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Bifidobacterium breve CCFM1179 Modulates Gut Microbiota and Improves Mild Cognitive Impairment: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT: Mild cognitive impairment (MCI) represents a critical transitional phase in Alzheimer's disease progression and constitutes a vital intervention window for delaying disease onset. With accelerating population aging, the demand for MCI prevention and management has become increasingly urgent, rendering the exploration of safe and effective early interventions of substantial clinical and societal importance. Building upon prior animal studies demonstrating the cognitive-enhancing potential of *Bifidobacterium breve* CCFM1179, this randomized, double-blind, placebo-controlled trial evaluated its clinical efficacy through a 12-week intervention in MCI subjects. Concurrently, alterations in gut microbiota, serum, and fecal metabolites were examined to elucidate potential mechanisms. Results demonstrated that compared with placebo, CCFM1179 significantly improved Montreal Cognitive Assessment scores, particularly in visuospatial/executive and naming domains, accompanied by elevated serum brain-derived neurotrophic factor (BDNF) levels. Gut microbiota α-diversity remained stable, yet β-diversity exhibited marked separation. Post-intervention abundance increased in genera including *Prevotella*, *Coprococcus*, and *Barnesiella*, whereas *Megamona*s, a genus associated with metabolic abnormalities, decreased. Metabolomic analyses revealed substantial alterations in both gut and serum metabolites, with differences predominantly concentrated in amino acid and tryptophan metabolic pathways. Notably, elevated levels of indole-3-propionic acid (IPA) and indole-3-carboxaldehyde (IAld) correlated positively with BDNF, while quinolinic acid decreased. These findings suggest that *Bifidobacterium breve* may enhance cognitive function by promoting protective indole-derived metabolic pathways and modulating gut-brain axis metabolic signaling. This study provides clinical evidence supporting microbiome-based interventions for MCI and highlights the potentially central role of tryptophan metabolism in cognitive improvement.

Keywords: Mild cognitive impairment; *Bifidobacterium breve;* Gut microbiota; Amino acids; Indole

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1. Introduction

With accelerating population aging, mild cognitive impairment (MCI) has progressively emerged as a global public health concern. MCI is widely recognized as an early stage of Alzheimer's disease (AD), wherein individuals maintain largely intact activities of daily living yet exhibit mild cognitive decline, including deficits in memory, executive function, and language[1]. Notably, approximately 10–15% of individuals with MCI progress to AD annually, whereas others may remain stable or even revert to normal functioning[2]. Consequently, early identification and intervention constitute a critical window for delaying cognitive decline and reducing AD progression risk.

The gut microbiota (GM) constitutes a complex, dynamic, and diverse ecosystem. Beyond altering its structure and function in response to disease states, dysbiosis may precipitate the onset and progression of certain conditions[3]. Research indicates significant differences in microbial composition between AD patients and cognitively normal controls. Similar GM alterations are observed in MCI, including *Bacteroides* reduction and *Escherichia* enrichment[4–6]. Mounting evidence suggests that the GM influences neurodegenerative diseases, including AD and Parkinson's disease, by modulating neural, endocrine, and immune pathways via the gut-brain axis[7]. The GM can affect amyloid deposition in the brain through multiple pathways, inducing pro-inflammatory factor release that impacts host inflammatory status. It also produces bioactive metabolites, including short-chain fatty acids and neurotransmitters, which directly or indirectly influence cognitive function by regulating immune cells (e.g., microglia) and vagal nerve signaling[8–10]. Notably, amino acid (AA) metabolism is emerging as a potentially significant contributor to AD and MCI pathological processes. For instance, the arginine-ornithine cycle influences cognition by regulating neuronal proliferation and cell death[11]. Tryptophan and its metabolites exert effects on cognitive function through both neurotoxic and neuroprotective mechanisms[12]. Consequently, modulating the GM and maintaining gut homeostasis may serve as potentially effective strategies for managing MCI.

Probiotic intervention, as an effective GM modulation approach, has demonstrated cognitive improvement in animal studies. For example, the SLAB51 probiotic complex, containing live strains including *Streptococcus thermophilus*, *Bifidobacterium lactis*, and *Lactobacillus paracasei*, improves impaired glucose metabolism in 3xTg-AD mice by restoring brain expression of key glucose transporters and insulin-like growth factor receptor β, thereby enhancing cognitive performance[13]. Preparations containing *Lactobacillus* effectively reduce amyloid accumulation in AD models, decrease mRNA levels of IL-6, IL-17A, and IL-22, increase BDNF mRNA expression, suppress neuroinflammation, and ultimately improve cognitive outcomes[14]. Clinical studies likewise demonstrate that probiotic supplementation can enhance cognitive indicators. In MCI subjects, *Bifidobacterium breve* A1 supplementation exhibited an 11.3-point improvement in RBANS scores compared to placebo, particularly in immediate memory, visuospatial/structural memory, and delayed memory domains[15]. Similarly, intervention with *Lactobacillus plantarum* C29-derived preparations altered the GM by increasing *lactobacilli* abundance, improved attention and verbal memory, and modestly elevated serum brain-derived neurotrophic factor

(BDNF) expression[16]. Despite these advances, most current clinical research primarily examines associations between the GM and cognitive phenotypes. Studies identifying key effectors responsible for cognitive improvement and elucidating underlying mechanisms remain limited. Further investigation from a metabolic perspective is needed to clarify interactions among microbiota, metabolism, and cognition.

Previous studies have showed that CCFM1179 may improve cognitive function in AD mice by promoting neurotransmitter (BDNF) production and modulating GM composition[17]. Building upon this foundation, the present study aims to evaluate the effects of *B. breve* CCFM1179 intervention on cognitive function in individuals with MCI. By integrating GM analysis with serum and fecal non-targeted and targeted AA metabolomics, we systematically explore potential associations among microbiota, metabolism, and cognitive abilities. This research seeks to provide theoretical basis and empirical evidence to support probiotic interventions for MCI.

2. Materials and Methods

2.1. Patient Recruitment and Ethical Statements

This study is a randomized, double-blind, placebo-controlled trial designed to evaluate the efficacy of intervention in patients with MCI. Inclusion criteria were based on the MCI diagnostic criteria established by Petersen et al. and Albert et al.[18,19], and the specific criteria include the following: 1) adults aged 60-80 years; 2) demonstrating mild memory impairment without substantial impact on activities of daily living; 3) scores between 24 and 28 on the Mini-Mental State Examination (MMSE) with no marked impairment in cognitive domains other than memory; 4) clinical presentation consistent with a diagnosis of MCI and meeting the cognitive characteristics of preclinical AD. Exclusion criteria are as follows: 1) a prior diagnosis of any type of neurodegenerative disease, such as Parkinson's disease or frontotemporal dementia; 2) presence of severe systemic diseases, including but not limited to heart disease, hepatic or renal failure, or major depressive disorder, which may impair cognitive function; 3) history of stroke, traumatic brain injury, or brain surgery; 4) pregnant or lactating women, and individuals with known allergies to the investigational medicinal product or bacterial strain.

