Journal of Health, Population and Nutrition

RESEARCH

Open Access

Gut flora reflects potential risk factors for cognitive dysfunction in patients with epilepsy

BingCong Hong[1*]

Abstract

Objective This cross-sectional study aims to analyze the differences in gut flora between patients with epilepsy with and without cognitive impairment and normal subjects.

Methods One hundred patients with epilepsy who came to our hospital from 2020.12 to 2022.12 (epilepsy group) were selected, and another 100 family members of the patients were selected as the control group (control group). Patients with epilepsy were further classified by the MMSE scale into 62 patients with combined cognitive impairment (Yes group) and 38 patients without cognitive impairment (No group). Detection of gut flora in feces by 16 S rRNA high-throughput sequencing. Logistic regression was used to analyze risk factors for cognitive dysfunction in patients with epilepsy.

Results There were more significant differences in the structure and composition of the gut flora between patients in the epilepsy group and the control group, but no significant differences in diversity analysis ($P > 0.05$). Actinobacteriota, *Faecalibacterium* and *Collinsella* were significantly lower in the Yes group than in the No group ($P < 0.05$), and the Alpha diversity index was numerically slightly smaller than in the No group, with the PCoA analysis demonstrating a more dispersed situation in both groups. Five metabolic pathways, including glycolysis and heterolactic fermentation, were upregulated in the Yes group. LEfSe analysis showed that five groups of bacteria, including Coriobacteriaceae and *Collinsella*, were selected as marker species for the presence or absence of comorbid cognitive impairment. Of these, *Collinsella*, Oscillospirales, and Ruminococcaceae have a greater impact on epilepsy combined with cognitive impairment.

Conclusion There was an imbalance in the gut flora of patients with epilepsy compared to healthy controls. The gut flora of patients with epilepsy with cognitive dysfunction differs significantly from that of patients without cognitive dysfunction. *Collinsella*, Oscillospirales, and Ruminococcaceae have a greater impact on epilepsy with cognitive dysfunction and can be used as an indicator for the observation of epilepsy with cognitive dysfunction.

Keywords Gut flora, Epilepsy, Cognitive dysfunction, Risk factors

*Correspondence:
BingCong Hong
84091749@qq.com
[1]Department of Neurology, Quanzhou First Hospital Affiliated to Fujian Medical University, Quanzhou 362000, China

Introduction

Epilepsy is a chronic syndrome of sudden, transient, recurrent episodes of central nervous system malfunction caused by abnormal over-discharge of neurons in the brain. There are approximately 70 million cases of epilepsy worldwide, with nearly 80% living in developing countries with limited medical, social and other resources [1]. According to epidemiological data in China, as of 2021, there are about 10 million cases of epilepsy in China, of which 30–40% of epilepsy patients are accompanied by varying degrees of cognitive dysfunction [2]. The main manifestations are memory loss, reduced attention and psychomotor speed, which can even cause mental retardation, seriously affecting the quality of life of patients and increasing the burden on their families and society. Early diagnosis and timely treatment of cognitive impairment in epilepsy is therefore important for improving the prognosis of patients.

The gut flora are microorganisms in the gastrointestinal tract that play a fundamental and important role in human biology [3]. The concept of the "microbe-gut-brain axis" has attracted a great deal of research interest in recent years. Many studies have shown that gut flora can be involved in the regulation of cognitive function through the "microbe-gut-brain axis" and is closely related to many diseases of the central nervous system, with alterations in patients with epilepsy, Parkinson's disease, Alzheimer's disease and others [4]. A large sample study found that the incidence of epilepsy was significantly higher in people with bowel stress syndrome than in normal people [5]. A comparison of pediatric refractory epilepsy with healthy infants of the corresponding age group revealed an imbalance in gut microecology in infants with refractory epilepsy [6]. Another study found a correlation between epileptic waves and bowel movement waves in the EEG of patients with epilepsy [7]. A study of intragastric treatment of mice with a mixture of antibiotics confirmed that dysbiosis of the gut flora, rather than a systemic antibiotic response, was responsible for cognitive dysfunction [8]. Multiple studies have found changes in the gut microbiome in various neurodegenerative diseases, suggesting that the development of these diseases has a clear impact on the gut microbiome [9, 10]. This correlation implies that gut microbial dysbiosis plays a crucial role in changes in cognitive function. Therefore, modulating gut microbes may also alter cognitive function in epilepsy patients. Based on this, we analyzed the structure of the gut flora in epileptic patients with and without cognitive dysfunction as well as in controls using various techniques such as high-throughput Illumina Miseq sequencing of 16 S rRNA. In order to find the possible factors influencing the development of cognitive dysfunction in epilepsy patients and to provide new ideas for improving the prognosis of cognitive function in epilepsy patients.

Many studies have explored the relationship between gut microbiota and cognitive function in neurological disorders. For instance, research has shown that gut dysbiosis is linked to Alzheimer's disease, Parkinson's disease, and epilepsy [6–10]. Despite these findings, there is limited understanding of the specific role of gut microbiota in cognitive impairment among epilepsy patients. This study aims to fill this gap by analyzing gut microbiota differences between epileptic patients with and without cognitive impairment. However, while this study focuses on the role of gut microbiota, it is important to acknowledge that cognitive decline in epilepsy is multifactorial. Factors such as seizure frequency, medication use, and psychiatric comorbidities also play significant roles.

Materials and methods

Patients' population

Patients with epilepsy who came to our hospital between 2020.12 and 2022.12 were selected, and all patients were required to be able to provide a qualified stool specimen and to cooperate in completing the questionnaire and signing the informed consent form. The study population was divided into 2 groups, the Epilepsy group (100 cases) and the healthy Control group (100 cases). The epilepsy group was subdivided into 2 subgroups, including the epilepsy group with combined cognitive impairment (Yes) and the epilepsy group without cognitive impairment (No).

Inclusion criteria

Epilepsy group, based on the International League Against Epilepsy criteria: brain disorders that meet any of the following conditions: (i) at least two unprovoked (or reflex) seizures>24 h apart; (ii) one unprovoked (or reflex) seizure with a probability of recurrence similar to the risk of recurrence after two unprovoked seizures (at least 60%) in the next 10 years; and (iii) diagnosis of epilepsy syndrome. Patients with epilepsy were included who met the diagnostic criteria for epilepsy. For the control group, healthy family members of patients with epilepsy were selected.

