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Research article

Unveiling the negative association of *Faecalibacterium prausnitzii* with ischemic stroke severity, impaired prognosis and pro-inflammatory markers

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ABSTRACT

Background: The correlation between acute ischemic stroke (AIS) and gut microbiota has opened a promising avenue for improving stroke prognosis through the utilization of specific gut bacterial species. This study aimed to identify gut bacterial species in AIS patients and their correlation with stroke severity, 3-month prognosis, and inflammatory markers. **Methods**: We enrolled 59 AIS patients (from June 2021 to July 2022) and 31 age-matched controls with similar cerebrovascular risk profiles but no stroke history. Fecal samples were analyzed using 16 S rDNA V3–V4 sequencing to assess α and β diversity and identify significant microbiota differences. AIS cases were categorized based on the National Institute of Health Stroke Scale (NIHSS) scores and 3-month modified Rankin Scale (mRS) scores. Subgroup analyses were performed, and correlation analysis was used to examine associations between flora abundance, inflammatory markers and stroke outcome.

Results: Significant differences in β-diversity were observed between case and control groups ($P < 0.01$). Bacteroides dominated AIS samples, while *Clostridia*, *Lachnospirales*, *Lachnospiraceae*, *Ruminococcaceae*, *Faecalibacterium*, and *Faecalibacterium prausnitzii* were prominent in controls. *Faecalibacterium* and *Faecalibacterium prausnitzii* were significantly reduced in non-minor stroke and 3-month poor prognosis groups compared to controls, while this difference was less pronounced in patients with minor stroke and 3-month good prognosis. Both *Faecalibacterium* and *Faecalibacterium prausnitzii* were negatively correlated with the NIHSS score on admission ($r = -0.48, -0.48, P < 0.01$) and 3-month mRS score ($r = -0.48, -0.44, P < 0.01$). Additionally, they showed negative correlations with pro-inflammatory factors and positive correlations with anti-inflammatory factors (both $P < 0.01$).

Conclusions: *Faecalibacterium prausnitzii* is negatively associated with stroke severity, impaired prognosis, and pro-inflammatory markers, highlighting its potential application in AIS treatments.

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1. Introduction

Stroke presents a significant global health burden, leading to considerable morbidity and mortality [1,2]. Acute ischemic stroke (AIS) is the predominant subtype, and the inflammatory response triggered by AIS can further aggravate damage to brain tissue. The human intestinal tract hosts a substantial portion (70%–80%) of immune cells, implying a potential role of gut microbiota in modulating the pro-inflammatory and anti-inflammatory activities of immune cells [3]. In recent years, increasing attention has been given to the role of gut microbiota beyond the confines of the gastrointestinal tract. Growing research on the "gut-brain axis" highlights the bi-directional communication between the gut and the central nervous system through gut microbiota [4]. Their potential relationship with stroke has become a subject of exploration. Emerging evidence suggests that gut microbiota is not only closely associated with the risk factors of stroke, such as hypertension, diabetes, obesity and atherosclerosis, but may also significantly impact the onset and prognosis of stroke [5]. Recent studies have demonstrated alterations in the composition and structure of gut microbiota in AIS patients [6]. Following AIS, there is a notable alteration in the composition of gut microbiota, characterized by a decrease in the proportion of probiotics and an excessive growth of harmful bacteria [7,8]. In addition, the damaged brain tissue after AIS leads to the activation of the immune inflammatory system through the release of inflammatory molecules and cell fragments. Gut microbiota plays an important role as an essential immune vector in this context [9]. Animal experiments have supported the notion that gut microbiota imbalance contributes to stroke development by mediating an immune inflammatory response [10,11].

As an adjustable factor, the gut microbiota warrants careful consideration and could potentially evolve into a promising target for therapy. However, the correlation between alterations in gut microbiota in patients with AIS and their stroke severity and prognosis, especially in conjunction with inflammatory markers, requires further investigation. Furthermore, it is imperative to identify significant microbial species that can be translated into effective clinical treatments.

This study aimed to explore the composition and structural characteristics of gut microbiota in AIS patients, with the expectation of identifying specific bacterial species displaying significant changes. Furthermore, we sought to analyze the correlation of these changes with stroke severity, 3-month functional prognosis, and inflammatory markers. By investigating the potential associations between gut microbiota and stroke outcome, we aimed to shed light on potential therapeutic interventions in the management of AIS.

2. Materials and methods

2.1. Subjects

We conducted this study at the First Affiliated Hospital of Dalian Medical University, enrolling 59 patients with newly diagnosed anterior circulation acute ischemic stroke (AIS) from June 2021 to July 2022, constituting the case group. On admission, patients were categorized into two subgroups based on their National Institute of Health Stroke Scale (NIHSS) score: minor stroke (NIHSS$\leq$5) and non-minor stroke (NIHSS$>$5) [12]. Additionally, based on their 3-month modified Rankin Scale (mRS) score post-stroke, they were further classified into either the good prognosis group (mRS 0–2) or the poor prognosis group (mRS 3–6) [13]. As a control group, we recruited 31 volunteers from the local Han population, carefully matched for age and gender, with similar cerebrovascular disease risk characteristics but no history of stroke events. Inclusion criteria for patients were as follows: (1) age $\geq$18 years old; (2) within 48 h of stroke onset; (3) long lived local Han population. To ensure the study's integrity, we excluded participants meeting any of the following criteria: (1) received antibiotics or probiotics within one month prior to inclusion; (2) suffering from digestive system diseases (e.g., chronic diarrhea and constipation), infectious diseases, malignant tumor, serious medical system disease, or immune-mediated inflammatory disease; (3) having special dietary habits (e.g., vegetarian diet); (4) presenting sequelae from previous stroke history. The sample size was determined according to previous studies [14–16].

