ORIGINAL RESEARCH

Gut Microbiota Dysbiosis and Inflammation Dysfunction in Late-Life Depression: An Observational Cross-Sectional Analysis

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Purpose: There are some challenges to diagnosis in the context of similar diagnostic criteria for late-life depression (LLD) and adult depression due to cognitive impairment and other clinical manifestations. The association between gut microbiota and inflammation remains unclear in LLD. We analyzed gut microbiota characteristics and serum inflammatory cytokines in individuals with LLD to explore the combined role of these two factors in potential biomarkers of LLD.

Methods: This was an observational cross-sectional study. Fecal samples and peripheral blood from 29 patients and 33 sex- and age-matched healthy controls (HCs) were collected to detect gut microbiota and 12 inflammatory factors. We analyzed differences in diversity and composition of gut microbiota and evaluated relations among gut microbiota, inflammatory factors, and neuropsychological scales. We extracted potential biomarkers using receiver-operating characteristic curve analysis to predict LLD utilizing the combination of the microbiota and inflammatory cytokines.

Results: Elevated systemic inflammatory cytokine levels and gut microbiota dysbiosis were found in LLD patients. Relative abundance of Verrucomicrobia at the phylum level and *Megamonas, Citrobacter*, and *Akkermansia* at the genus level among LLD patients was lower than HCs. Abundance of *Coprococcus, Lachnobacterium, Oscillospira*, and *Sutterella* was higher in LLD patients. Notably, IL6, IFNγ, Verrucomicrobia, and *Akkermansia* levels were correlated with depression severity. Our study identified IL6, *Akkermansia*, and *Sutterella* as predictors of LLD, and their combination achieved an area under the curve of 0.962 in distinguishing LLD patients from HCs.

Conclusion: This research offers evidence of changes within gut microbiota and systemic inflammation in LLD. These findings possibly help elucidate functions of gut microbiota and systemic inflammation in LLD development and offer fresh ideas on biomarkers for clinical practise in the context of LLD.

Keywords: late-life depression, gut microbiota, inflammation, inflammatory cytokines

Introduction

Late-life depression (LLD) is defined as a depressive disorder that occurs during old age (>60 years)[1] and includes both the first onset of depression during old age and depression that occurs prior and continues into or recurs during old age. This study focuses specifically on primary depressive disorders with first onset occurring at an older age (>60 years). With the increase in life expectancy worldwide, the prevalence of LLD has increased significantly, and approximately 4% of the world's elderly are diagnosed with LLD.[2] Although the diagnostic criteria for symptomatology of LLD and adult depression are similar, unlike depression occurring in younger adults, LLD is characterized by prominent physical symptoms, while emotional symptoms are inconspicuous and cognitive impairment more severe.[3,4] Moreover, research[4] suggests that LLD may be a precursor of dementia. Above all, it is suggested that LLD may have different pathological

mechanisms from adult depression. As a result, the low rates of treatment and treatment success present a great burden to patients and society. However, the current strategy for diagnosing LLD is mainly based on mental assessment, which is subjective to some extent and subject to misdiagnosis. Therefore, there is an urgent need for a clearer understanding of the pathogenesis of LLD and a better objective biomarker for LLD.

Pertinently, the microbiome–gut–brain axis has been widely studied lately and is considered a critical factor involved in the pathogenesis of neuropsychiatric diseases that involves bidirectional regulation of intestinal microbes and the brain through neuroanatomical, neuroimmune, and neuroendocrine pathways, microbial metabolites, and the blood–brain and intestinal mucosal barriers.[5] Countless studies on gut microbiota and aging have been done. More specifically, Zhuang et al[6] found that Lachnospiraceae abundance was decreased among Alzheimer's disease (AD) patients, with *Ruminococcaceae* abundance increased. For frail old people, *Akkermansia, Parabacteroides,* and *Klebsiella* levels are increased, but *Prevotella, Faecalibacterium, Megamonas, Roseburia*, and *Blautia* levels diminished.[7] However, changes in gut microbiota in LLD and the mechanisms leading to LLD remain unclear.

Inflammation is another process extensively studied in the context of psychoneurological disease pathogenesis. The interaction of peripheral immunoresponses with the central nervous system can lead to important alterations in central nervous system immunity. Cytokines and chemokines cross the blood–brain barrier by active transport[8] and stimulate microglia in the central nervous system, thereby activating immunoresponses. If immunoimbalance within peripheral and central nervous systems is maintained, immunoresponses will be amplified by immunoproteins and -cytokines, leading to neurodegeneration.[9] Studies have also shown that psychoneurotic diseases cause dysregulation of innate and adaptive immunoresponses, leading to changes in proinflammatory and anti-inflammatory cytokines.[10] Levels of IL6 and TNFα are tremendously increased among acute patients suffering from schizophrenia and bipolar mania.[11]

Both gut microbiota and inflammation are possibly vital for the pathogenesis of neuropsychiatric diseases. Some studies indicate that the gut microbiota promotes neuroinflammation through immunoactivation, thus causing disease.[12] However, many studies have found that the etiology of neuropsychiatric diseases is complex, and a single inflammatory pathway cannot completely explain the occurrence and development of disease.[13,14] Moreover, studies have found that not all gut microbes work through inflammatory pathways. For example, many types of *Lactobacillus* and *Bifidobacterium* directly generate γ-aminobutyric acid, a primary inhibitory neurotransmitter in brain.[15] Moreover, *Candida*, *Escherichia coli*, and *Enterococcus* produce the neurotransmitter serotonin, while some *Bacillus* strains produce dopamine.[15] Therefore, the gut microbiota can mediate bidirectional microbiome–gut–brain axis interactions to regulate host neurophysiology by producing excessive metabolites in the intestinal cavity and exchanging sensory information with the host. Furthermore, probiotics have been found to have both broad anti-inflammatory and antidepressant effects, further supporting the joint role of gut microbiota and inflammation in the pathophysiology of depression.[16] Therefore, combining gut microbiota with inflammation may reveal a more complete picture of LLD. Our aim could be particularly valuable to understand the gut microbial and inflammatory characteristic in LLD and to further identify biomarkers that might differentiate LLD and HC subjects. This study first explored the overall composition of systemic inflammatory cytokines in peripheral blood and the gut microbiota in LLD patients. Then, we analyzed the correlation among changed gut microbiota features, systemic inflammatory markers, and clinical characteristics of LLD patients to identify a relationship between the gut microbiota and inflammation. Additionally, we extracted potential biomarkers to predict LLD utilizing a combination of the microbiota and inflammatory cytokines.

Methods
Subjects

In total, 35 LLD patients and healthy controls (HCs) were enrolled in this observational cross-sectional analysis, eight (six LLD patients and two HCs) of which were not included because their stool samples did not meet the criteria due to the presence of diarrhea on the day of stool sample collection. Finally, data from a recruitment of 29 untreated LLD patients and 33 HCs from the Psychiatry Department of Zhejiang Provincial People's Hospital from January 2020 to December 2021 were analyzed. All subjects or their caregivers signed informed consent forms and completed questionnaires for collecting demographic information, sex, age, BMI, years of education, medical history, smoking, and

alcohol-use history included. This study complied with the guidelines of the Declaration of Helsinki and obtained approval from the Ethics Committee of Zhejiang Provincial People's Hospital (2019KY184).

