flavobacteriales;f__Weeksellaceae;g__Cloacibacterium	0.000033	0.001105	0.031
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcaceae_UCG-011	0.001219	0.000359	0.034
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella_9	0.004618	0.002231	0.043
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Muribaculaceae;g__EU455341_g	0.000000	0.000322	0.044
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Atopobiaceae;g__Olsenella	0.001096	0.000281	0.044
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__EF434341_g	0.000146	0.000000	0.044
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mollicutes_RF39;f__KF843164_f;g__KF843164_g	0.000889	0.000311	0.046
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Pseudoxanthomonas	0.000549	0.000039	0.048

Faecalibacterium prausnitzii is significantly reduced in frailer elderly, while the abundance of *Enterobacteriaceae* which can cause opportunistic infections is significantly increased in frailer individuals [23]. Jackson et al. have reported that frailty is negatively associated with the alpha diversity of gut microbiota. Species more abundant in the frail elderly included *Eubacterium dolichum* and *Eggerthella lenta*, while *Faecalibacterium prausnitzii* is less abundant in frail elderly [24]. *F. prausnitzii* is a key butyrate producer [35, 36] and has an anti-inflammatory effect on the gut [40]. The abundance of *F. prausnitzii* is decreased in patients with inflammatory bowel disease [41]. Likewise, Haran et al. have demonstrated that the nursing home elder with increasing frailty has lower abundances of butyrate-producing bacteria [42]. Nevertheless, the composition of the gut microbiota is greatly affected by diet, environment, and ethnicity [9–11],

and may exhibit varying patterns in different countries and geographic regions.

The dominant gut microbial phyla in human are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, in which *Firmicutes* and *Bacteroidetes* representing 90% of gut microbiota [43]. Supporting this notion, our results demonstrated that the common dominant phyla between the two groups were *Firmicutes*, *Bacteroidetes*, *Proteobacteria,* and *Actinobacteria, while* the common dominant genera included *Bac Bacteroides*, *Lac Lachnospiraceae UCG-008*, *Lac Blautia*, and *Rum Faecalibacterium*. Of note, there was no significant difference in the top-10 taxonomy categories and the abundances of gut microbiota at both phylum and genus levels between the two groups. According to the Shannon index and the Simpson index, we found that the alpha diversity of gut microbiota was similar between the

Fig. 7 The dendrogram of hierarchical clustering results, label A and B are the frailty and control groups, respectively
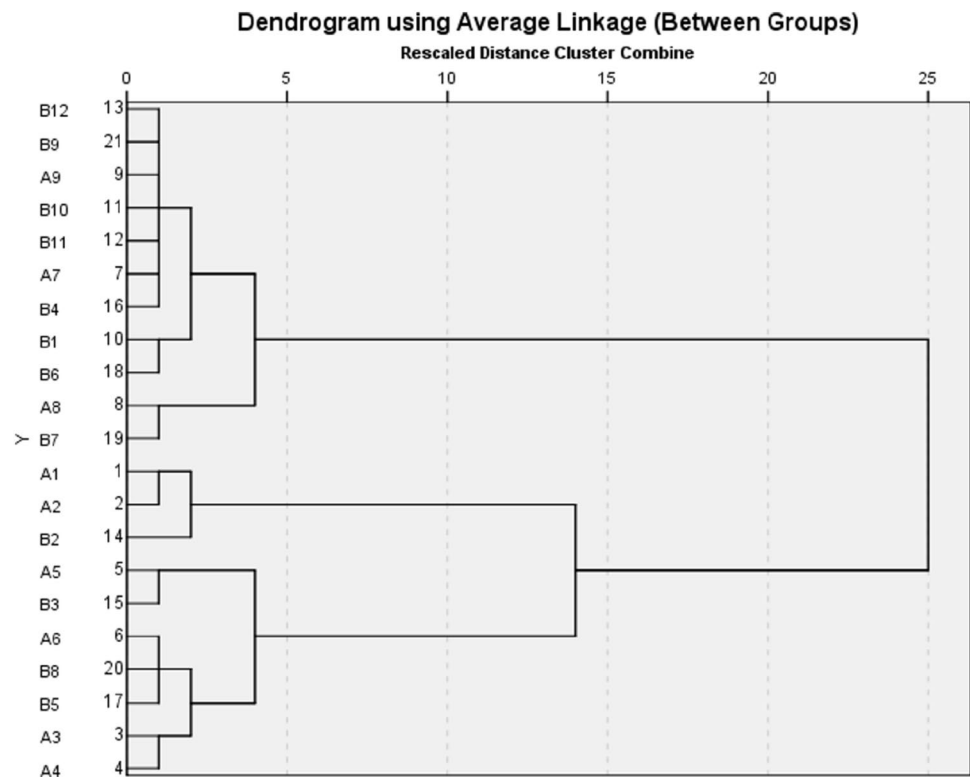
frailty group and the control group, which is inconsistent with Jackson et al.'s report [24]. This discrepancy may be attributed to the small sample size of this study. While in the beta diversity analysis, the frailty group had a relatively larger distribution area in both PCoA and NMDS plots as compared with the control group. One possible explanation might be that the various etiologies of frailty contributed to a larger difference in the beta diversity among the 15 frail elderly patients.