This study has received ethical approval from the Medical Ethics Committee of Yixing People's Hospital, Jiangsu Province, China (Ethical Review Number: Ethics Review 2024 Department 101-01). International ethical approval has been granted by the China Clinical Trial Registry (Registration Number: ChiCTR2500097969). All participants signed written informed consent forms prior to the study, and the research procedures strictly adhered to ethical review regulations to ensure the safety and privacy of research subjects were protected. Further details can be accessed at the following link: https://www.chictr.org.cn/showproj.html?proj=263002.

2.2. Sample size calculation and study design

Sample size calculations were performed using G*Power software (Figure S1), based on a published study, with the primary outcome being the estimated mean difference in Montreal Cognitive Assessment

(MoCA) scores. The calculations indicated that 20 subjects per group were required[20]. Considering an anticipated 25% dropout rate (including loss to follow-up or missing data), the final plan was to recruit 50 subjects; using an SPSS computer-generated randomization sequence, they were randomly allocated to two groups: the CCFM1179 group (n = 25) and the placebo group (n = 25). The allocation sequence was prepared by an independent researcher, and group assignments were concealed using coded labels.

This trial was conducted at Yixing People's Hospital in Jiangsu Province, China, from February to August 2025. The study flow diagram is shown in Figure 1. All subjects were instructed to take the *B. breve* CCFM1179 product with lukewarm water (≤ 37°C) daily, within 30 to 60 minutes after breakfast, over a 12-week trial period. Concurrently, the research team conducted regular follow-ups throughout the study to investigate and document subjects' dietary habits, lifestyle practices, and any adverse events. Furthermore, to ensure the efficacy and scientific rigor of the intervention, the trial duration and probiotic dosage were determined based on prior relevant studies[21].

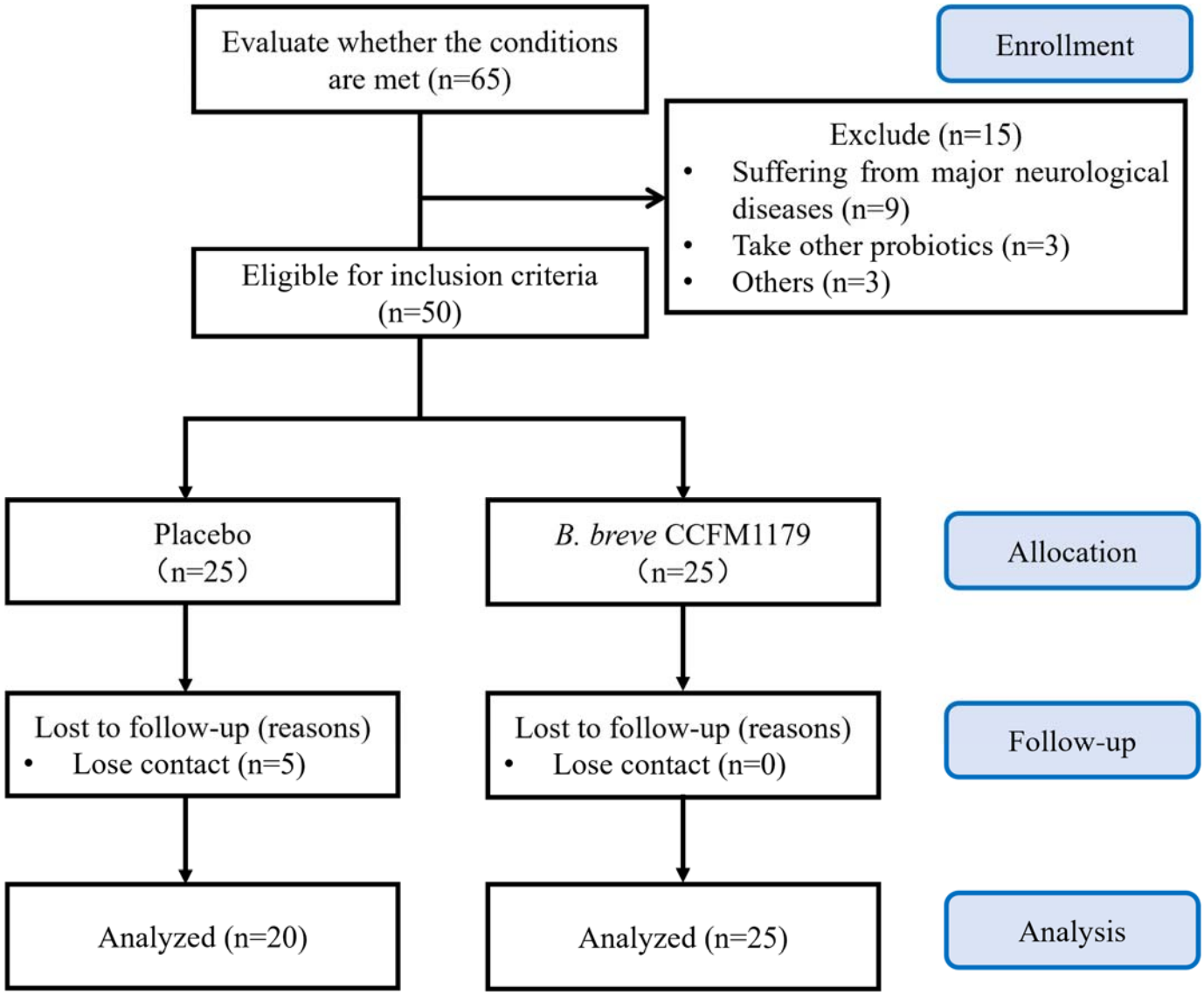

Figure 1. Flowchart of experimental research.

2.3. Cognitive, dietary intake assessment and study products

This trial utilized the MMSE and MoCA scales to evaluate subjects' cognitive function. The MMSE encompasses domains including orientation, attention, recall, language, and visual construction, with a maximum score of 30 points; the MoCA covers memory, attention, language, and executive function, also with a maximum score of 30 points. All subjects underwent MMSE and MoCA assessments prior to the trial and at its conclusion (12 weeks). These evaluations were conducted by trained, blinded investigators to ensure scoring accuracy and consistency.

Dietary intake was assessed at baseline using a semi-quantitative food frequency questionnaire (FFQ) that captured habitual intake over the preceding month. Nutrient intakes were calculated by multiplying consumption frequency by portion size and nutrient composition derived from food composition tables.