Inclusion criteria for the LLD group were age 60–85 years old and patients that had first met the criteria for major depression disorder (MDD) on the basis of the *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition after the age of 60 years. Exclusion criteria were 1) neurological disease, such as dementia, brain tumor, or stroke; 2) history of substance abuse or dependence; 3) depressive disorders secondary to other mental disorders, such as bipolar depression or schizophrenia; and 4) serious heart, lung, liver, kidney, blood system, or endocrine disease or tumors. The HC group denied neurological or mental disorders and had normal cognitive function, with a clinical dementia rating[17] score of 0 and Montreal Cognitive Assessment — Beijing version score ≥26.[18]

The exclusion criteria for gut microbiota sampling 1) major gastrointestinal surgery (including cholecystectomy and appendectomy) and major intestinal resection within the last 5 years; 2) concurrent inflammatory bowel disease, such as ulcerative colitis, Crohn's disease or uncertain colitis, irritable bowel syndrome, or other persistent, infectious gastro-enteritis, colitis or gastritis; 3) persistent or chronic diarrhea of unknown cause, recurrent *Clostridium difficile* infection, or *Helicobacter pylori* infection untreated; 4) previous gastrointestinal ulcers, bleeding, abdominal pain, dyspepsia, diarrhea, constipation, gastrointestinal polyps, dysplasia, or cancer; and 5) use of probiotics, prebiotics, antibiotics, drugs containing bismuth alkaline salicylate, and other components within 3 months prior to sampling.

Assessment of Depressive Symptoms

All patients underwent a medical history assessment and psychiatric examination by two highly trained psychiatrists. On the day of enrollment, depression severity was determined using the Chinese version of the 24-item Hamilton Depression Rating Scale (HDRS-24) and the Chinese version of the 30-item Geriatric Depression Scale (GDS-30). The HDRS-24 is the most widely used scale to assess depression in clinical practice.[19] Symptoms are scored on a five-point scale, ranging from asymptomatic to extremely severe symptoms (with a small number of questions ranging from 0 to 2) on seven subscales: anxiety/somatization, weight, cognitive impairment, circadian change, delay, sleep disturbance, and hope-lessness. According to Davis JM's delineation score, the cut-off points for the approval of HDRS-24 are as follows: >35 is considered severe, >20 is considered moderate, <8 indicates no depression.[19] The GDS-30 was used to assess depressive symptoms in the elderly.[20] Participants answered "yes" or "no" to each question. Scores range from 0 to 30, with 0–10 being normal, 11–20 mildly depressed, and 21–30 moderately to severely depressed.[21]

Analysis of Serum Inflammatory Factors

Venous blood (5 mL) was collected from every subject on an empty stomach in the morning, then these were centrifuged at 4°C for 15 min at 3000 r/min within 1 hour. The serum was aliquoted and stored at −80 °C. Levels of 12 inflammatory markers in plasma were measured using a Cytometric Bead Array human cytokine kit (BD, San Diego, CA, USA) assays according to the manufacturer's instructions. Markers comprised IL2, IL4, IL5, IL6, IL8, IL10, IL12p70, IL17, IL1β, IFNγ, IFNα, and TNFα.

Fecal Sample Collection and DNA Extraction

A sample of at least 3 g was collected from each subject, and sampling was repeated three times. DNA extraction was performed with nucleic acid extraction or purification reagent (Hangzhou Guhe: GHFDE100), and DNA concentration and quality were defined by a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), followed by agarose gel electrophoresis.

16S rRNA Sequencing

The V4 region of the bacterial 16S rRNA gene was amplified by PCR via forward primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Barcode uses a specific 7-bp sequence synthesized into the sequence. Phusion high-fidelity PCR master mix with HF buffer was employed for **a** 50 **μL**/25 μL PCR reaction system, with F/R primers before and after 3 μL (1 μL) each, **a** 10 μL DNA sample, and a ddH₂O complement to 50 μL. The PCR system was configured to perform PCR amplification with reaction conditions of predenaturation 98°C 30 s, 30 cycles; denaturation 98°C, 15 s; annealing 58°C, 15 s; and extension

72°C, 15 s, and final elongation at 72°C for 1 min. PCR product purification was done using AMPure XP Beads (Beckman Coulter, Indianapolis, IN) and quantification via a Qubit dsDNA HS assay kit. Following quantification, an Illumina Novaseq 6000 pin-END 2×150 bp platform was employed for sequencing (GUHE Info Technology, Hangzhou, China).

Sequence Data Processing and Bioinformatic Analysis

Data of every sample were split from original data based on barcode sequence and primer sequence. After deleting barcode and primer sequences, reads of every sample were spliced via Vsearch v2.4.4 for obtaining raw tag data. Meanwhile, sequence quality was controlled and filtered. Criteria for screening low-quality sequences were sequence <150 bp, mean quality values <20, sequences involving ambiguous base and sequences containing over 8 bp single-nucleotide repeats were discarded, and then the final effective tags were acquired.

OTU analysis uses Vsearch v2.4.4. This includes de-repeating sequences (–derep_fulllength), clustering (–cluster_fast,–id 0.97), and de-chimerism (–uchime_ref).[22] By default, sequences were clustered into OTUs with 97% similarity, and representative sequences of OTU were chosen via default parameters. Representative sequences were annotated by species on the basis of the SILVA128 database[23] through VSEARCH, and the OTU list was further generated. At each taxonomic level, the community composition of kingdom, phylum, class, order, family, genus, and species was counted. OTU abundance per sample and classification was recorded, and content in all samples < 0.001% of the total sequence of OTUs was removed.

Sequence data analysis primarily adopts QIIME and the R package (v3.2.0). QIIME software was employed for calculating the OTU-level alpha-diversity index, including the Chao1, ACE, Shannon, and Simpson indices. Alpha diversity refers to interarray variance analysis for comparison of OTU abundance and uniformity among samples. Beta-diversity analysis was done via Qiime software for calculating the UniFrac distance metric,[24] then a principal coordinate analysis (PCoA) map was used for analyzing the beta diversity of microbial community structure among diverse samples.

Monte Carlo permutation and Student's t-tests were employed for drawing a box plot for comparing differences in UniFrac distances between groups. Markers assessing intergroup differentiation of microbial community structure were evaluated via permutational analysis of variance and a method derived from "vegan" of the R pack. The R stats package Kruskal method was employed for comparing differences among different taxonomic levels, classes, orders, families, and genera between samples or groups. Linear discriminant analysis (LDA) effect size (LEfSe) analysis uses default LEfSe setting for detecting differences in taxonomic units between groups. The LDA score threshold was set to 2 and the P-value threshold to 0.05.

To gain an in-depth understanding of the differences in gut microbiota functional pathways between the LLD group and HCs, PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States version 1.1.3) analysis performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was applied to microbial function prediction. Here, we present only the third-level results.