Inclusion criteria for microbial studies: Referring to the Human Microbiology Project Consortium's criteria for gut microbial studies, the inclusion criteria for this experiment (epilepsy group and control group) were as follows: No hypertension, diabetes, digestive system, immune system or other diseases that may affect the stability of the gut flora; no history of drug use such as antibiotics, glucocorticoids, cytokines, high-dose probiotics and biologics within 6 months; no invasive gastroscopy, colonoscopy, barium meal gastrointestinal examination or other

digestive system-related surgeries within 6 months; no diarrhea or constipation within 6 months. No significant change in dietary habits and no history of alcohol abuse. This study is approved by the Ethics Committee of Quanzhou First Hospital. In accordance with the Declaration of Helsinki, written or oral informed consent was obtained from all patients prior to enrollment.

Exclusion criteria

(i) patients who have had epilepsy surgery or other intracranial surgery; (ii) patients diagnosed with intracranial tumors, sequelae of central nervous system infections, hypoxic encephalopathy, metabolic encephalopathy, autism, schizophrenia, depression, anxiety, Parkinson's disease, Alzheimer's disease and other neurological and psychiatric disorders other than epilepsy; (iii) patients who have had epilepsy dietary therapy within 3 years.

Data acquisition

Basic information collected included age, sex, height, weight, education, age at onset, duration of illness, time from onset to first visit, time from onset to first use of AEDs, type of seizure, frequency of seizures, and use of AEDs. EEG and other supportive tests such as cranial CT or MRI were not included in the treatment protocol.

Research methods

Cognitive function tests

The MMSE (Mini-mental state examination) scale was used to assess the cognitive function of patients with epilepsy who met the inclusion criteria and were assessed ≥ 1 week after their most recent seizure; the study staff were clinicians trained as medical psychologists and were proficient in the use of the MMSE scale. The MMSE scale assesses cognitive function in relation to the educational level of the study participants, with < 17 for illiterate people, < 20 for primary school students and < 24 for those with secondary school education and above being cognitively impaired, and the lower the score the worse the cognitive function.

Sample collection

The same person collected the faecal samples, provided the collection tools and explained to the participants the collection method and collection instructions. Faecal samples were collected between 7am and 9am each day, placed in ice boxes and transferred to the −80 °C freezer for freezing within 15 min and the time of collection was recorded.

16 S rRNA amplicon sequencing

16 S rRNA sequencing was performed using the Illumina NovaSeq6000 sequencing platform. Sample DNA was detected and quality extracted using 1% agarose gel electrophoresis. The 16 S V3V4 gene region was selected for PCR amplification and the PCR primer sequences were: forward primer 5′-CCTACGGGAGGCAGCAG-3′, reverse primer 5′-GACTACHVGGGTWTCTAAT-3′. QIIME2 was used for sequence quality control, denoising, clustering and diversity analysis. Alpha analysis was used to compare the diversity of the flora in the epilepsy and control groups, and the diversity of the flora in the epilepsy combined with cognitive dysfunction and epilepsy without cognitive dysfunction groups. Beta diversity was used to analyze the difference and concentration of the flora between the epilepsy and control groups, and the difference and concentration of the flora between epilepsy with cognitive impairment and epilepsy without cognitive impairment.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 statistical analysis software, and the measurement data were expressed as (±s) using the independent samples t-test; the count data were expressed as % using the $\chi 2$ test. Species composition and Alpha diversity results were statistically analyzed using a one-way ANOVA test. Risk factors for cognitive dysfunction in patients with epilepsy were analyzed using univariate and logistic regression. $P<0.05$ was used as a criterion to determine whether the difference was statistically significant.

Results

Comparison of general information on subjects

In our study, the age range of patients in the epilepsy group was 11 to 59 years with a mean age of (26.94±6.21) years, of which 54 were males. Subjects in the normal group ranged in age from 27 to 65 years with a mean age of (41.66±6.87) years, of which 41 were males. For detailed information, please refer to Table 1. Based on the results of the MMSE assessment, the epileptic subjects were divided into a cognitive impairment group (Yes) and a normal cognitive function group (No), with the Yes group containing 62 individuals and the No group containing 38 individuals.

Comparison of gut flora in patients with epilepsy and normal controls

Microbial composition and structural features

Amplicon sequence variants (ASVs) were obtained by clustering at 100% similarity, with 13,227 ASVs shared between the two groups of samples tested, 1965 unique to the control group and 1942 unique to the epilepsy group (Fig. 1A).

Figure 1B shows the histogram of relative abundance at the phylum level for the two groups of subjects. There was no significant difference in the relative abundance of each phylum between the two groups ($P>0.05$). The

Table 1 General information of subjects ($\overline{x}$ ±s)

General Information	Epilepsy($n=100$)	Control($n=100$)
Age(years)	26.94±6.21	41.66±6.87
Gender(Male/Female, No.)	54/46	41/59
Height (cm)	172.63±9.54	175.83±15.10
Weight (kg)	60.25±4.33	59.81±4.25
Educational level[No.(%)]		
Primary school	9(9%)	—
Junior high school	46(46%)	—
Senior high school	32(32%)	—
College level or above	13(13%)	—
Onset age(years)	15.34±6.77	—
Disease duration		
≤1 year	18(18%)	—
> 1 year and ≤ 5 years	37(37%)	—
> 5 years and ≤ 10 years	27(27%)	—
> 10 years and ≤ 20 years	16(16%)	—
> 20 years	2(2%)	—
Time from onset to first visit(Weeks)	3.36±0.56	—
Time between onset and first use of AEDs(Weeks)	4.88±0.63	—
Seizure type		
Partial seizure	32(32%)	—
General seizure secondary to partial seizure	11(11%)	—
General seizure	57(57%)	—
Seizure frequency		
Seizures within a week	18(18%)	—
Seizures within one month	22(22%)	—
Seizures within a quarter	31(31%)	—
Seizures within 6 months	29(29%)	—
AEDs usage(species)		
0	6(6%)	—
1	57(57%)	—
2	30(30%)	—
≥ 3	7(7%)	—

highest relative abundance of Firmicutes was over 50% (61.64% in the control group and 54.24% in the epilepsy group), followed by Bacteroidota (19.88% in the control group and 28.48% in the epilepsy group). The relative abundance of Firmicutes and Actinobacteriota were higher in the healthy control group than that in the epileptic group, and the relative abundance of Bacteroidota, Proteobacteria, Fusobacteriota, Verrucomicrobiota, Desulfobacterota, Synergistota, and Euryarchaeota in the healthy control group were lower than that in the epileptic group.

The results of the genus-level analysis (Fig. 1C) showed that among the top 10 genera in relative abundance, *Bacteroides* was significantly higher ($P<0.05$) in the YES group (16.06%) than in the NO group (7.57%), while the rest were not significantly different ($P>0.05$). The relative abundance of *Bacteroides*, *Prevotella*, *Megamonas*, *Roseburia*, and *Escherichia*, *Agathobacter* was higher in the YES group, while *Faecalibacterium*, *Collinsella*, *Bifidobacterium*, and *Subdoligranulum* were enriched in the NO group.