Additionally, the *B. breve* CCFM1179 used in this trial was provided by Jiangnan University. Subjects receiving the *B. breve* CCFM1179 formulation administered the product daily, with each gram of the preparation containing 5×10^9 colony-forming units of *B. breve* CCFM1179. The probiotic included maltodextrin and other protective components. The placebo formulation comprised an equivalent amount of maltodextrin without *B. breve* CCFM1179. Both formulations were manufactured by the Yangzhou Institute of Food Biotechnology in Jiangsu Province. They were packaged in powder form and subdivided into strip-type sachets (2 grams per sachet). The probiotic and placebo products were packaged identically in appearance, weight, and labeling. Both formulations were designed to be indistinguishable in taste, smell, and appearance to ensure effective blinding. All formulation products complied with the GB/T 29602 standard and were stored at temperatures below 4°C throughout the trial period.

2.4. Sample collection and clinical laboratory testing

All subjects with MCI underwent comprehensive health examinations at Yixing People's Hospital prior to intervention. Fasting blood samples and stool specimens were collected from participants on the first day and final day of the trial, respectively. Serum was separated via centrifugation (3500 rpm, 10 min) for clinical laboratory testing. Fecal samples were collected using sterile fecal collection tubes and stored at −80°C for subsequent multi-parameter analysis. Serum BDNF concentrations were determined using an ELISA kit (Enzyme-linked Biotechnology, Shanghai, China) according to the manufacturer's instructions. Serum biochemical parameters were measured using a Mindray biochemical analyzer (Mindray, Shenzhen, China), including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), and high-sensitivity C-reactive protein (hs-CRP).

2.5. Fecal microbiome analysis and functional prediction

DNA extraction from fecal samples was performed using the FastDNA Spin Kit (MP Biomedicals, Irvine, CA, USA) according to the manufacturer's protocol. Following the methodology described by Fang et al.[22], the 16S rRNA gene was amplified via polymerase chain reaction (PCR) using universal primers 341F/806R (V3-V4 region). The specific reaction system and conditions are detailed in Table S1. PCR products were purified and specific bands excised using the DNA Gel/PCR Purification Miniprep Kit

(Invitrogen Ambion, USA) following the manufacturer's instructions. High-throughput sequencing was performed on the Illumina MiSeq platform (MiSeq PE300, Illumina, San Diego, CA, USA). The raw data obtained underwent quality control, sequence denoising, assembly, and filtering using the QIIME 2 software platform. Microbial community α-diversity was assessed using Chao1, Shannon, and Simpson indices, and Observed_Species, while β-diversity was calculated based on Bray–Curtis distances and tested for significance via permutation-based multivariate analysis of variance (PERMANOVA). Differential microbial communities were screened using the Linear Discriminant Analysis Effect Size (LEfSe) method on the Galaxy platform (http://huttenhower.org/galaxy) and visualized via a phylogenetic tree. Principal component analysis (PCA) was performed based on genus-level relative abundance data to explore differences in community structure among samples. Additionally, the PICRUSt2 software was utilized to predict the potential functional characteristics of the gut microbiota.

2.6. Non-targeted metabolomics analysis of feces and serum and enrichment pathway exploration

A 100 μL serum sample was mixed with 400 μL of pre-chilled methanol-acetonitrile extraction buffer (1:1, v/v), vortexed for 30 seconds, and incubated at −20°C for 1 hour to precipitate proteins. Following centrifugation at 12,000 rpm and 4°C for 15 minutes. Collect the supernatant and dry it under vacuum. Dissolve 20 mg of lyophilized fecal sample in 200 μL of ultrapure water. Mix with 800 μL of pre-chilled methanol-acetonitrile extraction solvent (1:1, v/v). Add an appropriate amount of grinding beads for tissue disruption (65 Hz, 40 seconds), repeat this process three times. Incubate at -20°C for 1 hour to precipitate proteins. Centrifuge at 12000 rpm and 4°C for 15 minutes. The supernatant was collected and vacuum-dried. Subsequently, samples from both matrices were redissolved in acetonitrile-water (1:1, v/v) and centrifuged again under identical conditions before detection. The solvent system, gradient, mass spectrometry conditions, and statistical analysis for detection remained consistent with previous studies[23].

Simultaneously, equal volumes were withdrawn from test samples to prepare quality control (QC) samples. Periodic injections during analysis ensured detection system stability and signal intensity consistency.

2.7 Targeted metabolomics analysis of feces and serum

AA concentrations in samples were quantified using the Thermo Fisher Scientific Ultimate U-3000 UPLC system coupled with a high-resolution Q-Exactive mass spectrometer (Thermo Fisher Scientific). A 50 mg lyophilized fecal sample was dissolved in 900 μL of methanol-acetonitrile-water extraction solvent (2:2:1, v/v/v) and vortexed for 30 seconds. Grinding beads were added, and tissue was homogenized (65 Hz, 40 seconds) three times. After settling, the mixture was centrifuged at 15,000 rpm and 4°C for 15 minutes. The supernatant was collected and concentrated by vacuum centrifugation. Similarly, 100 μL of serum sample was mixed with 400 μL of pre-chilled methanol, incubated at -20°C for 1 hour to precipitate proteins, and centrifuged at 4000 rpm and 4°C for 20 minutes. The supernatant was transferred to sample vials for UHPLC Q-Exactive-MS analysis. Testing conditions and analytical methods followed previously reported procedures[24].

Quantification of tryptophan and its metabolites employed the same platform and associated parameters as the targeted AA analysis. The sample preparation workflow remained largely consistent, with minor adjustments to extraction solvent and protein precipitation steps. Specifically, fecal samples were mixed with 900 μL of methanol-water solution (1:1, v/v), while serum samples were mixed with 800 μL of methanol for precipitation. Subsequent centrifugation, concentration, redissolution, filtration, and quantification procedures were identical to those described above. Testing conditions and analytical methods followed previously reported procedures[25].

2.8 Outcomes

The primary outcome of this study was the change in MoCA score after the 12-week intervention. Secondary outcomes included changes in MMSE scores, biochemical indicators, BDNF levels, gut microbiota composition, and serum and fecal metabolomic profiles.

2.9 Statistical analysis

Statistical analysis was performed using SPSS 26 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 10.1.2 (GraphPad Software Co., Ltd., CA, USA). All data are expressed as mean ± standard deviation. Data normality was assessed using the Shapiro-Wilk test. For normally distributed data ($P > 0.05$), independent-samples t-tests or paired t-tests were used for between-group and within-group comparisons, respectively. For non-normally distributed data, the Wilcoxon signed-rank test was employed. For analyses involving multiple comparisons, p-values were adjusted using the Benjamini-Hochberg method to control the false discovery rate (FDR), and the resulting q-values were used to determine statistical significance. Statistical significance is defined as *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), and ****($P < 0.0001$).