Statistical Analysis

Statistical analysis was done via SPSS 25.0 and data visualization via GraphPad Prism 6. Data are displayed as means ± SD. Two-sided independent Student's t-tests were employed for data with normal distribution and Mann–Whitney U test for data with abnormal distribution. Statistical data are displayed as percentages, and the χ^2 test was used to group comparison. Correlations among neuropsychiatric scale scores, serum inflammatory factor levels, and gut microbe abundance were determined using Spearman correlation analysis. Since fitting of the regression model requires no correlation between the variables used as predictive variables, whether the altered gut microbiota and inflammatory factors can be used as predictive variables could be determined through the correlation results. Features that were not correlated could be included in the regression model. Logistic regression analysis was then applied to identify indicators among inflammatory factors and gut microbiota constituents that could be used to distinguish LLD patients from HCs. The value of inflammatory cytokines and gut microbiota characteristics in the identification of LLD was assessed via receiver-operating characteristic (ROC) curves. Two-sided $P<0.05$ was regarded as statistically significant.

Results

Clinical Characteristics

In sum, 29 LLD patients and 33 elderly HCs were included. The two groups showed no significant differences in sex, age, BMI, smoking, or alcohol consumption (Table 1). The HDRS-24 and GDS scores of LLD patients are shown in Table 1. Serum levels of the inflammatory cytokines IL2, IL4, IL5, IL6, IL8, IL10, IL12P70, IL17, IL1β, TNFα, IFNα, and IFNγ were tremendously higher among LLD group relative to HCs ($P<0.001$) (Table 1).

Comparative Analysis of Diversity Between Groups

Alpha diversity reflects the abundance and evenness of species within a given ecosystem. Chao1, Shannon, and Simpson indices were employed for measuring the intestinal microbial diversity of individuals in two groups. The Chao1 index in LLD group remained tremendously higher ($P=0.0431$) (Figure 1A). No significant differences between the groups on other indices were found (Figure 1B and C).

Beta-diversity analysis reflects the evolutionary relationship between each sample sequence, ie, abundance information, for calculating distance between samples as an indicator of significant microbial community differences between groups. In this study, we used PCoA (with unweighted UniFrac distance) for describing beta diversity. There existed tremendous structural differences between the LLD and HC groups in the first dimension of spatial distance (Figure 2A). The PCoA results showed the former two main factors explained 20.17% and 6.25% of the entire variation, respectively ($P<0.05$). As per Figure 2B, there existed tremendous differences in gut microbiota composition between LLD patients and HCs (Bray–Curtis: $R=0.129$, $P=0.001$).

Changed Microbiota Composition and Abundance in LLD Patients

Relative abundance of gut microbes was compared between two groups. At the phylum level, Verrucomicrobia abundance remained tremendously lower in the LLD group ($P=0.0148$, Figure 3A). At the genus level, *Megamonas*, *Citrobacter*, and *Akkermansia* levels were lower ($P<0.05$) in LLD patients than in HCs (Figure 3B–D). However, *Coprococcus*, *Lachnobacterium*, *Oscillospira*, and *Sutterella* ($P<0.05$) abundance remained higher in the LLD group (Figure 3E–H).

Table 1 Clinical data of participants

	LLD (n=29)	HCs (n=33)	*F/t*	*P*
Sex (F:M)	21:8	22:11	0.24	0.62
Age (years, mean ± SD)	68.83±6.56	69.03±6.08	0.13	0.90
BMI (kg/m, mean ± SD)	22.26±2.7	22±2	0.42	0.67
Smoking (n, %)	4 (13.8%)	7 (21.2%)	0.58	0.45
Drinking (n, %)	5 (17.2%)	4 (12.1%)	0.04	0.83
HRDS-24 (mean ± SD)	24.69±8.11	—		
GDS-30 (mean ± SD)	19.86±5.84	—		
IL2 (pg/mL, mean ± SD)	1.90±1.561	0.78±0.635	156	<0.001
IL4 (pg/mL, mean ± SD)	0.77±0.316	0.19±0.069	39	<0.001
IL5 (pg/mL, mean ± SD)	1.63±2.334	0.04±0.045	119	<0.001
IL6 (pg/mL, mean ± SD)	1.48±0.847	0.34±0.564	102	<0.001
IL8 (pg/mL, mean ± SD)	173.17±214.255	34.43±37.939	317	<0.001
IL10 (pg/mL, mean ± SD)	1.48±0.805	0.5±0.158	171	<0.001
IL12P70 (pg/mL, mean ± SD)	0.8±0.372	0.14±0.053	97.5	<0.001
IL17 (pg/mL, mean ± SD)	1.37±0.933	0.17±0.137	84	<0.001
IL1β (pg/mL, mean ± SD)	3.04±2.323	0.32±0.273	131	<0.001
TNFα (pg/mL, mean ± SD)	1.5±0.84	0.34±0.313	143	<0.001
IFNγ (pg/mL, mean ± SD)	3.61±8.254	0.56±0.897	220.5	<0.001
IFNα (pg/mL, mean ± SD)	1.47±0.792	0.49±0.456	166.5	<0.001