Microbial diversity analysis and its differences

We analyzed Alpha and Beta diversity in subjects, in order to assess changes in gut microbial diversity. Alpha diversity statistics showed no significant differences ($P>0.05$) in shannon, simpson, chao1, and ace indices between the two sample groups (Table 2), with the epilepsy group having slightly higher values of shannon and ace indices than the control group. Bray-Curtis based PCoA analysis (Fig. 2A) showed no significant difference between the two sample groups ($P>0.05$). It is suggested that the diversity of the gut flora of the epileptic group did not differ significantly from that of its relatives, the healthy controls. LEfSe results showed that only *slackia* of Actinobacteriota was the hallmark species of the epileptic group (Fig. 2B).

Comparison of gut flora in patients with and without cognitive dysfunctional epilepsy

Microbial composition and structural features

The two groups tested shared 8618 ASVs, 3419 unique to the Yes group and 2734 unique to the No group (Fig. 3A).

Figure 3B shows the histogram of relative abundance at the gate level between the two groups. The results show that the relative abundance of Bacteroidota, Desulfobacterota, Fusobacteriota, Proteobacteria and Synergistota were all higher in the Yes group than in the No group, but the differences were not statistically significant ($P>0.05$). The relative abundance of Firmicutes, Actinobacteriota, Verrucomicrobiota, and Euryarchaeota were all lower than that of the No group, with Actinobacteriota showing a significant difference between the two groups ($P<0.05$).

The results of the genus level analysis (Fig. 3C) showed that, among the top 10 genera in relative abundance, the relative abundance of *Bacteroides*, *Prevotella*, *Megamonas*, *Escherichia-Shigella*, *Roseburia* and *Agathobacter* were all higher in the Yes group than in the No group, but there was no significant difference between the groups ($P>0.05$). The relative abundances of *Faecalibacterium*, *Collinsella*, *Bifidobacterium* and *Subdoligranulum* were all lower than those of the No group, with *Faecalibacterium* and *Collinsella* significantly different between the two groups ($P<0.05$).

Analysis of microbial diversity and its differences

To assess the changes in gut microbial diversity in the two groups of patients with epilepsy, we analyzed the Alpha and Beta diversity of the patients. the Alpha diversity statistics showed that the Yes group samples were slightly smaller than the No group in terms of the

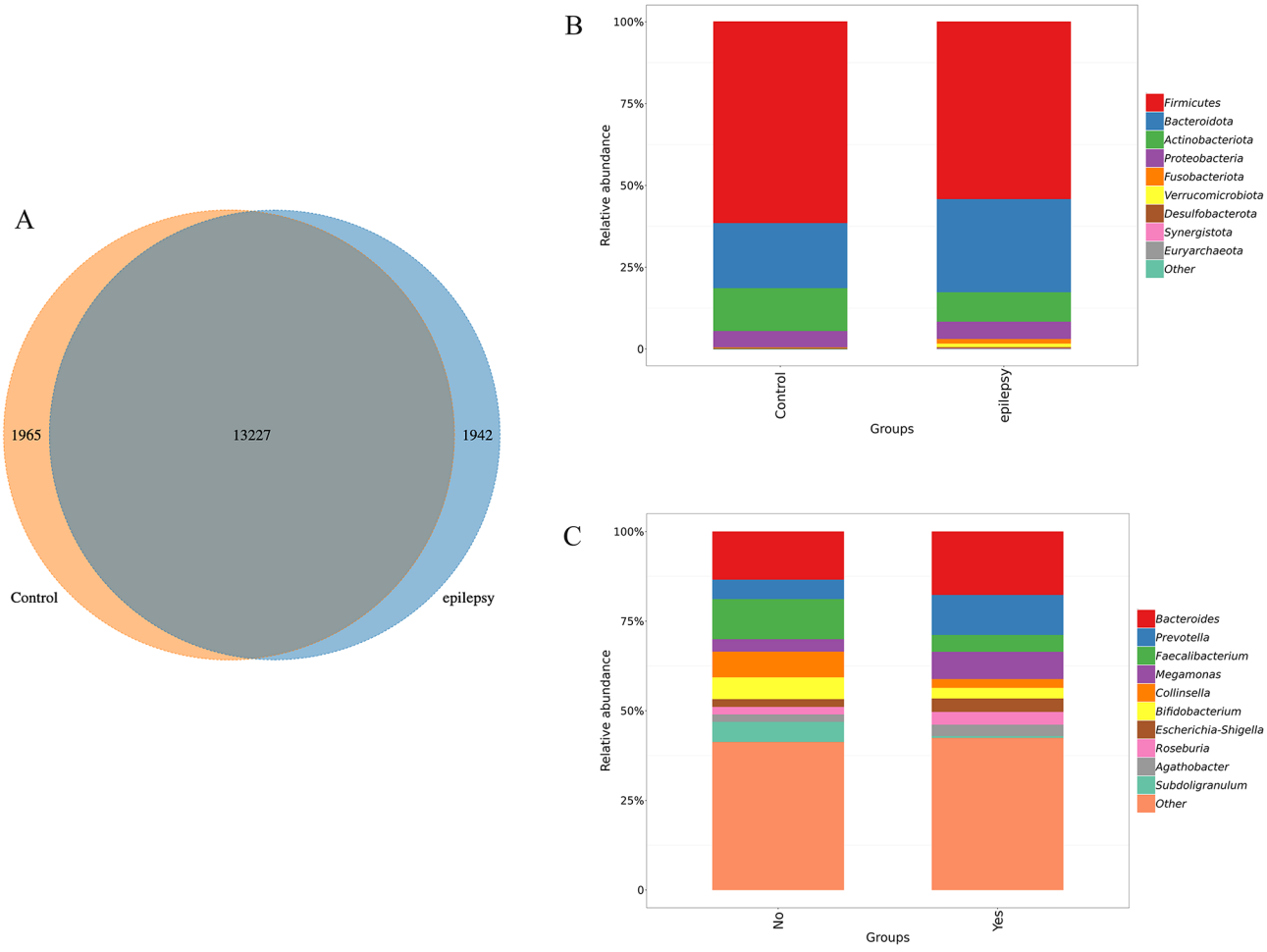

Fig. 1 Microbial composition and structural characteristics of the epileptic and normal groups. **(A)** Venn diagram of ASVs for both groups of subjects; **(B)** Relative abundance of each group at the door level for both groups of subjects; **(C)** Relative abundance of the top 10 genera ranked at the genus level in the two groups of subjects

Table 2 Statistical analysis of alpha diversity in both groups

Diversity Index	shannon	simpson	chao1	ace
control groups	10.59±0.68	1.00±0.00	4228.93±954.48	4081.38±1064.71
epilepsy groups	10.74±0.41	1.00±0.00	3889.09±854.85	4207.30±1128.56
P value	0.498	0.231	0.145	0.764

values of shannon, simpson, chao1 and ace indices, but there was no significant difference between the groups ($P>0.05$) (Table 3). The Bray-Curtis PCoA analysis (Fig. 4A) showed that there was a small amount of overlap between the two sets of samples, with an overall greater dispersion. To explore the taxa that differed between the two patient groups, we performed LEfSe analysis, which revealed Coriobacteriaceae, *Collinsella*, Coriobacteriia, Coriobacteriales, Oscillospirales, and Ruminococcaceae as the marker species in the No group (Fig. 4B).