3. Results

3.1 No significant differences in baseline characteristics among subjects

Based on inclusion and exclusion criteria, this study initially enrolled 50 subjects. Subsequently, five subjects were lost to follow-up due to reasons including loss of contact. No adverse events were reported in either group during the 12-week intervention, and the probiotic intervention was well tolerated (Table S2). Ultimately, the placebo group comprised 20 subjects, while the CCFM1179 group comprised 25 subjects. As shown in Table 1, the mean age of participants in the placebo group was 71.25 ± 9.31 years, with a body mass index (BMI) of 22.73 ± 2.20. The CCFM1179 group had an average age of 72.16 ± 11.38 years and a BMI of 23.61 ± 5.79. As shown in Table 2, at baseline, no significant differences existed between the two groups in cognitive status or physiological indicators ($P > 0.05$). In addition, no significant differences were observed between the two groups in dietary intakes at baseline ($P > 0.05$, Table S3).

Table 1. Baseline characteristics of participants.

	Placebo (n=20)	CCFM1179 (n=25)
Age	71.25±9.31	72.16±11.38
Sex ratio (male/female)	8/12	13/12

BMI (Kg/m^2)	22.73±2.20	23.61±5.79
Hypertension, n (%)	6 (30%)	10 (40%)
Diabetes, n (%)	5 (25%)	6 (24%)
Hyperlipidemia, n (%)	7 (35%)	6 (24%)
Family history of AD, n (%)	3 (15%)	3 (12%)
Primary school and below, n (%)	5 (25%)	6 (24%)
Junior middle school, n (%)	8 (40%)	9 (36%)
Senior school or technical secondary school, n (%)	6 (30%)	7 (28%)
College and above, n (%)	1 (5%)	3 (12%)

Table 2. Cognitive and physiological indicators of participants.

	Placebo	CCFM1179	P-Value
TG (mmol/L)	1.95 ± 1.33	1.30 ± 0.54	0.174^{NS}
TC (mmol/L)	4.74 ± 1.14	4.64 ± 1.15	0.753 ^{NS}
HDL (mmol/L)	1.19 ± 0.32	1.22 ± 0.28	0.680 ^{NS}
LDL (mmol/L)	2.62 ± 0.73	2.64 ± 0.93	0.928 ^{NS}
AI	3.08 ± 0.78	2.90 ± 0.87	0.454 ^{NS}
FPG (mmol/L)	5.62 ± 1.62	5.80 ± 1.00	0.197 ^{NS}
hs-CRP (mg/L)	0.96 ± 1.11	1.61 ± 2.39	0.193 ^{NS}
MMSE	23.05 ± 4.55	23.12 ± 5.05	0.878 ^{NS}
MoCA	19.70 ± 6.03	18.92 ± 5.35	0.492 ^{NS}

NS, not significant; N, number of participants; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AI, atherogenic index; FPG, fasting plasma glucose; hs-CRP, high-sensitivity C-reactive protein; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

3.2 B. breve CCFM1179 intervention improves cognitive status in mild cognitive impairment subjects

This study employed the MMSE and MoCA scales to assess cognitive function, with evaluations conducted at baseline and at the end of the study period (Figure 2). Analysis of the MMSE scale revealed that after 12 weeks of intervention, the placebo group's score increased from 23.05 ± 4.55 to 24.20 ± 5.24, while the CCFM1179 group's score rose from 23.12 ± 5.05 to 24.16 ± 5.77. Both groups demonstrated overall improvement, with significant within-group differences ($P < 0.05$, Figure 2A). MoCA scale analysis revealed that the placebo group's scores increased from 19.70 ± 6.03 to 19.80 ± 5.76 after intervention, but no significant difference was observed ($P > 0.05$). In contrast, CCFM1179 intervention resulted in a significant increase in scores from 18.92 ± 5.35 to 21.16 ± 6.22 ($P < 0.001$, Figure 2B). Notably, within the MoCA scoring criteria, CCFM1179 intervention resulted in significant improvements in visuospatial and executive function domains, with scores rising from 2.36 ± 1.32 to 3.24 ± 1.36 ($P < 0.001$, Figure 2C). Similarly, naming ability scores increased from 2.44 ± 0.65 to 2.76 ± 0.44 ($P < 0.05$, Figure 2D). Regarding delayed recall, although the CCFM1179 group did not show a significant within-group change, a significant improvement was observed compared with the placebo group ($P < 0.05$, Figure 2E), indicating a modest positive effect. However, no significant differences were observed in attention, language, abstraction, or orientation ($P > 0.05$, Figure 2F–I).