**Notes**: *P*-values from two-sided *t*-test or x^2 test.
Abbreviations: HDRS-24, 24-item Hamilton Depression Rating Scale; GDS-30, 30-item Geriatric Depression Scale.
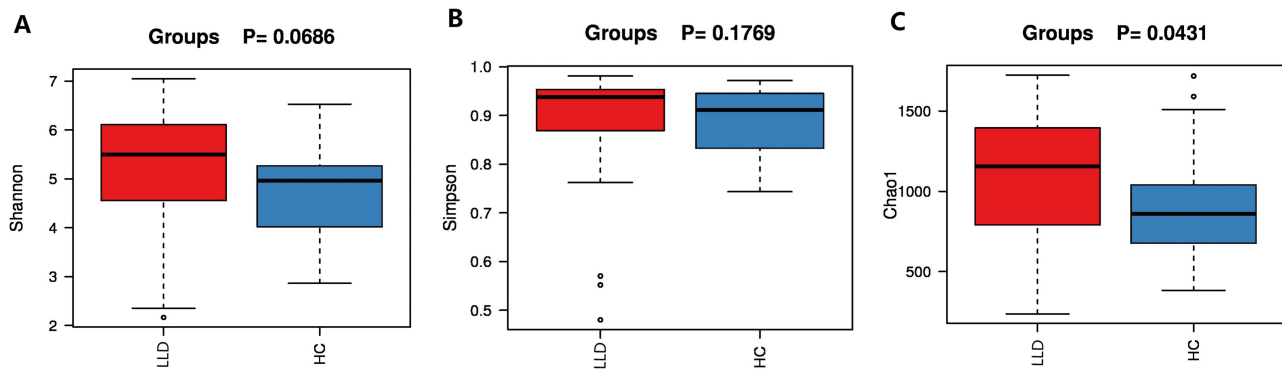
Figure 1 Alpha diversity of gut microbiota in LLD group and HCs.
Notes: Gut microbiota alpha-diversity indices in LLD and HCs. Box plots display differences among microbiome-diversity indices between LLD and HCs via (**A**) Shannon diversity index, (**B**) Simpson index, and (**C**) Chao1 index. Each box plot denotes medians, interquartile ranges, and minimum and maximum values.
Abbreviations: LLD, late-life depression; HCs, healthy controls.
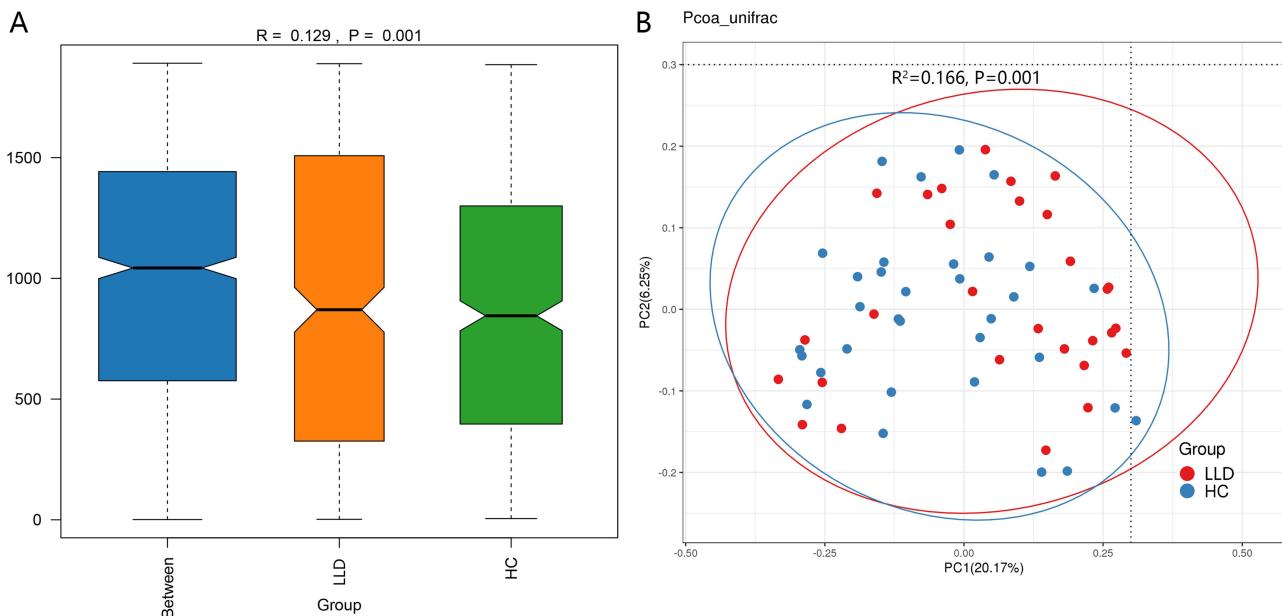
**Figure 2** Beta diversity of gut microbiota in LLD group and HCs.
Notes: Gut microbiota beta-diversity indices in LLD and HCs. Level of similarity between gut microbial communities detected in LLD (orange) and HCs (green) was evaluated through (**A**) unweighted analysis of similarity (ANOSIMs) and (**B**) principal coordinate (PC) analysis (according to UniFrac distance matrix). ANOSIM *R* values display community variations between the compared groups. One symbol denotes one sample.
Abbreviations: LLD, late-life depression; HCs, healthy controls.

LEfSe analysis can further identify species or communities (ie, biomarkers) that have significant differences among samples. LEfSe analysis revealed 23 characteristic species with tremendous difference in relative abundance between two groups ($P<0.05$, LDA >2; Figure 4B). At the phylum level, Verrucomicrobia was enriched in HCs, whereas at the genus level, the biological markers *Akkermansia*, *Megamonas*, and *Citrobacter* were identified in HCs. Biomarkers of the LLD group at the genus level were *Lachnobacterium*, *Phyllobacterium*, *Dorea*, *Oscillospira*, *Coprococcus, Sutterella*, and *Acinetobacter* ($P<0.05$, LDA >2; Figure 4A and B).