2.2. Clinical data and inflammatory indicators collection

Participants' demographic information and medical history were recorded through face-to-face interviews within 24 h of admission. The morning after admission, fasting peripheral venous blood samples were collected and promptly sent to the hospital clinical laboratory for analysis. We assessed interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and conducted blood cell counts. Consequently, we calculated the platelet to lymphocyte ratio (PLR), the neutrophil to lymphocyte ratio (NLR), the monocyte to lymphocyte ratio (MLR) and the platelet to neutrophil ratio (PNR).

Fecal samples collection and 16 S rDNA V3–V4 region sequencing.

Within 72 h after admission, approximately 2 g of fresh and clean feces was collected from patients who had not received antibiotics or probiotics. Fecal samples from the controls were collected during the same period. The collected fecal samples were immediately frozen in a liquid nitrogen tank and then stored in a −80 °C refrigerator for preservation. Once all samples were obtained, they underwent unified gut microbiota 16 S rDNA V3–V4 region sequencing. DNA extraction from the samples was performed using the cetyltrimethyl ammonium bromide (CTAB) method, and the purity and concentration of the extracted DNA were assessed through agarose gel electrophoresis. Subsequently, an appropriate amount of sample DNA was taken and diluted to 1 ng/μL with sterile water. The diluted genomic DNA was then used as a template to amplify the 16 S V3–V4 region using specific primers with barcode. Enzyme labeled quantitative method was employed to mix the PCR products in equal amounts based on their concentrations. After thorough mixing, the PCR products were subjected to 2% agarose gel electrophoresis to detect the target strip, and the gel recovery kit provided by Qiagen Company was used to recover the products. For library construction, the TruSeq® DNA PCR-Free Sample Preparation Kit was used, and the constructed library was quantified using Qubit and Q-PCR. Finally, NovaSeq6000 was employed for the sequencing

process, enabling comprehensive analysis of the gut microbiota composition in the collected samples.

2.3. Analysis of the gut microbiota

The initial data underwent filtering and splicing to obtain relevant and reliable information. Subsequently, the sequences were clustered into Operational Taxonomic Units (OTUs) with 97% consistency. Based on the OTUs clustering results, species annotation was performed to obtain the corresponding species information and the abundance distribution of species at various levels of classification (phylum, class, order, family, genus, species). Following this, both α-diversity and β-diversity analyses were conducted to compare different groups. The α-diversity analysis was employed to assess the richness and evenness of microbiota within each sample, using parameters such as observed_species, chao1, ACE, shannon, simplson, goods_coverage and PD_whole_tree. Meanwhile, the β-diversity analysis was used to investigate potential differences in microbiota composition among groups, visualized through Principal Co-ordinates Analysis (PCoA). Microbiota with significant differences in abundance among groups were identified using Linear discriminant analysis Effect Size (LefSe) and Metastat. In the final step, the correlation between the relative abundance of microbiota and stroke severity, 3-month prognosis and inflammatory markers were evaluated.

2.4. Statistical analysis

All statistical analyses were performed using SPSS (version 26.0), Qiime (Version 1.9.1), and R (Version 4.0). The significance level was set at p value < 0.05. Continuous variables with a normal distribution, confirmed by Kolmogorov-Smirnov tests, were presented as mean values with standard deviation (SD). For non-normally distributed variables, median values with interquartile range [IQR] were reported. Two-group difference was assessed using either the Mann-Whitney U test or the independent-samples t-test. Categorical variables were expressed as frequency and percentage, and analyzed using Fisher's exact test or chi-square test. To examine the bivariate correlation, Spearman correlation analysis was utilized.

3. Results

3.1. The baseline clinical characteristics of participants

The baseline characteristics of both AIS patients and controls were meticulously documented and subjected to comprehensive analysis, as detailed in Table 1. The patient cohort exhibited an average age of 67.66 ± 8.94 years, comprising 42 males (71.2%), while the control group displayed an average age of 67.13 ± 6.30 years, with 17 males (54.8%). Notably, no statistically significant disparities were observed in terms of age, gender distribution, or stroke risk factors between the two groups. These risk factors included hypertension, diabetes, coronary heart disease, atrial fibrillation, as well as smoking and alcohol consumption ($P > 0.05$). This underscores the comparability of baseline characteristics between the two cohorts.