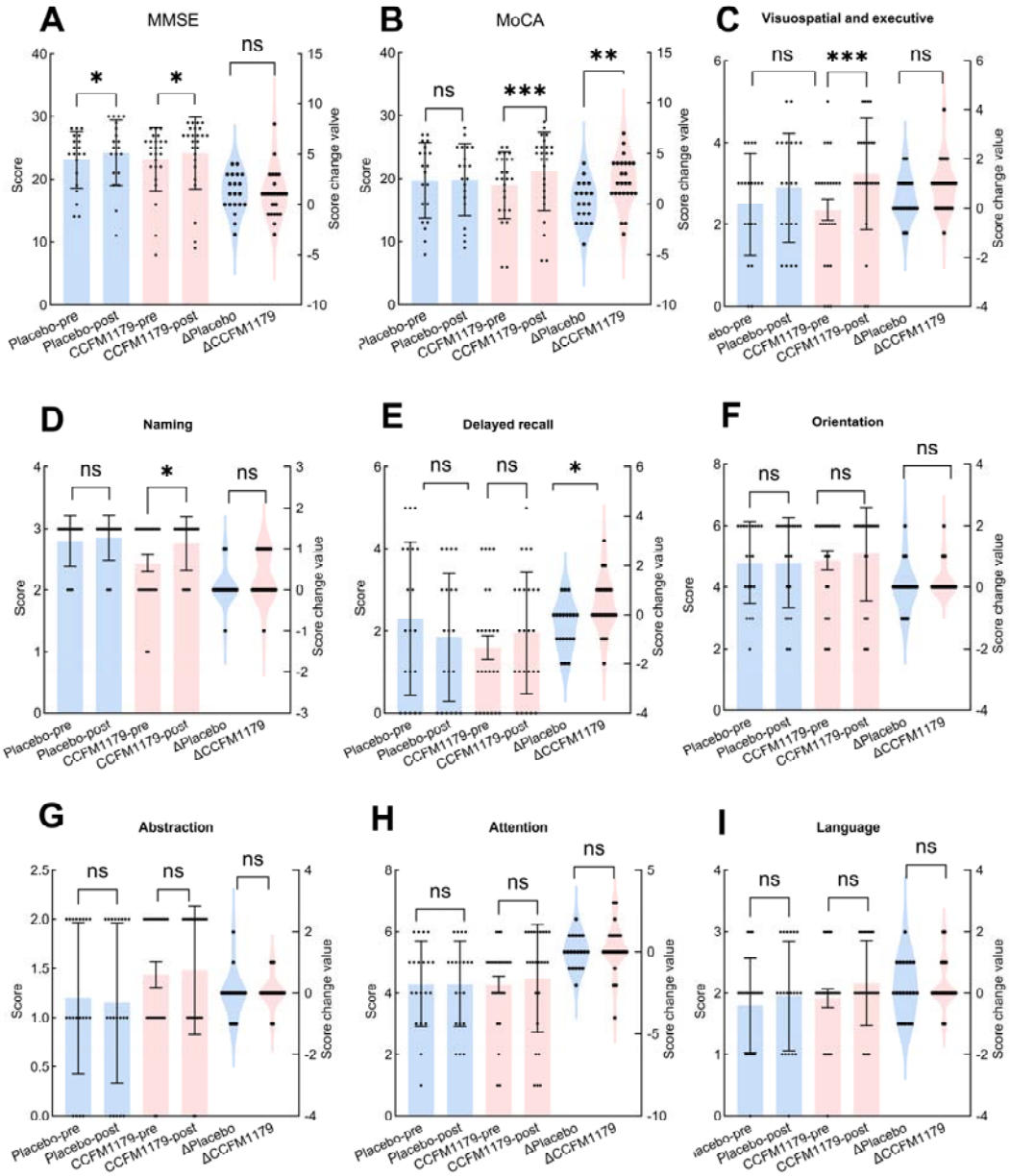

Figure 2. Effect of *B. breve* CCFM1179 on cognitive scores of participants (A–B) and influence on MoCA scoring criteria. (C) Visuospatial and executive; (D) Naming; (E) Delayed recall; (F) Orientation; (G) Abstraction; (H) Attention; (I) Language. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and ns, not significant.

3.3 B. breve CCFM1179 intervention elevates brain-derived neurotrophic factor levels

After a 12-week intervention, multiple metabolic and neuro-related indicators were compared between the placebo and CCFM1179 groups (Figure 3). No significant differences were observed in lipid parameters, including TG, TC, LDL-C, and the atherogenic index ($P > 0.05$, Figure 3A-C, E). However, HDL-C levels significantly decreased in the placebo group post-intervention ($P < 0.001$, Figure 3D), whereas the CCFM1179 group exhibited a downward trend without reaching statistical significance, suggesting that probiotic intervention may exert a stabilizing effect on HDL-C maintenance. Regarding metabolic and inflammatory markers, no significant pre-to-post changes in FPG or hs-CRP were observed within or between groups ($P > 0.05$, Figure 3F-G). Notably, BDNF levels increased to varying degrees in both groups. The CCFM1179 group demonstrated significant elevation after 12 weeks of intervention ($P < 0.05$), whereas

the placebo group exhibited no significant change ($P > 0.05$, Figure 3H), suggesting that CCFM1179 may potentially promote neuroplasticity and cognitive function.

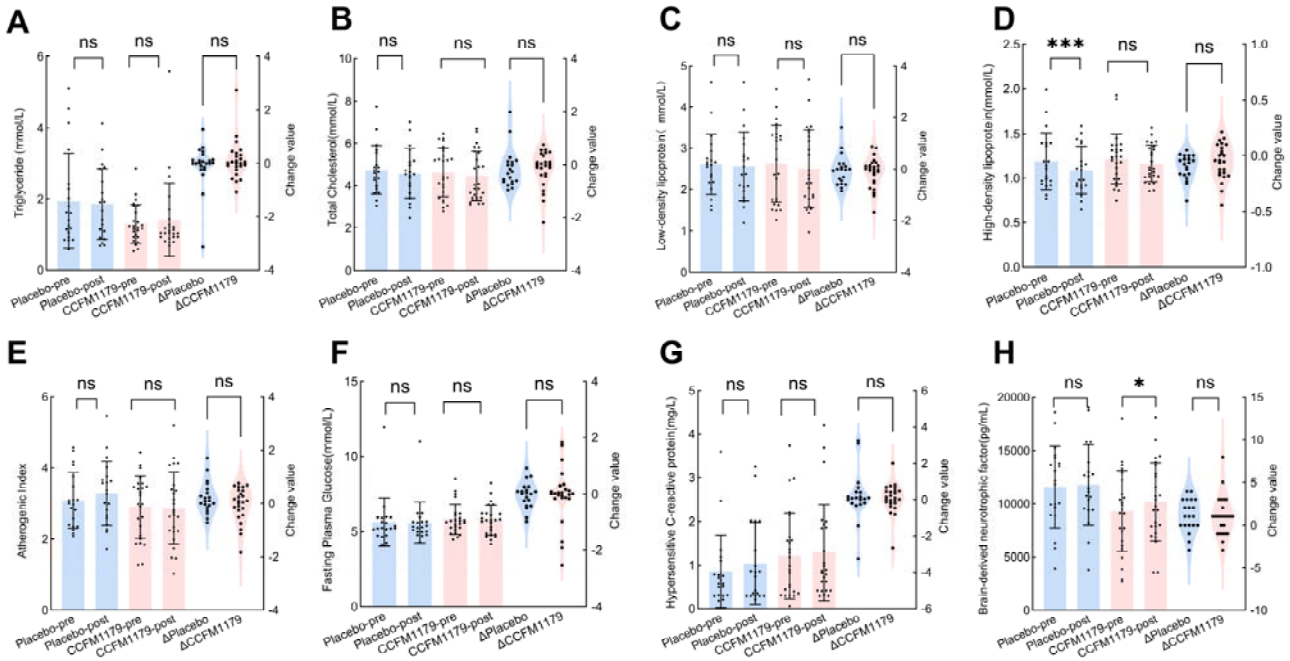

Figure 3. Effects of *B. breve* CCFM1179 on blood indicators in participants. (A) Triglyceride; (B) Total cholesterol; (C) Low-density lipoprotein; (D) High-density lipoprotein; (E) Atherogenic index; (F) Fasting plasma glucose; (G) High-sensitivity C-reactive protein; (H) Brain-derived neurotrophic factor. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ns, not significant.