Functional forecasts

We analyzed the enrichment of intestinal flora in the METACYC metabolic pathway in both groups of patients based on PICRUSt2 functional predictions (Fig. 5). A total of six metabolic pathways were significantly different between groups ($P<0.05$), namely: P341-PWY glycolysis V_Pyrococcus, PWY-2941 L-lysine biosynthesis II, P124-PWY Bifidobacterium shunt, P122-PWY heterolactic fermentation, ARGORNPROST-PWY arginine ornithine and proline interconversion, PWY-6471 peptidoglycan biosynthesis IV_Enterococcus faecium. The abundance of P124-PWY Bifidobacterium shunt was up-regulated in the No group, while the abundance of the remaining metabolic pathways were up-regulated in the Yes group.

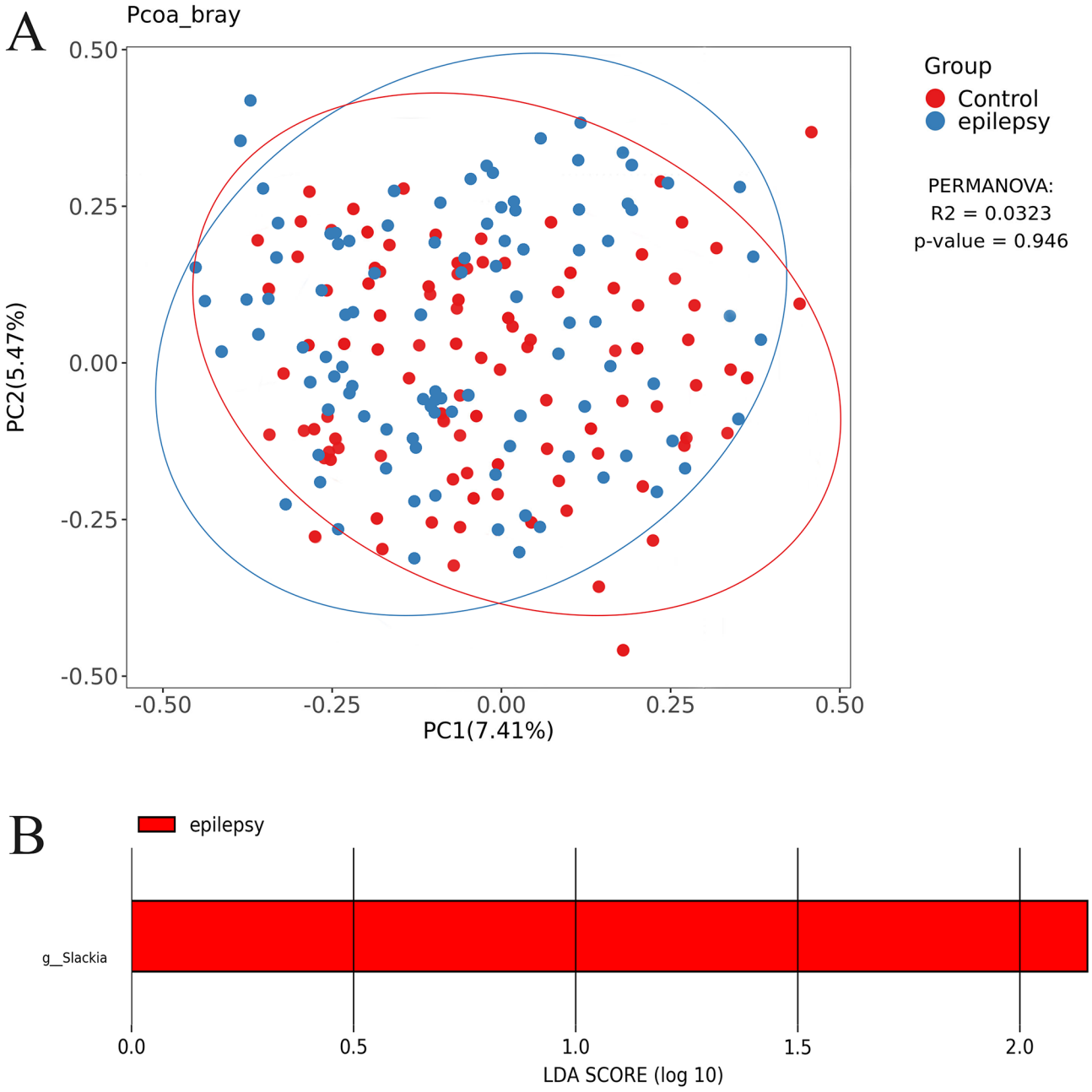

Fig. 2 Analysis of microbial diversity in the epileptic and normal groups and its differences. **(A)** PCoA analysis of gut microorganisms in two groups of subjects; **(B)** Markers of between-group differences in LEfSe analysis in two groups of subjects

Logistic regression analysis of factors influencing cognitive dysfunction in patients with epilepsy

Six intergroup marker species from the Yes group of patients with combined cognitive impairment epilepsy in the LEfSe analysis were analyzed with logistic regressions against the No group of patients with no cognitive impairment epilepsy (Table 4). The results showed that *Collinsella*, Oscillospirales, and Ruminococcaceae had a greater effect on combined cognitive impairment in epilepsy. The logistic regression analysis has been updated to include additional factors such as education level, duration of epilepsy, use of ASM, types of epilepsy, seizure frequency, co-existent psychiatric illness, age, and gender. This provides a more comprehensive understanding of the risk factors.