3.2. Comparison of gut microbiota between AIS patients and controls

To investigate the differences in gut microbiota between AIS patients and controls, we employed 16 S rDNA V3–V4 region sequencing of gut microbiota, and performed α-diversity and β-diversity analyses to understand the richness and composition of microbiota in both groups. We also conducted Linear Discriminant Analysis Effect Size (LefSe) to identify bacteria with significant abundance differences. The α-diversity analysis did not reveal any statistically significant difference in gut microbiota between AIS patients and controls ($P > 0.05$, Table 2). However, the β-diversity analysis demonstrated a significant difference between the two groups, leading to clear separation in the Principal Coordinates Analysis (PCoA) ($P < 0.01$, Fig. 1A).

The microbiota in both groups primarily consisted of three phyla, namely *Firmicutes*, *Bacteroidota* and *Proteobacteria.* Linear discriminant analysis Effect Size (LefSe) results indicated that AIS patients exhibited a high abundance of *Bacteroides* at the levels of phylum, class, order, family, and genus. In contrast, *Clostridia*, *Lachnospirales*, *Lachnospiraceae*, *Ruminococcaceae*, *Faecalibacterium* and *Faecalibacterium prausnitzii* were the dominant bacteria in the control group (Fig. 1B and C).

Table 1
Baseline clinical characteristics of participants.

	Patients (n = 59)	Controls (n = 31)	p-value
Age, y, mean ± SD	67.66 ± 8.94	67.13 ± 6.30	0.769
Sex, male, n (%)	42 (71.2)	17 (54.8)	0.121
Hypertension, n (%)	40 (67.8)	18 (58.1)	0.359
Diabetes mellitus, n (%)	19 (32.2)	11 (35.5)	0.754
Coronary heart disease, n (%)	10 (16.9)	3 (9.7)	0.351
Atrial fibrillation, n (%)	17 (28.8)	4 (12.9)	0.090
Smoking, n (%)	22 (37.3)	12 (38.7)	0.895
Alcohol intake, n (%)	13 (22.0)	6 (19.4)	0.767

Table 2
The α-diversity analysis of gut microbiota between patients and controls.

α-diversity	Patients (n = 59)	Controls (n = 31)	*p*-value
observed_species	490 (435–640)	524 (426–617)	0.835
shannon	5.43 (4.82–6.01)	5.22 (4.81–5.72)	0.377
simpson	0.93 (0.87–0.96)	0.92 (0.84–0.95)	0.607
chao1	554.49 (497.59–726)	582 (508.92–688.16)	0.668
ACE	551.25 (503.84–748.27)	590.26 (502.16–715.37)	0.815
goods_coverage	0.998 (0.997–0.998)	0.998 (0.997–0.998)	0.392
PD_whole_tree	43.06 (35.81–50.46)	44.36 (33.11–50.04)	0.839