## Comparison of Predicted Gut Microbiota Function Between Groups

To reveal the differences in gut microbiota functions between groups, PICRUSt analysis was employed. Through PICRUSt functional analysis of the 16S rRNA gene sequence, three levels of functional gene enrichment in KEGG pathways were observed. At level 3, we found 38 metabolic pathways with tremendous difference between groups
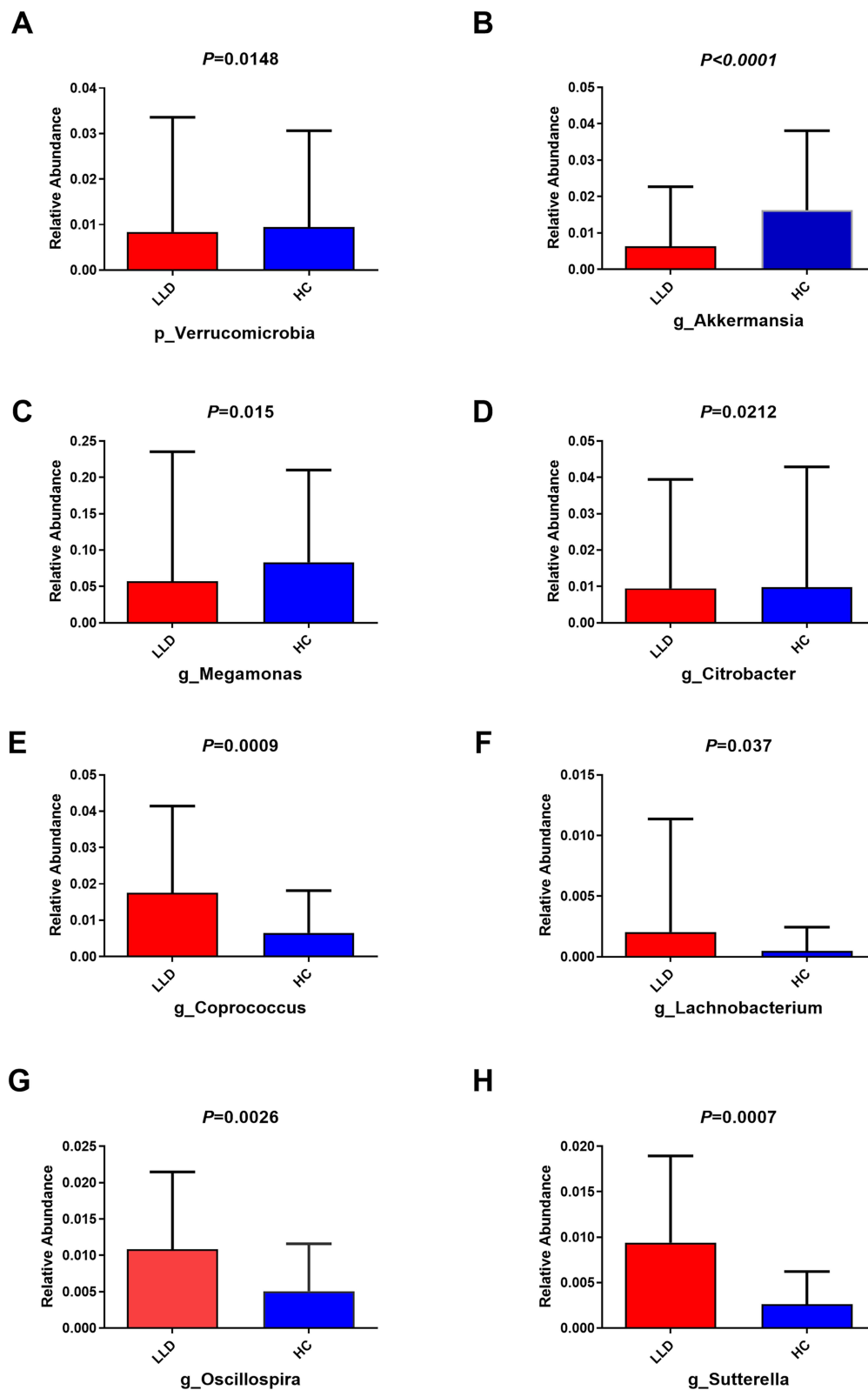
Figure 3 Altered microbiota composition and abundance distribution among LLD patients.
Notes: Altered microbiota composition and abundance distribution in LLD patients. At phylum level, (**A**) Verrucomicrobia abundance was greatly lower in LLD relative to HCs. At genus level, (**B**) *Akkermansia*, (**C**) *Megamonas* and (**D**) *Citrobacter* levels were lower in LLD relative to HCs. (**E**) *Coprococcus*, (**F**) *Lachnobacterium*, (**G**) *Oscillospira* and (**H**) *Sutterella* abundance remained higher in LLD relative to HCs.
Abbreviations: LLD, late-life depression; HCs, healthy controls.
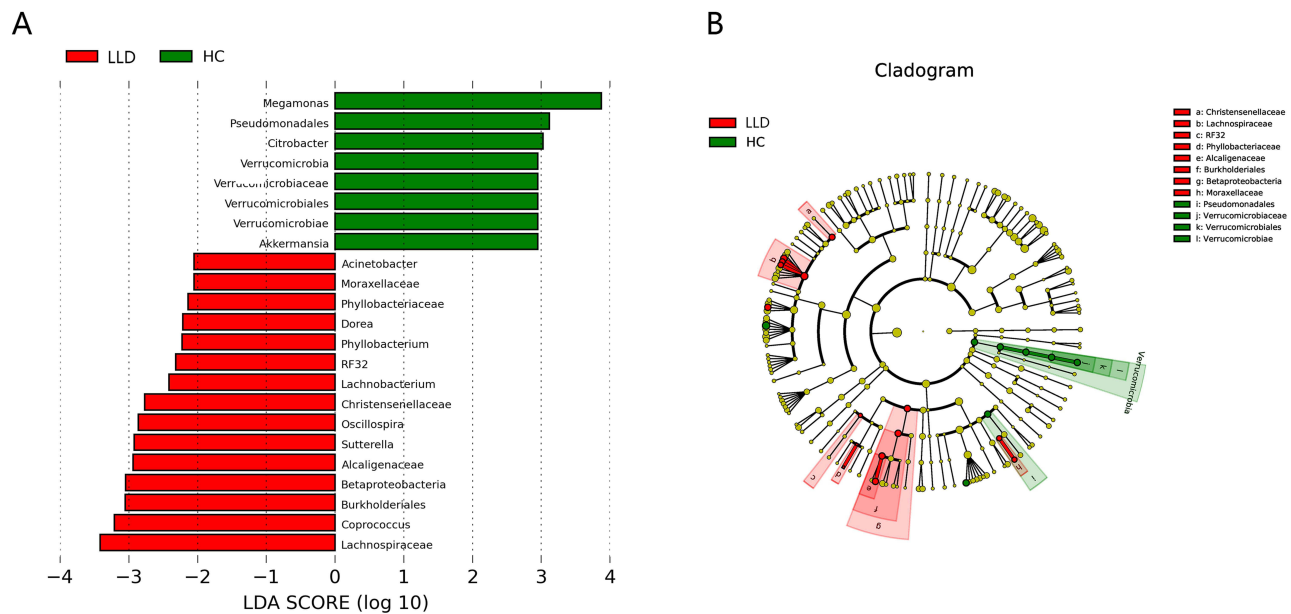
Figure 4 LEfSe analysis of LLD patients and HCs.
Notes: Taxonomic representation of great difference in relative abundance between LLD and HCs. (**A**) LDA scores for bacterial taxa with differential abundance between LLD and HCs. Positive and negative LDA scores denotebacterial taxa enriched in LLD and HCs separately. Taxons with *P*<0.01 (Wilcoxon rank-sum test) and LDA >2 are displayed. (**B**) Cladogram-generated LEfSe indicating difference in bacterial taxa between subjects with LLD and HCs. Nodes in red denote taxa enriched in LLD relative to HCs, while nodes in green denote taxa enriched in HCs relative to LLD.
Abbreviations: LLD, late-life depression; HCs, healthy controls.

(*P*<0.05, Figure 5). For example, LLD patients showed enrichment of amino acid–related enzymes, aminoacyl-tRNA biosynthesis, and other pathways, but glutathione metabolism and ubiquinone biosynthesis pathways were less enriched.

Correlation Between Gut Microbiota Changes and Clinical Indicators

For determining whether alterations in gut microbiota are completely associated with inflammation, and since fitting of the linear regression model requires that there should be no correlation between variables used as predictors, it can be determined whether intestinal microbes and inflammatory factors can be used as predictors through the correlation findings. Verrucomicrobia and *Akkermansia* levels were negatively related to HDRS and GDS scores *(P*<0.05; Table 2, Figure 6). Both IL6 (*r*=0.4380, *P*=0.0175) and IFNγ (*r*=0.4251, *P*=0.0215) levels had positive correlations with GDS scores (Table 2 and Figure 6). Moreover, *Megamonas* abundance was negatively correlated with IL1β, IL4, IFNγ, and IL17 levels (*P*<0.05; Table 2 and Figure 6).

Combined Analysis of Gut Microbiota Composition and Inflammatory Factor Levels Can Improve the Accuracy of LLD Diagnosis