Discussion

The human intestinal flora is complex in composition, containing over a hundred species of bacteria with a total of approximately 104 microorganisms, which can

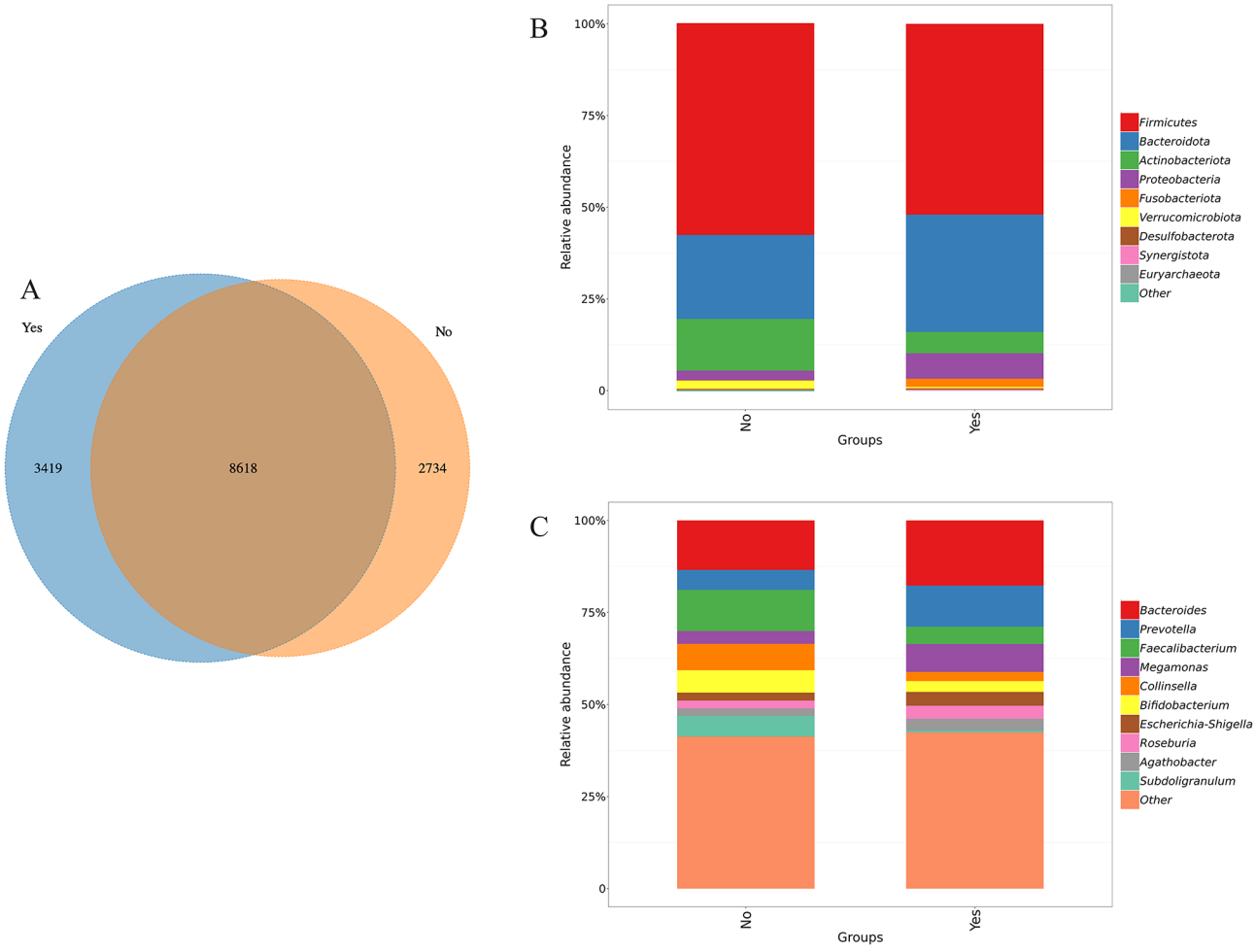

Fig. 3 Microbial composition and structural characteristics of the two groups of patients. **(A)** Venn diagram of ASVs in both groups; **(B)** Relative abundance of each group at the portal level for both groups of patients; **(C)** Relative abundance of the top 10 genera in both groups

Table 3 Statistical analysis of alpha diversity in both groups

Diversity Index	shannon	simpson	chao1	ace
Yes groups	10.62±0.45	1.00±0.00	3669.71±737.10	4031.52±1230.01
No groups	10.93±0.26	1.00±0.00	4273.00±1020.03	4488.56±1006.31
P value	0.193	0.138	0.904	0.502

produce more than 1.5 kg of biomass [11] and are important in maintaining a dynamic metabolic ecological balance. In recent years an increasing number of animal and clinical studies have shown that gut microbes are associated with various psychiatric, neurological and neurodegenerative disorders such as epilepsy, demyelinating diseases of the central nervous system, Parkinson's disease and Alzheimer's disease. Changes in gut microbes, with or without a central role in the pathophysiology of these psychiatric and neurological disorders, have increasingly become a hot topic of research in the psychiatric and neurological fields [4]. The interaction of gut flora with the gut and brain is known as the "gut flora-gut-brain axis" [12], where gut flora contributes to the maturation of the neuroendocrine system, regulating neural circuits and stress behavior [13]. Studies have shown that the overproduction of certain metabolites synthesised by gut microbes, such as short-chain fatty acids (SCFAs) and p-cresol sulfates, interferes with microglia function and triggers misfolding of alpha-synuclein, which can accumulate inside neurons and cause damage [14]. Thus alterations in the composition of the gut microbiota, i.e. dysbiosis, may affect the motor, cognitive and emotional functions of the brain through the gut flora-gut-brain axis pathway [15].

Epilepsy is a clinical syndrome caused by highly synchronized abnormal discharges of central neurons due to multiple factors. The pathogenesis of epilepsy