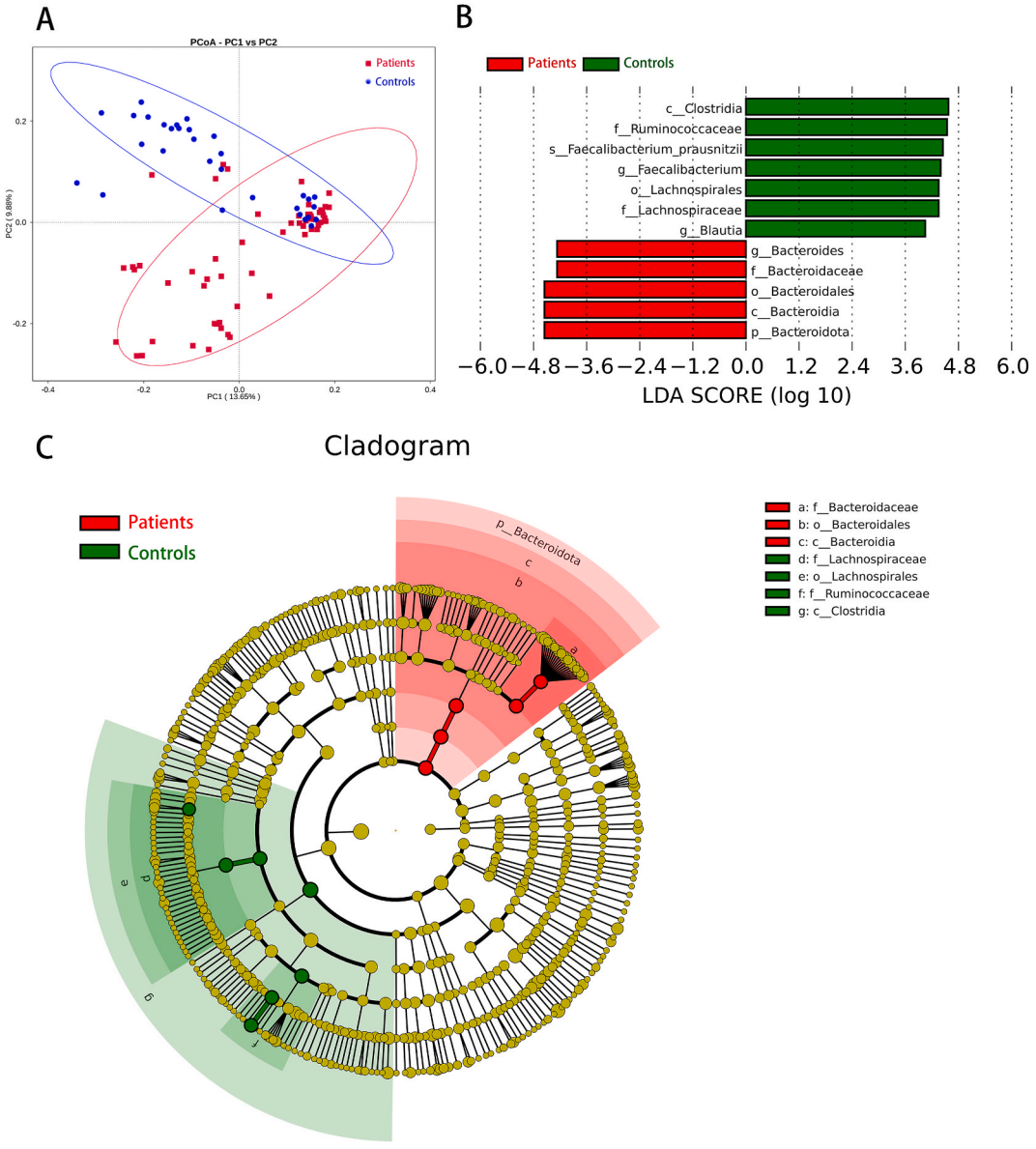

Fig. 1. Comparative Analysis of Gut Microbiota Between Acute Ischemic Stroke Patients and Controls. (A) Principal Coordinates Analysis highlights a significant divergence in the composition of gut microbiota between the two groups. (B, C) Linear Discriminant Analysis Effect Size underscores the distinctive microbiota profile of AIS patients, characterized by elevated Bacteroides abundance across phylum, class, order, family, and genus levels. Conversely, the control group showcases dominance in *Clostridia*, *Lachnospirales*, *Lachnospiraceae*, *Ruminococcaceae*, *Faecalibacterium*, and *Faecalibacterium prausnitzii* populations.

3.3. Analysis of gut microbiota between minor and non-minor stroke and controls

The case group was further divided into two subgroups based on stroke severity: 28 cases in the minor stroke (NIHSS $\leq$5) and 31 cases in the non-minor stroke (NIHSS >5) categories. We aimed to compare the gut microbiota in the minor and non-minor stroke patients as well as the controls. Through α-diversity and β-diversity analyses, we explored whether there were significant differences in microbiota composition among the three groups. No significant difference was observed in the α-diversity among the three groups ($P > 0.05$), but the β-diversity showed a significant difference ($P < 0.001$). PCoA illustrated that the minor stroke group occupied an intermediate position between the non-minor stroke group and the controls (Fig. 2A).

Given the relevance of genus and species-level classifications for differential microbial screening, we focused our analysis on these lower-level taxonomic categories. Compared to the controls, the non-minor stroke group exhibited significantly reduced levels of genera such as *Faecalibacterium*, *Blautia*, *Agathobacter*, *Subdoligranulum*, as well as species including *Faecalibacterium prausnitzii* ($P < 0.01$). Additionally, they showed increased levels of genera like *Parabacteroids*, *Akkermansia* and *Christensenellaceae_R-7_group*, as well as species like *Bacteroides dorei*, *Akkermansia muciniphila* and *Bacteroides caccae* ($P < 0.05$, Fig. 2B and C). However, these significant differences were not observed when comparing the minor stroke group to either the non-minor stroke group or the controls ($P > 0.05$).

3.4. Analysis of gut microbiota between 3-month good prognosis and poor prognosis and controls

To investigate the association between gut microbiota and 3-month prognosis after stroke, the 3-month functional prognosis after stroke was classified into two categories: good prognosis with 32 cases (mRS 0–2) and poor prognosis with 27 cases (mRS 3–6). There was no significant difference in the α-diversity among the good prognosis, the poor prognosis and the control group ($P > 0.05$), but the β-diversity was significantly different ($P < 0.001$). PCoA revealed that the 3-month good prognosis group was positioned between the 3-month poor prognosis group and the controls (Fig. 3A).

At the genus level, the representation of the top 30 relative abundances of microbiota across the three groups is depicted in Fig. 3B. To delve into bacteria exhibiting notable disparities between groups, we employed MetaStat for analyzing microbiota relative abundances and generating abundance distribution box plots for distinct species among the groups. We specifically focused on elucidating the top 4 bacteria characterized by substantial abundance discrepancies between the groups. Our findings revealed that, in contrast to both the control and 3-month good prognosis groups, the 3-month poor prognosis cohort exhibited marked reductions in