3.4. B. breve CCFM1179 intervention alters gut microbiota composition and promotes enrichment of beneficial bacterial genera

Through 16S rRNA gene sequencing, this study evaluated changes in GM diversity among participants before and after intervention. Analysis of α-diversity revealed no significant differences in Chao1, Shannon, and Simpson indices, or observed species, between pre- and post-intervention samples ($P > 0.05$, Figure 4A-D), indicating that CCFM1179 intervention did not significantly affect microbial abundance or within-group diversity. β-diversity analysis further revealed that following placebo and CCFM1179 interventions (Figure 4E-F), the principal coordinate analysis (PCoA) plots based on Bray-Curtis distances showed distinct spatial separation compared to pre-intervention levels. PERMANOVA analysis revealed statistically significant intergroup differences ($P < 0.05$), indicating that CCFM1179 intervention substantially alters GM structure in MCI individuals.

LEfSe analysis, an algorithm specifically designed for high-dimensional biomarker discovery and interpretation, identifies and characterizes differential features across multiple biological conditions. This study performed LEfSe analysis on post-intervention samples from the placebo group and the CCFM1179 group (Figure 4G), revealing significant differences in GM composition between the placebo and CCFM1179 groups after treatment ($P < 0.05$). At the genus level, 12 differentially abundant genera were identified, with 7 enriched following placebo intervention and 5 enriched following CCFM1179 intervention (Figure 4H). Compared with the placebo group, the relative abundances of *Prevotella 2*, *Prevotella 9*,

Coprococcus 3, *Barnesiella*, and *Streptococcus* significantly increased after CCFM1179 intervention, whereas *Tyzzerella 4*, *Lachnoclostridium*, *Flavonifractor*, *Megamonas*, and *Sulfitobacter* were significantly decreased (Figure 4I).

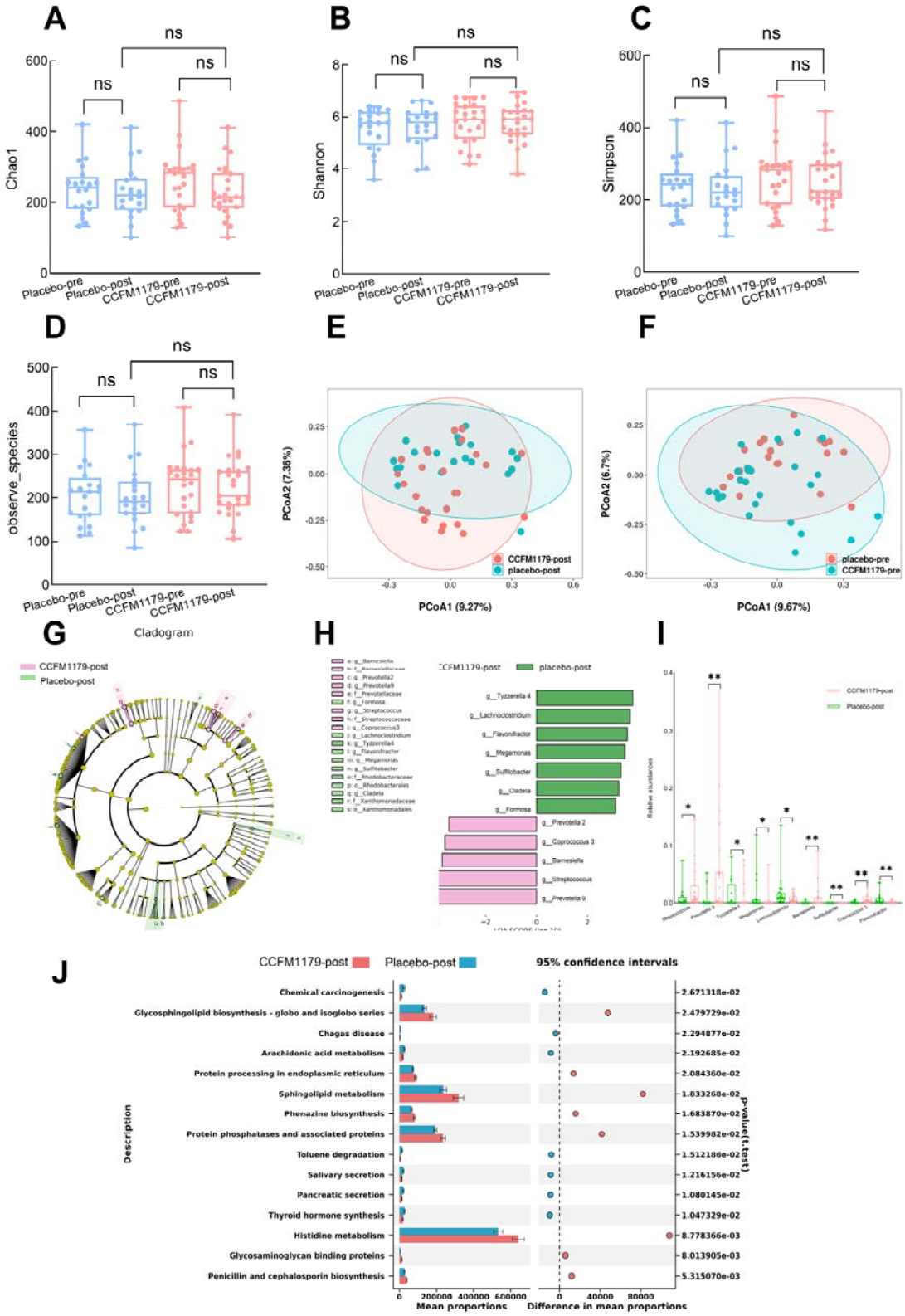

Figure 4. Effects of *B. breve* CCFM1179 on the GM of participants. (A-D) α-diversity indices, including Chao1, Shannon, Simpson, and observed species; (E-F) PCoA analysis with PERMANOVA post-processing test; (G) Linear discriminant analysis effect size; (H) LDA results of the GM (log(LDA score) > 3, FDR-adjusted $P < 0.05$); (I) Relative abundance of specific bacteria at genus level; (J) PICRUSt-predicted functional metabolic pathways (Welch's t-test, $P < 0.05$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

To elucidate functional differences in microbial communities across groups, we employed PICRUSt2 for functional prediction, revealing significant alterations in KEGG pathways of the GM following CCFM1179 intervention (Figure 4J). Compared with the placebo group, pathways including histidine metabolism, sphingomyelin metabolism, and endoplasmic reticulum protein assembly were relatively enriched in the CCFM1179 group. Notably, these pathways are closely associated with neurotransmitter synthesis, membrane lipid homeostasis, protein folding, and neuroendocrine regulation. Conversely, pathways related to inflammation and oxidative stress, such as arachidonic acid metabolism and chemotaxis, were relatively elevated in the placebo group, suggesting that CCFM1179 may mitigate neuroinflammatory responses by downregulating pro-inflammatory metabolic pathways.