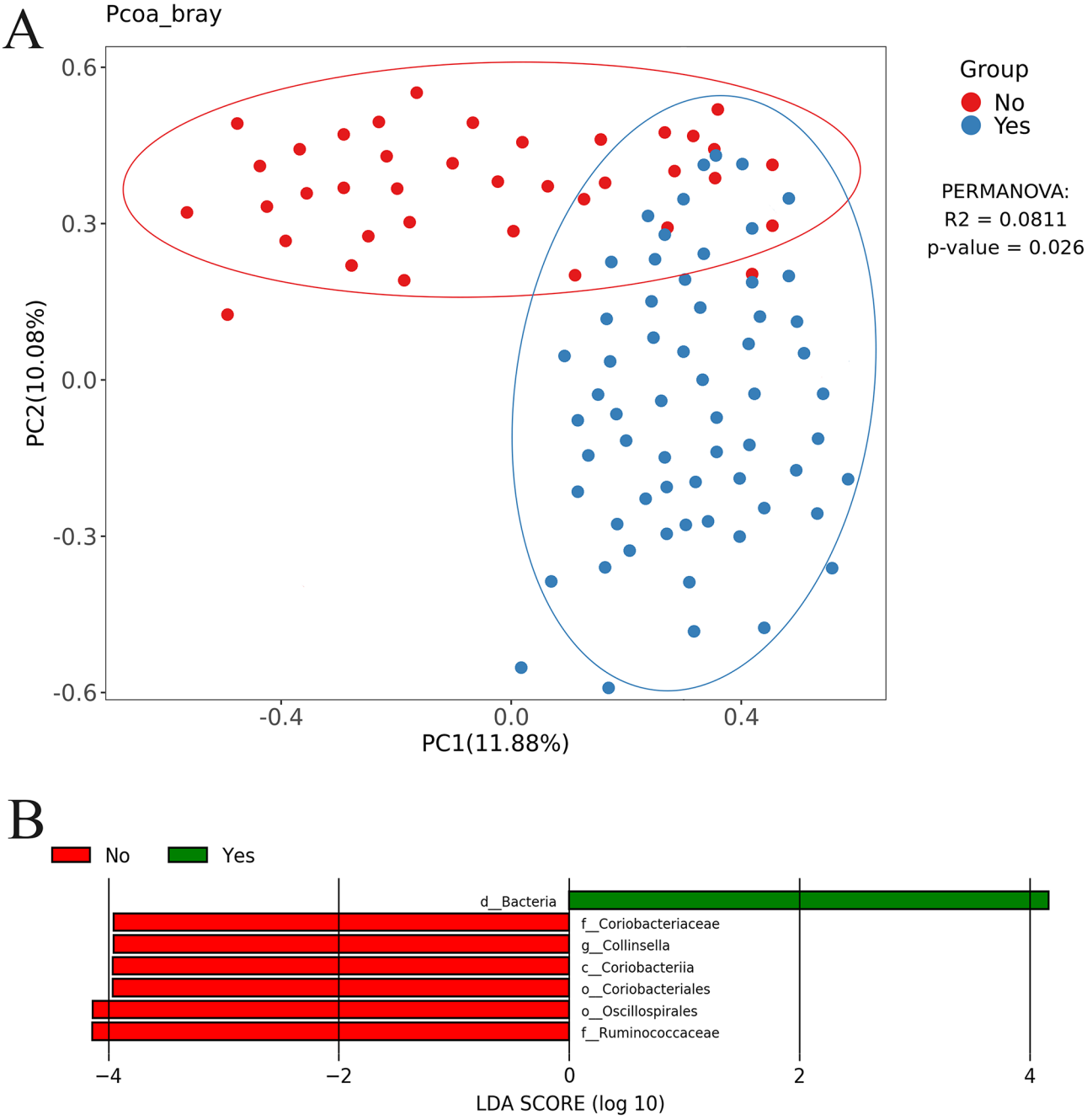

Fig. 4 Analysis of microbial diversity and its differences between the two groups of patients. **(A)** PCoA analysis of intestinal microorganisms in two groups of patients; **(B)** Markers of between-group differences in LEfSe analysis between the two groups of patients

is complex and not yet fully elucidated, with an imbalance of inhibitory-excitatory neurotransmitters thought to be an important factor in seizures. The mechanisms by which intestinal flora affect the development of epilepsy are still not specifically understood. According to current research evidence, intestinal flora can produce GABA, glutamate and 5-hydroxytryptamine, as well as their precursors (e.g. tryptophan), which affect the excitatory-inhibitory balance of nerve cells [16]. This suggests a potential clinical diagnostic and therapeutic value of intestinal flora in brain disorders.

This study began with a comparative analysis of the gut microbiota of patients with epilepsy and their healthy family members. The probability of developing gastrointestinal disorders increases with age, and other studies have shown that a high proportion of gastrointestinal disorders coexist with neurodegenerative diseases, suggesting that dysbiosis of the gut flora can influence the

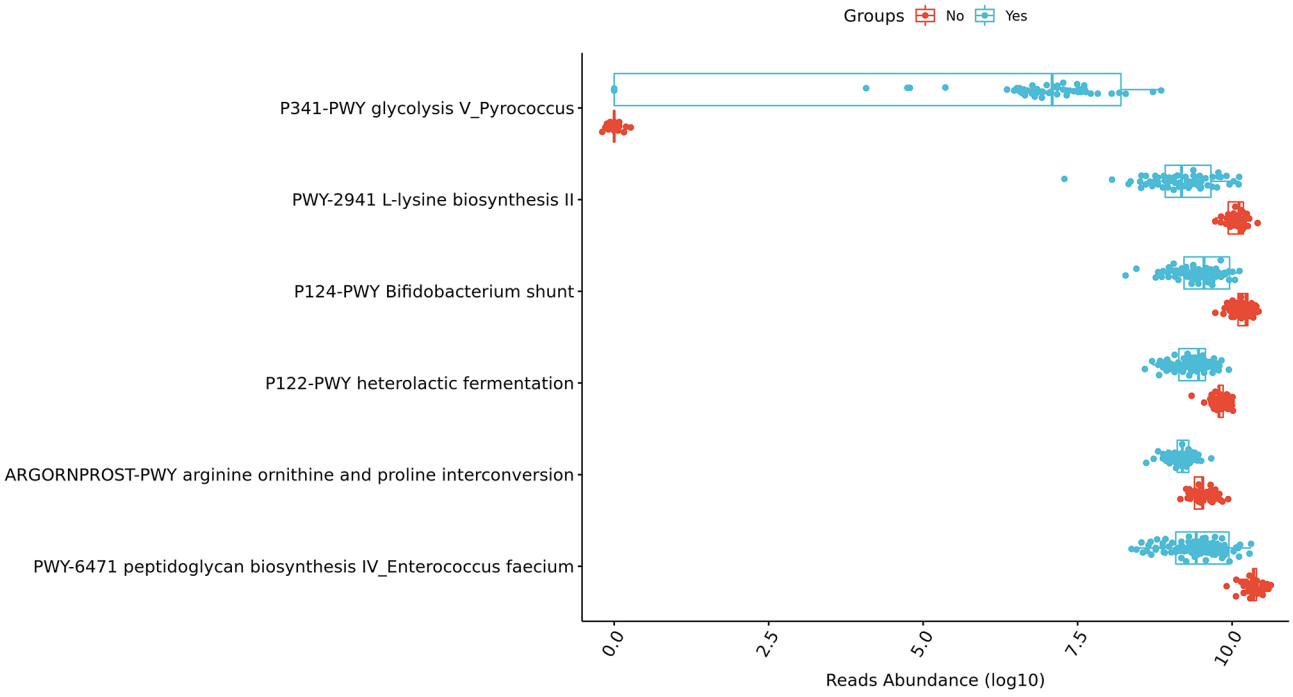

Fig. 5 Metabolic pathways that differed significantly between groups

Table 4 Logistic regression analysis of cognitive dysfunction in patients with epilepsy

Independent variable	β	SE	WaldX^2	df	*P*	OR(95%CI)
Coriobacteriaceae	0.172	0.455	0.143	1	0.239	1.188
Collinsella	0.603	0.164	13.519	1	0.000	1.828
Coriobacteriia	0.470	0.570	0.680	1	0.410	1.600
Coriobacteriales	0.042	0.659	0.004	1	0.813	1.043
Oscillospirales	0.596	0.252	5.594	1	0.005	1.815
Ruminococcaceae	0.413	0.382	1.169	1	0.026	1.511

development and progression of neurological disorders [17]. Significant differences in the composition of the gut flora between patients with epilepsy and healthy volunteers have been shown [18]. The results showed that the composition of the gut flora of the members of the epileptic group differed from that of the normal control group in terms of relative abundance at both the gate level and the genus level, suggesting a possible dysbiosis of the gut flora in the epileptic group. There was no significant difference in intestinal flora diversity between the two groups of subjects in this study. Analysis of the reasons for this may be due to the fact that the two groups of subjects, as family members, shared similar lifestyles, dietary habits and living environments, and therefore there was no significant difference in intestinal flora.