Comparing the gut microbiota at the genus level between the two groups, we found that *Gemella*, *Lachnoanaerob-aculum*, *Eubacterium[U1]_ruminantium_group*, *Azospira*, *Tyzzerella*, *Cloacibacterium*, *and EU455341_g* genera were significantly less abundant in the frailty group, while the *Acetanaerobacterium*, *Catenibacterium*, *[Ruminococcus]_torques_group*, *DQ801572_g*, *Ruminococcaceae_UCG-011*, *Prevotella_9*, *Olsenella*, *EF434341_g*, *KF843164_g*, *and Pseudoxanthomonas* were more abundant in the frailty group as compared with the frailty group. Family *Moraxel-laceae* was more abundant in the frailty group. These results indicated that the composition of gut microbiota shows different patterns in different countries and geographic regions. Of them, genera *Prevotella*, *Olsenella*, *Catenibacterium*, and *Tyzzerella* are reported to be associated with a lifetime risk of cardiovascular disease [44]. On the other hand, *Olsenella* bacteria are involved in endodontic infections in humans [45]. The pathogenic role of these microbiomes may be involved in the development of frailty in the elderly.

However, it is unknown whether these relationships are the causes or consequences of frailty, and the underlying mechanism remains to be further investigated.

Reduction in microbiota-related metabolic capacity, such as lower short-chain fatty acids (SCFAs) levels may be associated with age-related frailty [46]. SCFAs mainly include acetic acid, propionic acid, and butyric acid, which stimulate the secretion of mucin by inducing hypoxia-inducible factor (HIF) in the intestinal epithelial cells, thereby strengthening the intestinal mucosal barrier. Meanwhile, SCFAs promote IL-8 release, accelerating repair of the intestinal mucosal barrier and modulate inflammation, oxidative stress [47]. High frail individuals are reported to have a decrease in the abundance of *Eubacteria*, *Faecalibacterium*, and *Lacto-bacilli* [48]. We also found that *Eubacteria* was less abundant in the frailty elderly ($p = 0.023$); however, there was no significant difference in *Lactobacilli and Faecalibacte-rium* between the two groups. *Eubacterium* is considered as protective colonic bacteria by producing SCFAs (especially butyrate) [49] and modulating the immune system [50]. Thereby, a decrease in *Eubacterium* abundance may negatively affect gut health or induce systemic effects. The *Eubacterium* decreased in the frail elderly may support the protective effect of SCFAs on human guts. However, the detailed mechanism is needed to be further investigated. Our result showed that genus *Prevotella* was more abundant in the frail group, which is in line with van Tongeren et al.'s report [23]. For other bacterial genera with an abundant

change in our study, their potential correlations with the development of frailty remain to be elucidated.

Several limitations of this study should be pointed out. First, the sample size of this study is small, and the findings of this study should be validated in a large study. In addition, hospitalization may have an impact on gut microbiota composition due to exposure to nosocomial pathogens, disruption of usual dietary and lifestyle habits, presence of acute diseases and need for acute treatments. Nevertheless, this study was conducted in the elderly health care ward of our hospital, and all patients were admitted to a single ward. In addition, the patients with acute infectious diseases, antibiotics or probiotics were excluded. Thus, the impact of hospitalization could be minimized. Moreover, we cannot determine whether frailty is the cause, consequence or bystander of the changes in gut microbiota diversity and composition. All these studies should be addressed in the future study.

In summary, our study demonstrated that frailty can change the abundance and composition of gut microbiota in Chinse frail late elderly hospitalized patients, further highlighting an association between the gut microbiota alterations and development of frailty. Our finding may provide a theoretical basis for further study on the pathological mechanism for the development of frailty.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Statement of human and animal rights This study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of the First Affiliated Hospital, Sun Yat-Sen University. Written informed consent was obtained from the patient.

Informed consent The written informed consent was waived by the IRB due to the retrospective nature of this study.

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