3.5. B. breve CCFM1179 alters metabolic levels in subjects with MCI

Using a non-targeted metabolomics approach, we characterized fecal metabolites in the study populations. During LC-MS monitoring, QC samples were injected at regular intervals to assess system stability. As shown in Figure 5A, all QC samples clustered tightly, indicating that the non-targeted fecal metabolomics method demonstrated high analytical stability and reliability. OPLS-DA revealed clear separation between the placebo and CCFM1179 groups after intervention (Figure 5C), indicating substantial alterations in fecal metabolite composition. Based on the thresholds of fold change > 2 and FDR-adjusted $P < 0.05$ ($q < 0.05$), 5 significantly differential metabolites were identified (Figure 5E), including elevated levels of ornithine, α-eleostearic acid, and Oleic acid, as well as reduced levels of quinolinic acid (QA). The detailed differential metabolites with FDR-adjusted q-values are presented in Supplementary Table S4.1. Notably, QA is a metabolite associated with neuroinflammation and excitotoxicity. Further pathway enrichment analysis using the SMPDB database revealed that these metabolites were primarily enriched in pathways related to spermine and spermidine biosynthesis, arginine and proline metabolism, and tryptophan-related metabolic processes (Figure 5E), suggesting that CCFM1179 intervention may modulate the host intestinal metabolic state by regulating these key metabolic pathways.

To further investigate the systemic metabolic effects of *B. breve* CCFM1179 intervention, non-targeted metabolomics analysis was also conducted on serum samples. As shown in Figure 5B, PCA revealed tight clustering of QC samples, demonstrating that the non-targeted serum metabolomics workflow exhibited excellent analytical stability and reliably reflected the serum metabolic landscape of participants. OPLS-DA analysis further showed clear separation between the placebo and CCFM1179 groups, indicating that probiotic intervention induced pronounced alterations in systemic metabolic profiles (Figure 5D). Based on the thresholds of fold change > 2 and FDR-adjusted $P < 0.05$ ($q < 0.05$), a total of 9 significantly differentially expressed metabolites were identified (Figure 5F), primarily including upregulation of azelaic acid, nonanoic acid, dodecanedioic acid, N-acetyl-L-tryptophan, and L-tyrosine, alongside downregulation of paraxanthine and others. The detailed differential metabolites with FDR-adjusted q-values are presented in Supplementary Table S4.2. Pathway enrichment analysis using the SMPDB database revealed that the

enriched metabolic pathways primarily involved caffeine metabolism, thyroid hormone synthesis, catecholamine biosynthesis, and phenylalanine and tyrosine metabolism (Figure 5F).

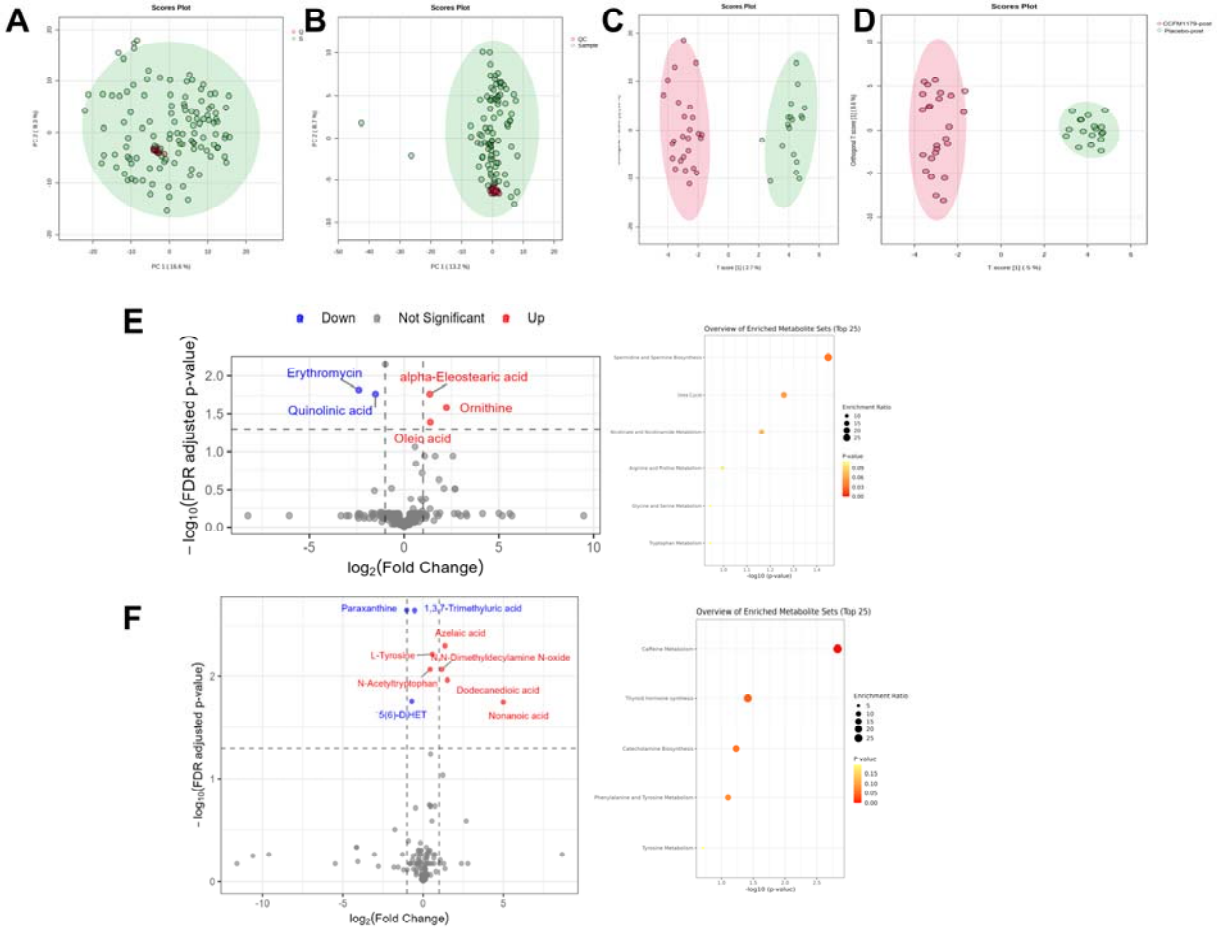

Figure 5. (A) Quality control evaluation of fecal untargeted metabolomics. (B) Quality control evaluation of serum untargeted metabolomics. (C) Changes in fecal metabolic signatures between Placebo-post and CCFM1179-post groups. (D) Changes in serum metabolic signatures between Placebo-post and CCFM1179-post groups. (E) Fecal metabolic markers and differential metabolic pathways between Placebo-post and CCFM1179-post groups. (F) Serum metabolic markers and differential metabolic pathways between Placebo-post and CCFM1179-post groups.