Cognitive dysfunction is a common complication of epilepsy, which manifests as complete or partial impairment of different cognitive domains such as attention, memory, naming, visuospatial ability, and executive function ability [19], and as a type of neurodegenerative disorder that severely affects the quality of life of patients. The development of neurodegenerative diseases is accompanied by changes in the intestinal flora and its associated metabolites [20]. This study further divided the patients in the epilepsy group into a Yes group with combined cognitive dysfunction and a No group without cognitive dysfunction by using the MMSE scale. The comparison showed that the relative abundance of gut flora at the phylum level and genus level differed more significantly between the two groups in both value and ranking, and the Beta diversity of the two groups showed a more scattered profile, indicating differences in the structure and composition of the gut flora between the two groups of patients. Thus, gut flora could be an important factor in the evaluation of cognitive impairment in combination with epilepsy.

Gut flora gene function was assessed by PICRUSt2 analysis in the epilepsy group with and without cognitive impairment. High lactate/glucose ratios have been reported to lead to central fatigue, cognitive impairment and some visible seizures [21]. The findings that glycolysis V and heterotactic fermentation are richly upregulated in

patients with cognitive dysfunction are consistent with previous results, suggesting that glycolysis and lactate play an important role in the regulation of neurological disorders. Peptidoglycan is a major component of the cell wall of bacteria, Enterococcus faecium, which is a normal flora of the human body and also an important pathogen for opportunistic infections, but no studies have yet shown an association with epilepsy or cognitive dysfunction. The reason for the upregulation of the peptidoglycan biosynthesis IV_Enterococcus faecium metabolic pathway in patients with combined cognitive impairment in this study is unclear and needs to be further explored.

This study demonstrated the species of difference between the two groups with and without comorbid cognitive dysfunction in epilepsy by LEfSe analysis and further analyzed the correlation between species of difference and comorbid cognitive dysfunction in epilepsy by logistics regression. *Collinsella*, *Oscillospirales*, and *Ruminococcaceae* produce short-chain fatty acids (SCFAs) [22], which are key signaling molecules in the gut-brain axis and can cross the blood-brain barrier (BBB) to regulate neuronal and microglial functions. SCFAs have been shown to reduce neuroinflammation and improve blood-brain barrier integrity, suggesting their potential in preventing neurodegenerative diseases [23]. However, these bacteria may also have detrimental effects in the context of epilepsy combined with cognitive impairment. Further research is needed to elucidate the complex roles of these microorganisms in different neurological conditions. Wenzel et al. [24] found that SCFAs reduced IL-1β, the monocyte chemotactic protein MCP-1/CCL2, TNF-α and cytotoxic production by immunostimulant THP-1 cells, inhibiting neuroinflammation during the development of Alzheimer's disease. Liu et al. [25] found that SCFAs upregulated the expression of the tight junction proteins Occluding and Z0-1 in the brains of mice with septic encephalopathy to increase blood-brain barrier integrity, downregulated the expression of the inflammatory pathways JNK and NF-κBp65, and reduced inflammatory factors, alleviating neuroinflammation and reducing neuronal degeneration and behavioral deficits in mice. The above evidence suggests that short-chain fatty acids may be a potential target for the prevention of neurodegenerative diseases, but the mechanisms by which the dynamics of SCFAs interact with cognitive function need further investigation.

A significant limitation of this study is the exclusive use of the Mini-Mental State Examination (MMSE) to assess cognitive impairment. While the MMSE is a widely used screening tool, it primarily assesses certain cognitive domains and may not detect subtle or multifactorial cognitive impairments in epilepsy patients. It also has educational and ceiling biases, which can misclassify cognitive status. Future research should incorporate a broader battery of neuropsychological tests and account for demographic variables to provide a more comprehensive assessment of cognitive impairment in epilepsy patients. Despite its limitations, the MMSE offered a practical screening method. Besides, the high prevalence of cognitive impairment in our epilepsy cohort may be due to factors such as the severity of epilepsy, duration of the disease, and comorbid conditions. Further research is needed to explore these associations.

Conclusion

In summary, there was an imbalance in the gut flora of patients with epilepsy compared to healthy controls, but no significant differences were found. The intestinal flora of patients with combined cognitive dysfunction epilepsy differed more significantly from those without cognitive dysfunction epilepsy, with *Collinsella*, Oscillospirales, and Ruminococcaceae having a greater impact on combined cognitive impairment epilepsy, which can be used as one of the observation indicators for combined cognitive impairment epilepsy.

Acknowledgements
This study was conducted by a single investigator, with clinical evaluation, enrollment, and assessments conducted independently.

Author contributions
BingCong Hong wrote the main manuscript text, prepared figures and review the manuscript.

Funding
This study was supported by The Fujian Medical University Launch Fund Project (project number: 2019QH1252).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study is approved by Quanzhou First Hospital (Quan Yilun [2019] No. 185). Signed informed consent were also obtained from all participants.

Consent for publication
The work described has not been published previously.

Competing interests
The authors declare no competing interests.