Logistic regression was first applied to determine gut microbiota constituents and inflammatory factors that could be used to distinguish LLD patients from HCs. The results showed tremendous differences in IL6 (*P*<0.001), *Akkermansia* (*P*=0.018), and *Sutterella* (*P*=0.003) levels between LLD and HCs. Then, potential discriminative value of these classifiers was evaluated using ROC curves. We found that when gut microbiota features were used alone as a predictor, the AUC was 0.746 (95% CI 0.621–0.871, Figure 7). When inflammatory factors were used as a predictor, the AUC was 0.893 (95% CI 0.909–0.9777, Figure 7). However, when the two were used in combination as predictors, the AUC was 0.962 (95% CI 0.92–1, Figure 7). Combined analysis of gut microbiota composition and inflammatory factor levels can improve the accuracy of LLD diagnosis.
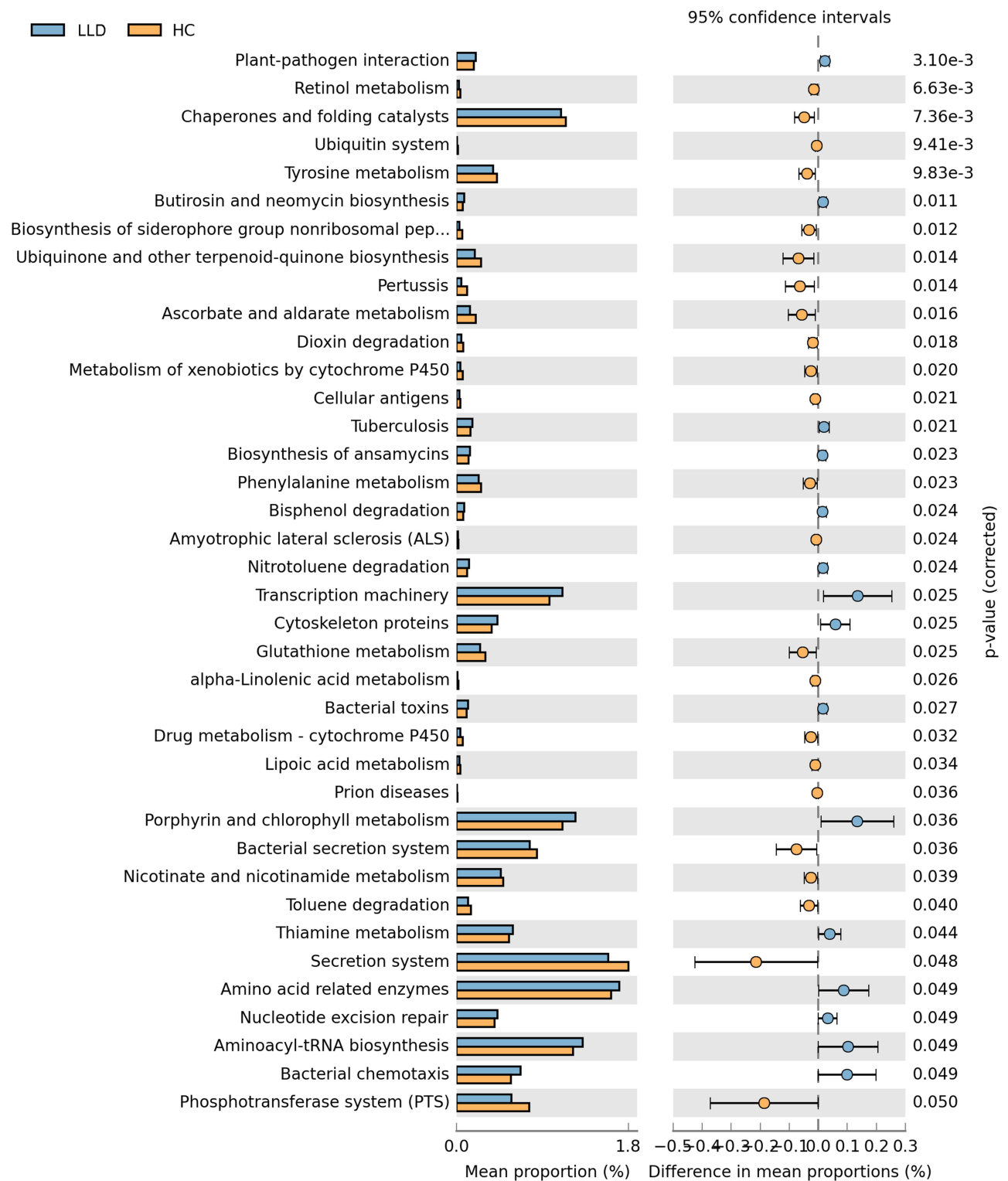
Figure 5 PICRUSt-based examination of functions of gut microbiome in LLD patients and HCs.

Notes: The left of the figure displays abundance ratios between the groups. The middle section displays proportional variation in functional abundance within 95% CI. *P* values at the right.

Abbreviations: LLD, late-life depression; HCs, healthy controls.

Table 2 Correlations among changed gut microbiota, neuropsychiatric assessment and systemic inflammation markers

Interactions		r	P
Gut microbiota × neuropsychiatric assessment			
Verrucomicrobia	HRDS-24	−0.3804	0.0418
Akkermansia	HRDS-24	−0.7086	<0.0001
	GDS-30	−0.5513	0.0019
Gut microbiota × systemic inflammation			
Megamonas	IL1β	−0.5706	0.0012
	IL4	−0.4286	0.0203
	IFNγ	−0.4578	0.0125
	IL17	−0.3944	0.0343
Systemic inflammation × neuropsychiatric assessment			
IL6	GDS-30	0.4380	0.0175
IFNγ	GDS-30	0.4251	0.0215

Abbreviations: HDRS-24, 24-item Hamilton Depression Rating Scale-24; GDS-30, 30-item Geriatric Depression Scale.

Discussion

This is the initial research for revealing gut microbiome characteristics and systemic inflammation in LLD patients and to conduct a combined analysis of the two to identify biomarkers of LLD. We explored characteristics in gut microbiota and systemic inflammatory factors in patients with LLD and explored their association with depression severity. Elevated systemic inflammatory factor levels, increased taxonomic richness, and changed composition of gut microbiota were found among patients suffering from LLD. IL6 and IFNγ levels and Verrucomicrobia and *Akkermansia* abundance were related to depression severity. Moreover, *Megamonas* abundance was negatively correlated with IL1β, IL4, IFNγ, and IL17 levels. More importantly, IL6, *Akkermansia* and *Sutterella* levels were predictors of LLD. Notably, the combination of gut microbiota and inflammatory factors had greater diagnostic power as a potential biomarker for LLD.