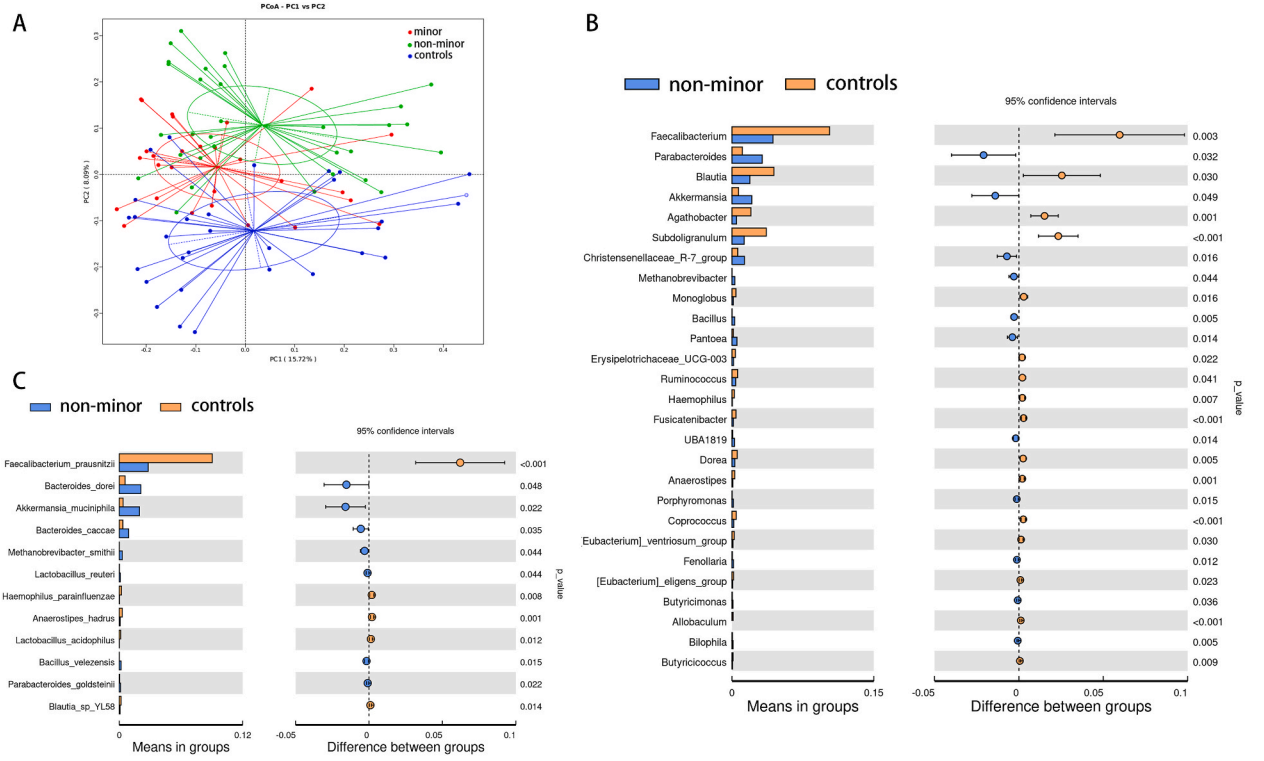

Fig. 2. Comparative Analysis of Gut Microbiota Among Minor Stroke, Non-Minor Stroke, and Control Groups. (A) Principal Coordinates Analysis highlights a significant dissimilarity in gut microbiota composition across the three groups, with the minor stroke group (n = 28, NIHSS $\leq$5) occupying an intermediary position between the non-minor stroke group (n = 31, NIHSS >5) and controls. (B) T-test reveals genus-level distinctions between the non-minor stroke and control groups. (C) T-test unveils species-level differences between the non-minor stroke and control groups.