Conflict of interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

Received: 9 May 2024 / Accepted: 4 September 2024
Published online: 28 September 2024

References

1. Trinka E, Kwan P, Lee B, Dash A. Epilepsy in Asia: Disease burden, management barriers, and challenges. Epilepsia. 2019;60(Suppl 1):7–21. https://doi.org/10.1111/epi.14458.
2. Ding D, Zhou D, Sander JW, Wang W, Li S, Hong Z. Epilepsy in China: major progress in the past two decades. Lancet Neurol. 2021;20(4):316–26. https://doi.org/10.1016/S1474-4422(21)00023-5.
3. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, Long-Smith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019;99(4):1877–2013. https://doi.org/10.1152/physrev.00018.2018.
4. Vendrik KEW, Ooijevaar RE, de Jong PRC, Laman JD, van Oosten BW, van Hilten JJ, Ducarmon QR, Keller JJ, Kuijper EJ, Contarino MF. Fecal microbiota transplantation in neurological disorders. Front Cell Infect Microbiol. 2020;10:98. https://doi.org/10.3389/fcimb.2020.00098.
5. Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, Schwierzeck V, Utermöhlen O, Chun E, Garrett WS, McCoy KD, Diefenbach A, Staeheli P, Stecher B, Amit I, Prinz M. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18(7):965–77. https://doi.org/10.1038/nn.4030.
6. Wang JW, Zhou Q, Dai WK, et al. Changes in the intestinal flora of infants and children with refractory epilepsy. Chin J Microecology. 2017;29(5):502–5. https://doi.org/10.13381/j.cnki.cjm.201705002.
7. Nikiforova AS. Stress-induced gastrointestinal motility is responsible for epileptic susceptibility. Med Hypotheses. 2014;82(4):442–51. https://doi.org/10.1016/j.mehy.2014.01.020.
8. Fröhlich EE, Farzi A, Mayerhofer R, Reichmann F, Jačan A, Wagner B, Zinser E, Bordag N, Magnes C, Fröhlich E, Kashofer K, Gorkiewicz G, Holzer P. Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. Brain Behav Immun. 2016;56:140–55. https://doi.org/10.1016/j.bbi.2016.02.020. Epub 2016 Feb 23.
9. Cirstea MS, Yu AC, Golz E, Sundvick K, Kliger D, Radisavljevic N, Foulger LH, Mackenzie M, Huan T, Finlay BB, Appel-Cresswell S. Microbiota Composition and Metabolism are Associated with gut function in Parkinson's Disease. Mov Disord. 2020;35(7):1208–17. https://doi.org/10.1002/mds.28052.
10. Cox LM, Maghzi AH, Liu S, Tankou SK, Dhang FH, Willocq V, Song A, Wasén C, Tauhid S, Chu R, Anderson MC, De Jager PL, Polgar-Turcsanyi M, Healy BC, Glanz BI, Bakshi R, Chitnis T, Weiner HL. Gut Microbiome in Progressive multiple sclerosis. Ann Neurol. 2021;89(6):1195–211. https://doi.org/10.1002/ana.26084.
11. Kuziel GA, Rakoff-Nahoum S. The gut microbiome. Curr Biol. 2022;32(6):R257–64. https://doi.org/10.1016/j.cub.2022.02.023.
12. Bauer KC, Huus KE, Finlay BB. Microbes and the mind: emerging hallmarks of the gut microbiota-brain axis. Cell Microbiol. 2016;18(5):632–44. https://doi.org/10.1111/cmi.12585.
13. De Vadder F, Grasset E, Mannerås Holm L, Karsenty G, Macpherson AJ, Olofsson LE, Bäckhed F. Gut microbiota regulates maturation of the adult enteric nervous system via enteric serotonin networks. Proc Natl Acad Sci U S A. 2018;115(25):6458–63. https://doi.org/10.1073/pnas.1720017115.
14. Wiefels MD, Furar E, Eshraghi RS, Mittal J, Memis I, Moosa M, Mittal R, Eshraghi AA. Targeting Gut Dysbiosis and Microbiome metabolites for the development of therapeutic modalities for neurological disorders. Curr Neuropharmacol. 2022 Oct;3. https://doi.org/10.2174/1570159X20666221003085508.
15. Doifode T, Giridharan VV, Generoso JS, Bhatti G, Collodel A, Schulz PE, Forlenza OV, Barichello T. The impact of the microbiota-gut-brain axis on Alzheimer's disease pathophysiology. Pharmacol Res. 2021;164:105314. https://doi.org/10.1016/j.phrs.2020.105314.
16. Roth W, Zadeh K, Vekariya R, Ge Y, Mohamadzadeh M. Tryptophan metabolism and Gut-Brain Homeostasis. Int J Mol Sci. 2021;22(6):2973. https://doi.org/10.3390/ijms22062973.
17. Westfall S, Lomis N, Kahouli I, Dia SY, Singh SP, Prakash S. Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis. Cell Mol Life Sci. 2017;74(20):3769–87. https://doi.org/10.1007/s00018-017-2550-9.
18. Russo E. The gut microbiota as a biomarker in epilepsy. Neurobiol Dis. 2022;163:105598. https://doi.org/10.1016/j.nbd.2021.105598.
19. Wang L, Chen S, Liu C, Lin W, Huang H. Factors for cognitive impairment in adult patients with epilepsy. Brain Behav. 2020;10(1):e01475. https://doi.org/10.1002/brb3.1475. Epub 2019 Dec 21.
20. Cai YY, Wu YX, Li FT, Xie T, Wang YZ, Zhang MY, Dai YF, Zheng F, Yue H. Advances in the study of intestinal flora and its metabolites in relation to neurodegenerative diseases. Chin J Appl Chem 2023,40(03):309–16.https://doi.org/10.19894/j.issn.1000-0518.220161
21. Kann O. Lactate as a supplemental fuel for synaptic transmission and neuronal network oscillations: potentials and limitations. J Neurochem. 2023 Jun;13. https://doi.org/10.1111/jnc.15867.
22. Xie J, Li LF, Dai TY, Qi X, Wang Y, Zheng TZ, Gao XY, Zhang YJ, Ai Y, Ma L, Chang SL, Luo FX, Tian Y, Sheng J. Short-chain fatty acids produced by Ruminococcaceae Mediate α-Linolenic acid promote intestinal stem cells proliferation. Mol Nutr Food Res. 2022;66(1):e2100408. https://doi.org/10.1002/mnfr.202100408.
23. Yissachar N, Zhou Y, Ung L, Lai NY, Mohan JF, Ehrlicher A, Weitz DA, Kasper DL, Chiu IM, Mathis D, Benoist C. An intestinal Organ Culture System uncovers a role for the Nervous System in Microbe-Immune Crosstalk. Cell. 2017;168(6):1135–e114812. https://doi.org/10.1016/j.cell.2017.02.009.
24. Wenzel TJ, Gates EJ, Ranger AL, Klegeris A. Short-chain fatty acids (SCFAs) alone or in combination regulate select immune functions of microglia-like cells. Mol Cell Neurosci. 2020;105:103493. https://doi.org/10.1016/j.mcn.2020.103493.
25. Liu J, Jin Y, Ye Y, Tang Y, Dai S, Li M, Zhao G, Hong G, Lu ZQ. The neuroprotective effect of short chain fatty acids against Sepsis-Associated Encephalopathy in mice. Front Immunol. 2021;12:626894. https://doi.org/10.3389/fimmu.2021.626894.

Publisher's note