We found increased levels of both proinflammatory cytokines (eg, IL6, IFNγ, and TNFα) and anti-inflammatory cytokines (eg, IL4 and IL10) in LLD. We speculate that there are various mechanisms regulating cytokine-production changes in the body during LLD and that each cytokine is involved in activation of and participation in multiple downstream signaling pathways, exerting complex synergistic or antagonistic effects that eventually lead to the emergence of clinical manifestations. More importantly, we found that IL6 and IFNγ levels had positive correlations with LLD disease severity, suggesting that these two inflammatory factors participate in LLD occurrence and development. IL6 is a proinflammatory cytokine expressed in neurons and glial cells that is vital for immune and acute-phase responses.[25] Some scholars have proposed that high IL6 levels can promote serotonin degradation and reduce serotonin production, which may impair neural plasticity and lead to structural brain abnormalities, such as hippocampal and frontal lobe atrophy,[10,26] which have been confirmed to be associated with LLD and LLD-induced cognitive impairment. Consistently with our study, many others have found elevated IL6 levels among patients suffering from LLD that are positively related to disease severity.[27–29] In contrast, the results of studies on IFNγ in MDD are inconsistent. Zhang et al[30] found that IFNγ levels increased in mice with depression-like features and were associated with cognitive deficits. However, Buspavanich et al[31] found a reduction in IFNγ levels among MDD patients. Pertinently, IFNγ is a proinflammatory factor participating in central nervous system inflammation and activates microglia in the brain to induce
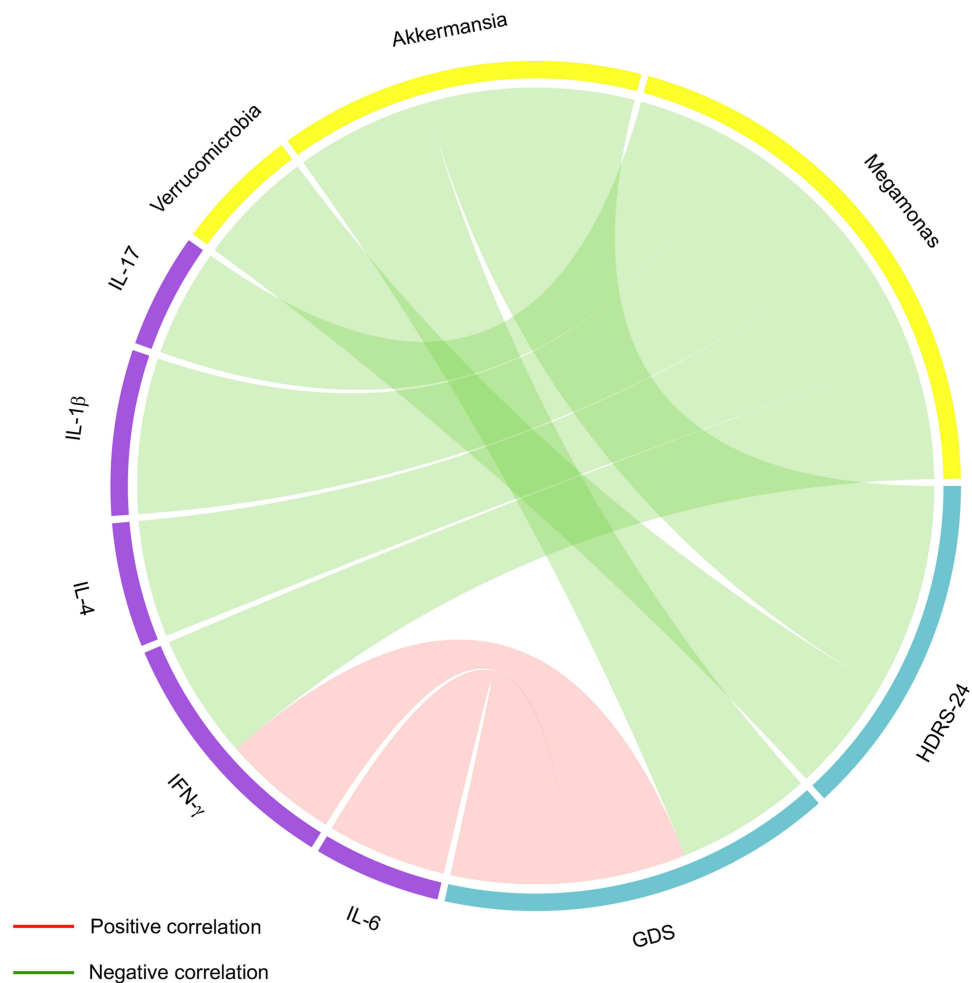
Figure 6 Connections among changed gut microbes, clinical variables, and inflammatory factors identified by Spearman correlation.
**Notes**: Connections among changed gut microbiota, neuropsychiatric assessment, and systemic inflammation identified by Spearman correlation. Correlations with *P*<0.05 are displayed.
Abbreviations: HDRS-24, 24-item Hamilton Depression Rating Scale; GDS-30, 30-item Geriatric Depression Scale.

proinflammatory responses.[32] Studies have shown IFNγ-activated microglia alter the hippocampal neurogenic niche, inhibit the proliferation of neural stem and progenitor cells, and facilitate the apoptosis of immature neurons, thereby causing depression symptoms and cognitive deficits in mouse.[30] Our study confirms this idea clinically and demonstrates IFNγ levels are related to LLD severity. In brief, this research reveals that LLD is an inflammatory state where proinflammatory and anti-inflammatory cytokines coexist and that IL6 and IFNγ are related to disease severity.

In our study, the Chao1 index in the LLD group remained tremendously higher than the HC group, indicating that richness of gut microbiota among LLD patients remains greater than in HCs. However, the Shannon and Simpson indices did not differ tremendously between the groups, indicating that diversity of gut microbiota did not differ between the two. Our findings are similar to previous studies on MDD,[33,34] but other studies on LLD have revealed that richness and diversity in intestinal microbiota showed no difference between patients and controls.[35] We hypothesize the augmented gut microbiota richness among LLD patients is possibly associated with the increase in the levels of harmful bacteria rather than in those of the core microbiota in the gut. However, notably, the gut microbiota is a dynamic community influenced by factors like genetics, diet, age, stress, geography, and health status. Therefore, the characteristics of the diversity and richness of gut microbiota in LLD patients need to be further studied.

Beta-diversity analysis revealed tremendous changes in gut microbiota compositions in LLD patients. We discovered microbes with decreased abundance were mainly probiotics, and most of the bacteria with increased abundance were
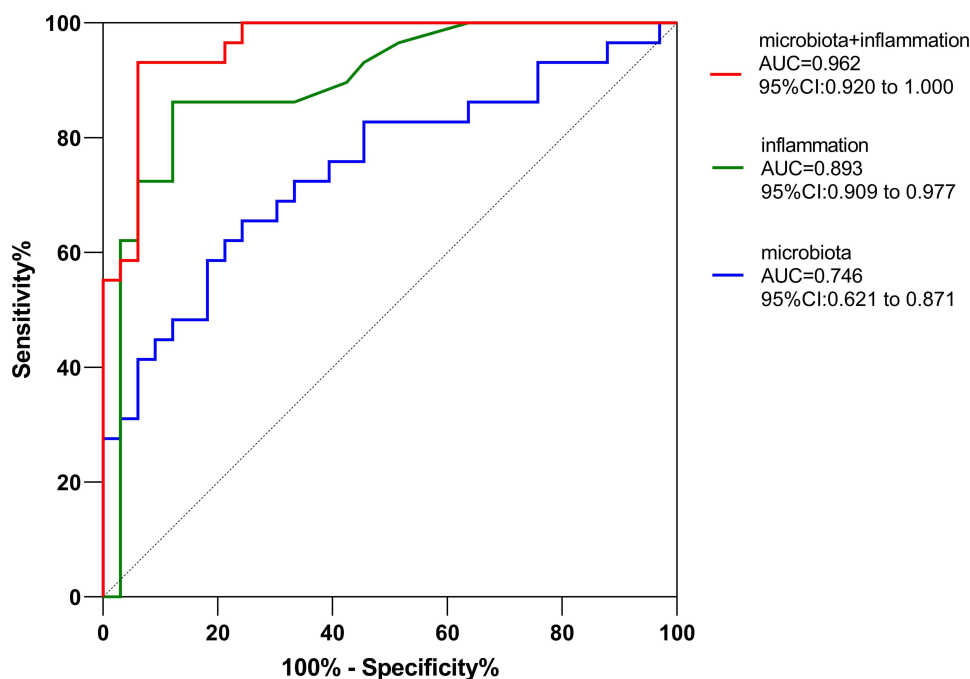
Figure 7 Receiver-operating characteristic curve analysis of sensitivity and specificity of differentially abundant genera as diagnostic factors for LLD.
Notes: The ROC generated using microbiota features is marked in blue, inflammatory factor levels marked in green, and microbiota features combined with inflammatory factor levels marked in red.
Abbreviation: LLD, late-life depression.

involved in protein and fat metabolism. Verrucomicrobia and *Akkermansia* are strains of the same family. Verrucomicrobia includes a small group of bacteria found in the human gut, and a recent study showed that *Akkermansia* are beneficial bacteria that can potentially be used in next-generation therapies.[36] Previous research has revealed Verrucomicrobia abundance decreases among patients suffering from anxiety and depression,[37] and an increase in *Akkermansia* abundance can decrease anxiety and enhance cognition in middle-aged mice.[38] This research matches preceding research and complements studies among elderly populations.[7] Notably, *Akkermansia* abundance remained tremendously decreased among patients suffering from LLD and related to depression severity, and logistic regression showed that *Akkermansia* abundance is a risk factor that can be used to predict the probability of LLD. However, there was no correlation between *Akkermansia* abundance and inflammatory factor levels. This finding reveals *Akkermansia* may have no function in LLD pathogenesis through inflammatory pathways. Interestingly, Wang et al[39] found that through direct interactions between the *Akkermansia* FF protein Amuc_1100 and TLR2, *Akkermansia* promoted expression of the 5-HT synthesis rate–limiting enzyme Tph1 in RIN-14B cells and decreased expression of serotonin-reuptake transporters in Caco-2 cells, hence increasing the biosynthesis and extracellular availability of 5-HT, which suggested that *Akkermansia* can affect LLD by regulating the release of neurotransmitters directly through the intestinal barrier. Further studies are needed in the context of LLD.