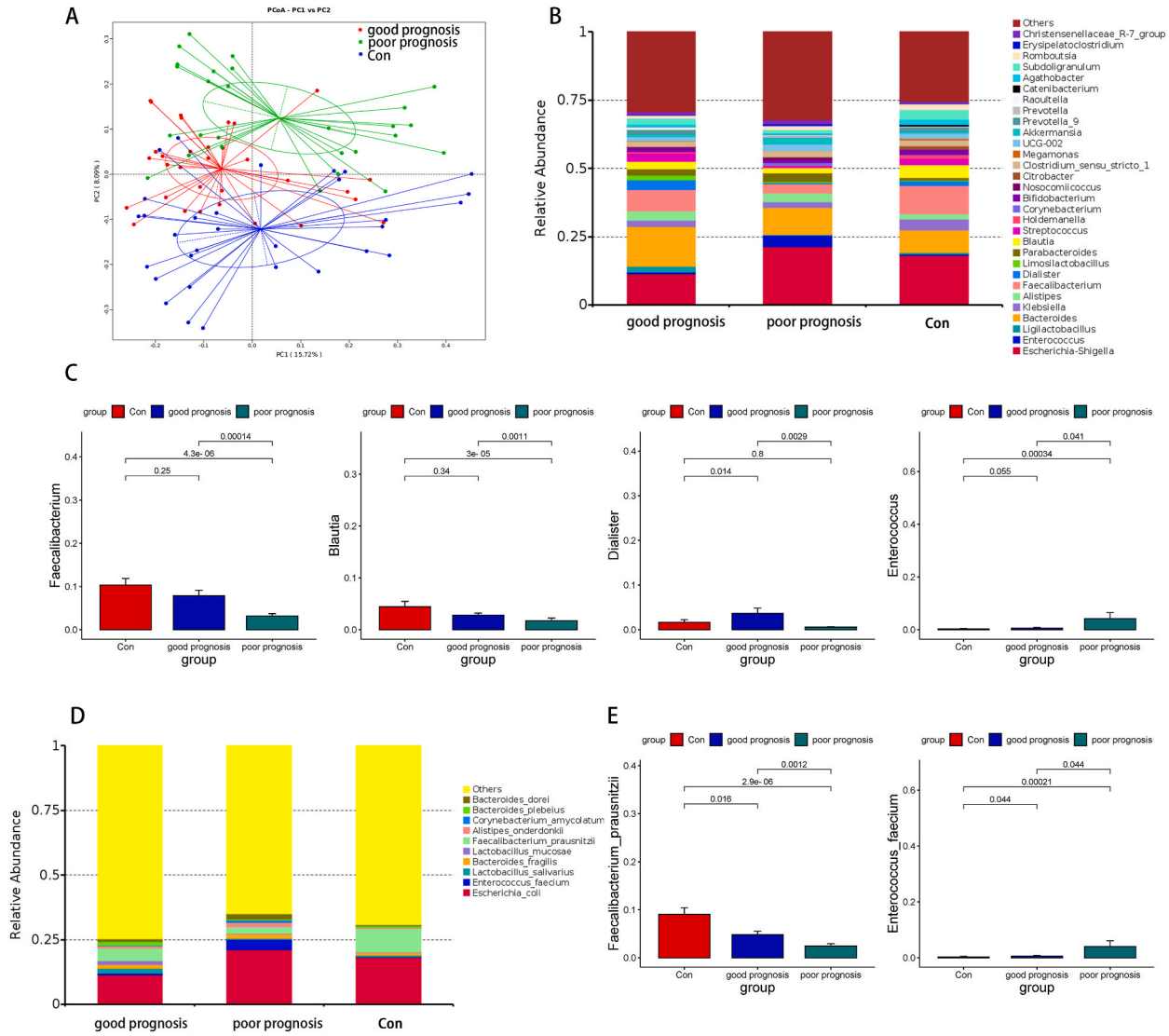

Fig. 3. Comparative Analysis of Gut Microbiota Among 3-Month Good Prognosis, Poor Prognosis, and Control Groups. (A) Principal Coordinates Analysis illustrates the positioning of the 3-month good prognosis group (n = 32, modified Rankin Scale (mRS) 0–2) between the 3-month poor prognosis group (n = 27, mRS 3–6) and controls. (B) The top 30 relative abundance of microbiota at the genus level among the three groups. (C) The top 4 microbiota with significantly different relative abundance among the 3-month poor prognosis, 3-month good prognosis and control groups at the genus level (Wilcoxon rank sum test). (D) The top 10 relative abundance of microbiota at the species level among the three groups. (E) The relative abundance of differential bacterial species belonging to the diverse genera mentioned above among the three groups (Wilcoxon rank sum test).

Faecalibacterium and *Blautia*, along with elevated levels of *Enterococcus*. Interestingly, these bacterial variations were less prominent when comparing the 3-month good prognosis group to the control group ($P > 0.05$) (Fig. 3C). Furthermore, the relative abundance of *Dialister* in the 3-month poor prognosis group was notably lower than that in the 3-month good prognosis group. While investigating relevant species belonging to these diverse genera, we observed a significant decrease in the relative abundance of *Faecalibacterium prausnitzii*, accompanied by a significant increase in *Enterococcus faecium* in AIS patients compared to controls, with the difference being more pronounced in the 3-month poor prognosis group (Fig. 3D and E).

3.5. Correlation of gut microbiota with stroke severity, prognosis and systemic inflammatory markers

We conducted correlation analysis between the top 10 bacteria with relatively high abundance and stroke severity, 3-month prognosis and systemic inflammatory markers at the genus and species levels in all AIS patients. The results indicated that both *Faecalibacterium* and *Faecalibacterium prausnitzii* were negatively correlated with NIHSS score at admission ($r = -0.48$, -0.48, $P <$

0.01) and 3-month mRS score ($r = -0.48$, -0.44, $P < 0.01$). In addition, *Faecalibacterium* and *Faecalibacterium prausnitzii* displayed negative correlations with the pro-inflammatory markers like NLR, MLR, and IL-6 (all $P < 0.01$), and a positive correlation with the anti-inflammatory marker PNR (all $P < 0.01$). Conversely, *Enterococcus* and *Enterococcus faecium* did not show significant correlations with NIHSS score at admission, 3-month mRS score and systemic inflammatory markers ($P > 0.05$) (Fig. 4A and B).

4. Discussion

In this study, we used 16 S rDNA sequencing to compare the distinct attributes of gut microbiota between acute ischemic stroke (AIS) patients and controls. Furthermore, we conducted a comprehensive analysis focusing on disparities within patients with minor stroke, non-minor stroke, as well as those exhibiting either a favorable 3-month prognosis or an unfavorable prognosis, in comparison with control subjects. The outcomes of our investigation revealed pronounced deficiencies in *Lachnospiraceae*, *Ruminococcaceae*, *Faecalibacterium*, and *Faecalibacterium prausnitzii* in AIS patients. Conversely, opportunistic pathogens, particularly *Enterococcus*, were found to be enriched, especially within the subgroup of patients afflicted with non-minor stroke and those with a 3-month poor prognosis. Remarkably, *Faecalibacterium* and *Faecalibacterium prausnitzii* emerged as bacteria exhibiting the most substantial distinctions both at the genus and species levels. These entities exhibited a compelling negative correlation with stroke severity and the levels of inflammatory markers, underscoring their potential significance in AIS pathogenesis.