We further found that *Megamonas* abundance was correlated with levels of IL1β, IL4, IL17, and IFNγ, which are mostly proinflammatory factors. *Megamonas* belongs to the phylum Firmicutes, which comprises important short-chain fatty acids producing bacteria that produce acetic acid, propionic acid, and lactic acid by fermenting various carbohydrates. Of note, the abundance of *Megamonas* has been shown to differ in various studies of depression.[40] Zheng et al[41] found that *Megamonas* abundance decreased in patients with depression, and another study showed that *Megamonas* abundance decreased in elderly patients with frailty,[7] which is consistent with our study. Research has revealed that butyrate is vital for inhibiting intestinal inflammation: it can change intestinal permeability and influence regulatory T-cell activity to exert anti-inflammatory effects.[42] Therefore, we speculate that in patients with LLD, *Megamonas* reduces butyrate output, thus influencing regulatory T-cell activity and inducing proinflammatory cytokine release, which triggers local gut inflammation and drives LLD through the microbe–gut–brain axis. Based on the results regarding the

aforementioned inflammatory factors, we hypothesize that *Megamonas* may mediate the occurrence and development of LLD by upregulating IFNγ production, but further in-depth mechanistic research is still needed. Other studies have shown that *Megamonas* is involved in the release of inflammatory factors in psoriasis,[43] cancer pain,[44] and other diseases, but the underlying mechanism remains to be explored.

Furthermore, we found that *Coprococcus*, *Lachnobacterium*, *Oscillospira*, and *Sutterella* levels were elevated at the genus level in LLD patients. Most of these bacteria with increased levels are related to protein and fat metabolism. For example, studies have shown that elevated *Oscillospira* levels may be related to increased protein metabolism.[40] *Coprococcus* has a positive correlation with obesity.[45] Increased protein metabolism mediated by the microbiota involves fermentation or bacterial protein degradation, a process possibly involving the transfer of essential amino acids from host to microorganism and leading to the production of toxic products like ammonia, putrescine, and phenol, which in turn cause intestinal barrier disruption and neuroinflammation. More remarkably, through logistic analysis, *Sutterella* is predictive of LLD. *Sutterella* is an important intestinal commensal bacterium.[46] Research has revealed that *Sutterella* abundance is significantly increased among patients suffering from MDD and generalized anxiety disorder.[47,48] Many studies have found that *Sutterella* is positively correlated with obesity, as well as with weight and fat gain.[49–51] The bidirectional relation of obesity and depression is well established, and research has revealed hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis bringing about maladaptive release of cortisol may be a common mechanism for both.[52] Therefore, we speculate that *Sutterella* might affect cortisol release by affecting the HPA axis, thus triggering LLD.

Through correlation analysis, we found that not all alterations in gut microbiota were related to inflammation, suggesting that some alterations in gut microbiota possibly have pathological functions in LLD through other pathways. PICRUSt analysis revealed activation of multiple signaling pathways related to gut microbiota, including amino acid metabolism, glutathione metabolism, and the HPA axis, which suggests that some gut microbes may also participate in the development of LLD through other noninflammatory pathways. Thus, we conclude that gut microbiota and inflammation interact in the pathogenesis of LLD, but there are also disease-causing processes that are independent of this mechanism.

A single indicator cannot fully explain the pathogenesis of LLD, nor can it be used to predict LLD more accurately. Therefore, we used both gut microbiota and inflammatory index to be biomarkers of LLD. Initially, we excluded the gut microbes associated with inflammatory factors through correlation analysis and then extracted IL6, *Akkermansia* and *Sutterella* as predictors through logistic regression. The ROC curve was employed for assessing sample classification accuracy. We found that the combination of inflammatory cytokines and gut microbiota could improve LLD diagnosis accuracy. Our research therefore provides a new approach for the distinction of LLD. Previous studies have demonstrated that gut microbes or inflammatory cytokines can be used as noninvasive biological markers to identify neuropsychiatric disorders, such as bipolar disorder,[53] methamphetamine abuse,[54] and schizophrenia.[55] However, there is little research on the combined analysis of the two factors. Some studies have reported that other biological markers can be used in conjunction with inflammatory markers to more accurately predict neuropsychiatric disorders. For instance, Carniel and da Rocha[56] found that BDNF and inflammatory factors can be used as predictors of MDD. In addition, Wei et al[57] demonstrated that the combination of the systemic inflammation response index, neutrophil:high-density lipoprotein ratio, and monocyte:high-density lipoprotein ratio can improve the effectiveness of bipolar disorder diagnosis, though preceding research has primarily emphasized functions of gut microbiota and inflammation, with little attention given to gut microbiota functions in causing mental health disorders through other mechanisms. We believe that this component of noninflammatory microbiota should not be ignored in the prediction of the onset of LLD and should be included in the diagnostic model with inflammation indicators to predict LLD more accurately. However, biomarkers based on intestinal microbial markers and inflammatory factors need to be validated in larger samples.

There exist certain shortcomings of this research. Primarily, gut microbiota can be influenced by a variety of living environments, and although the subjects in this research were not allowed to use antibiotics or probiotics, none of them was given a standardized diet. Second, the research only contained small clinical samples of the elderly. Therefore, the sample size needs to be further expanded. Third, this was a cross-sectional study, and longitudinal research may provide stronger evidence.

Conclusion

Together, our findings reveal that alterations in gut microbiota and inflammatory cytokines in LLD contribute to the development of LLD. Most importantly, the combination of gut microbiota and inflammatory markers has improved power as a potential biomarker for LLD. These discoveries thus offer a deeper awareness of gut microbiota functions on LLD pathogenesis and elucidate new strategies for LLD diagnosis and treatment.

Abbreviations

LLD, late-life depression; HCs, healthy controls; ROC, receiver-operating characteristic; MDD, major depressive disorder; AD, Alzheimer's disease; HDRS-24, 24-item Hamilton Depression Rating Scale; GDS-30, 30-item Geriatric Depression Scale; ELISA, enzyme-linked immunosorbent assay; QIIME, Quantitative Insights Into Microbial Ecology; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; ANOSIM, analysis of similarities; LDA, linear discriminant analysis; LEfSe, LDA effective size; PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States.

Ethics Approval and Consent

This research obtained approval from the Ethics Committee of Zhejiang Provincial People's Hospital (2019KY184). Consent was informed and signed by all subjects or their caregivers.

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Disclosure

The authors report no conflicts of interest in this work.

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