We found no significant difference in α-diversity between AIS patients and controls, indicating that the overall richness and evenness of gut microbiota were comparable. These findings contrast with previous studies reporting lower α-diversity of gut microbiota in AIS patients, which has been attributed to the intestinal barrier destruction after AIS, resulting in the “leakage" of intestinal bacteria [17,18]. Interestingly, conflicting reports on the impact of AIS on gut microbiota, including Yin et al.’s findings of increased α-diversity in AIS patients [19] and Li et al.’s results of no difference [16], underscore the complexity of this relationship. The interplay between AIS and gut microbiota appears to be multifaceted, warranting further investigation into the factors contributing to the variability in α-diversity changes.

The β-diversity between AIS patients and controls was significantly different, indicating distinct compositions of gut microbiota in the two groups. Consistent with previous research, Yin et al. reported increased abundance of opportunistic pathogenic bacteria such as *Enterobacter*, *Megasphaera*, *Oscillibacter* and *Desulfovibrio* in patients with large atherosclerosis type ischemic stroke and transient ischemic attack than healthy controls, alongside decreased levels of symbiotic and beneficial bacteria such as *Prevotella* and *Faecalibacterium* [19]. Furthermore, other studies have documented an increased abundance of *Lactobacillus ruminis* and *Akkermansia* in AIS patients [16,20]. These findings collectively demonstrate significant alterations in the composition of gut microbiota in AIS patients, indicating that the impact of AIS extends beyond brain damage and involves perturbations in the gastrointestinal system, leading to dysbiosis. Nonetheless, the varying findings among different studies emphasize the intricate nature of the interplay between AIS and gut microbiota. Hence, subgroup analyses are warranted to decipher the individualized responses of gut microbiota to AIS, considering the complexity of this interaction.

Notably, Yin et al. found that patients with moderate-to-severe stroke (NIHSS>4) exhibited lower levels of *Bacteroides* and higher levels of *Escherichia/Shigella* compared to those with mild stroke (NIHSS ≤4) [19]. Another study described a significant increase in the abundance of *Roseburia* in minor stroke (NIHSS ≤3) group and higher *Enterococcus* levels in non-minor stroke group (NIHSS>3), though the α-diversity of gut microbiota showed no difference between the minor and non-minor stroke groups [21]. Furthermore, Sun et al. found that the α-diversity of the 3-month poor prognosis group was lower than that of the 3-month good prognosis group. The 3-month poor prognosis group displayed an enrichment of pathogenic bacteria such as *Enterococcus* and a reduction of beneficial bacteria such as *Ruminococcaceae* and *Faecalibacterium* [8]. However, the preceding study did not encompass a comprehensive comparison and analysis of diverse subgroups in relation to healthy controls. Additionally, it omitted the identification and

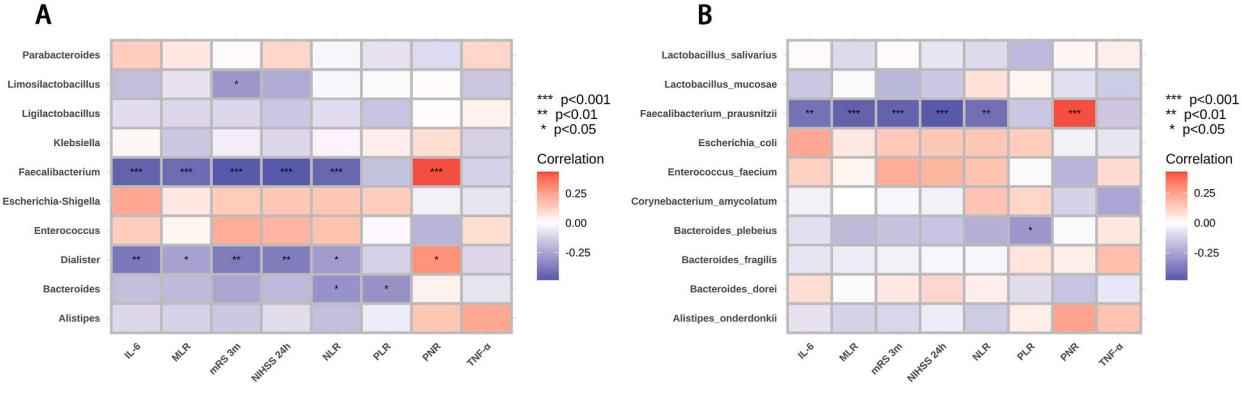

Fig. 4. Correlation Analysis Between the Top 10 Relative Abundance Genera (A) and Species (B) with NIHSS Score at Admission, 3-Month mRS Score, and Inflammatory Markers. IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-α; PLR, Platelet-to-Lymphocyte Ratio; NLR, Neutrophil-to-Lymphocyte Ratio; MLR, Monocyte-to-Lymphocyte Ratio; PNR, Platelet-to-Neutrophil Ratio; NIHSS, National Institute of Health Stroke Scale; mRS, Modified Rankin Scale.

examination of distinct bacterial species variations. Furthermore, the investigation did not delve into the correlation existing between imbalances within gut microbiota and the systemic inflammatory response.

We compared different subgroups of the AIS patients with the controls and found no statistical difference in the α-diversity. However, the β-diversity analysis suggested distinct composition and abundance of gut microbiota among the groups. At the levels of genus and species, *Faecalibacterium* and *Faecalibacterium prausnitzii* were significantly reduced in the 3-month poor prognosis group compared to that in the 3-month good prognosis group and controls. An important finding of the study is the relationship between the abundance of *Faecalibacterium* and *Faecalibacterium prausnitzii* and stroke severity, 3-month prognosis and systemic inflammation. We observed a negative correlation between the relative abundance of *Faecalibacterium* and *Faecalibacterium prausnitzii* and both the NIHSS score at admission and the 3-month mRS score. This suggests that as the stroke severity increases, the relative abundance of *Faecalibacterium* and *Faecalibacterium prausnitzii* decreases, and the 3-month prognosis tends to worsen. Additionally, the immune inflammatory response in the body, as reflected by NLR, MLR, and IL-6, showed an inverse relationship with the relative abundance of *Faecalibacterium* and *Faecalibacterium prausnitzii*. In contrast, a positive correlation was observed between the relative abundance of these bacteria and PNR, indicating that reduced *Faecalibacterium* and *Faecalibacterium prausnitzii* levels are associated with a more pronounced inflammatory response in the body. Therefore, our results suggest a negative correlation between *Faecalibacterium* and *Faecalibacterium prausnitzii* and both stroke severity and a poor 3-month prognosis. We hypothesize that the absence of *Faecalibacterium* and *Faecalibacterium prausnitzii* may have a detrimental effect on the prognosis of AIS patients and is closely related to the inflammatory response. However, it is necessary to expand the sample size and exclude confounding factors to further confirm this hypothesis.

The species *Faecalibacterium prausnitzii* emerged as the bacterium exhibiting the most significant differences among the groups studied here. It belongs to the genus *Faecalibacterium* and family *Ruminococcaceae* within the order *Clostridiales* and the phylum *Firmicutes*. It is one of the important butyrate-producing bacteria and represents one of the most abundant and important anti-inflammatory commensal bacteria in human intestine [22]. Notably, butyrate, produced by these bacteria, plays a pivotal role in post-stroke recovery by regulating immune cells, including lymphocytes and microglia, both systemically and within the brain [23]. The attention garnered by butyrate-producing bacteria in recent years stems from their significant impact on promoting intestinal homeostasis and influencing stroke progression through their role in maintaining the integrity of the intestinal barrier and exerting immune regulatory and anti-inflammatory effects [24,25]. We observed a significant negative correlation between *Faecalibacterium* and *Faecalibacterium prausnitzii* and pro-inflammatory factors, such as IL-6, NLR, and MLR. This finding supports the notion that *Faecalibacterium* and *Faecalibacterium prausnitzii* may mediate immune inflammatory responses after AIS, a hypothesis also substantiated by recent animal experiments. In a notable study conducted by Lee et al. [26], transplantation of butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, was found to ameliorate neurological deficits and inflammatory reactions after stroke in mouse model of middle cerebral artery occlusion. This intervention resulted in increased content of butyrate in the intestine, brain, and plasma. Moreover, the regulatory T lymphocytes (Treg) with protective effects in the intestine and brain increased, while the pro-inflammatory cytokine IL-17^{+}γδT cells in the brain decreased. These findings suggest that butyrate-producing bacteria affect the immune inflammatory response and promote stroke recovery by regulating the balance of Treg/IL-17^{+}γδT in the brain. In light of these exciting results, investigations into the neuroprotective potential of butyrate-producing bacteria hold promise for identifying new therapeutic targets for AIS patients. Interventions such as transplantation of butyrate-producing bacteria and supplementation of butyrate may prove effective in alleviating post-stroke inflammation and improving stroke outcome.

Our study has several limitations that should be acknowledged. First, it was conducted at a single center, which may restrict the generalizability of the findings to a broader population. Second, despite our efforts to assemble a relatively homogeneous research population, consisting of local Han individuals with stable eating habits, and excluding participants with recent use of antibiotics, probiotics, steroids, and immunosuppressive drugs, it is challenging to completely eliminate the potential influence of dietary factors or other medications on gut microbiota. To address these limitations and to achieve a more profound understanding of the relationship between gut microbiota and AIS, it is essential to conduct more comprehensive and well-designed longitudinal studies in the future.

5. Conclusions

In conclusion, we reveals that *Faecalibacterium prausnitzii* is negatively associated with stroke severity, impaired prognosis, and pro-inflammatory markers, highlighting its potential application in AIS treatments.

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None.

Ethical approval

This study and data collection received approval from the Ethics Committee of the First Affiliated Hospital of Dalian Medical University (Approval No.: PJ-KS-KY-2021-120). All participants provided written informed consent.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yayin Luo: Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Geng Chang:** Software, Methodology, Investigation, Formal analysis, Data curation. **Guangxiang Yu:** Software, Methodology, Investigation, Formal analysis, Data curation. **Yanan Lin:** Software, Methodology, Investigation, Formal analysis, Data curation. **Qiuyi Zhang:** Software, Methodology, Investigation, Formal analysis, Data curation. **Zhe Wang:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Jie Han:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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