3.6. B. breve CCFM1179 remodels amino acid and tryptophan metabolism while enhancing indole metabolite production

Based on preliminary untargeted metabolomics findings, most differential metabolites observed following *B. breve* CCFM1179 intervention primarily clustered within AA metabolic pathways, particularly those involving tryptophan metabolism. Therefore, targeted analyses of AAs and tryptophan-related metabolites were further performed in the placebo and CCFM1179 groups post-intervention (Figure 6). For fecal AAs (Figure 6A), differences were primarily observed in proline (Pro), arginine (Arg), tyrosine (Tyr), and ornithine (Orn). Following CCFM1179 intervention, Pro, Arg, and Orn all showed significant decreases, whereas Tyr exhibited a significant increase. No statistically significant differences were observed in the remaining AAs ($P > 0.05$), indicating that CCFM1179 can alter the local metabolite profile by modulating intestinal AA metabolic pathways. In contrast, serum AA alterations were primarily concentrated in serine (Ser), valine (Val), and alanine (Ala) (Figure 6B). Compared with the placebo group, levels of these three

AAs were significantly reduced following *B. breve* CCFM1179 intervention, suggesting that intake of this strain may suppress the production or circulating levels of certain serum AAs to some extent.

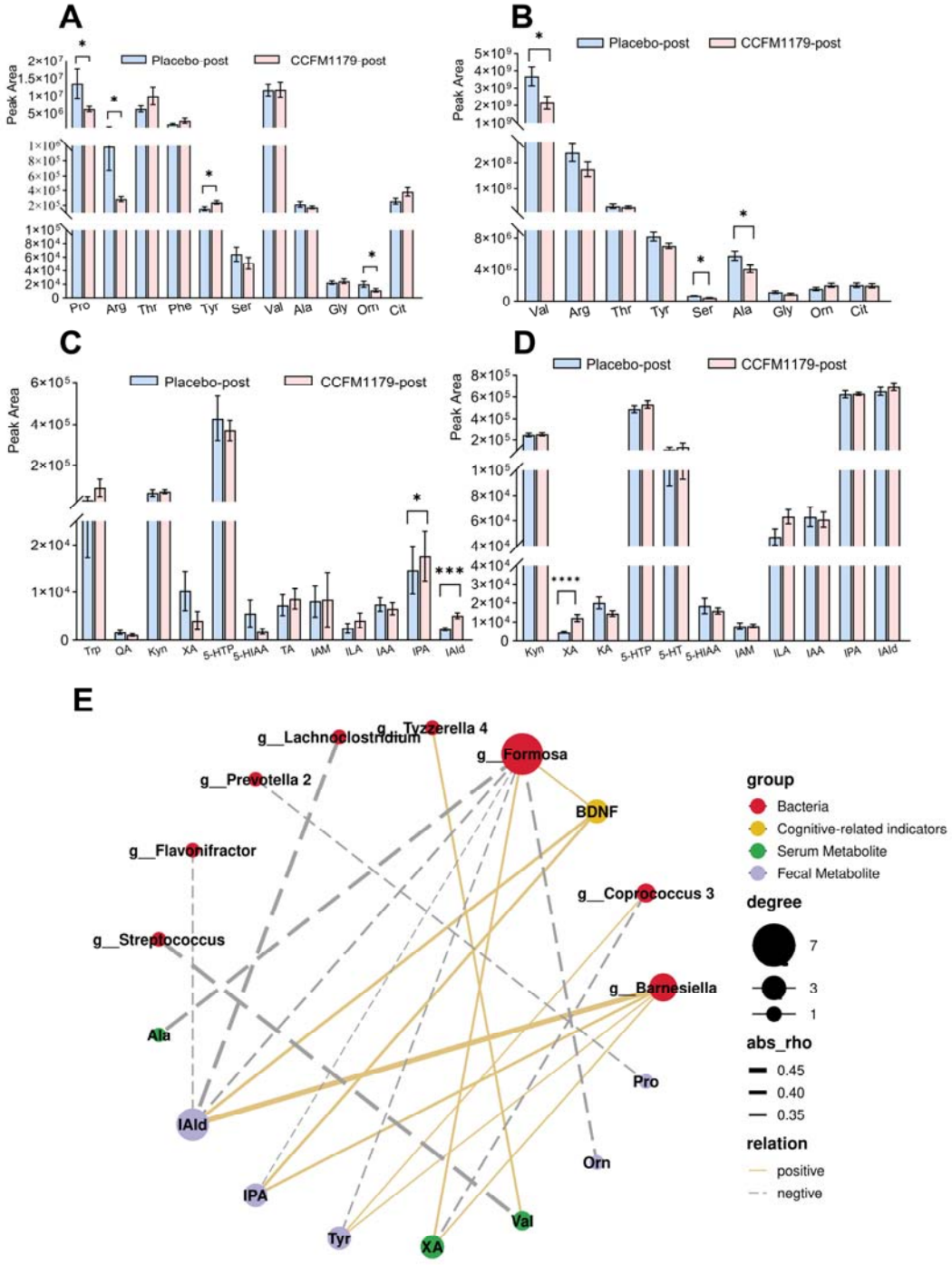

Figure 6. (A–B) Fecal and serum AA peak areas in Placebo-post and CCFM1179-post groups. (C–D) Fecal and serum tryptophan and its related metabolite peak areas in Placebo-post and CCFM1179-post groups. (E) Correlation network among AAs, tryptophan-related metabolites, gut microbiota, and cognitive indicators. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

Tryptophan undergoes metabolism through three primary pathways in the host and gut: the serotonin, kynurenine, and indole-related pathways[26]. Consequently, we focused on whether metabolic changes were induced in these key pathways. In fecal samples (Figure 6C), levels of indole-3-propionic acid (IPA) and

indole-3-carboxaldehyde (IAld) were significantly increased following intervention, whereas quinolinic acid (QA) showed a slight decrease. No significant differences were detected in other tryptophan metabolites, indicating that CCFM1179 primarily redirects microbial tryptophan metabolism toward the indole pathway. Additionally, serum analyses revealed significant differences in xanthurenic acid (XA) levels, indicating that peripheral metabolism is also affected to some extent (Figure 6D).

Furthermore, correlation network analysis was conducted to explore potential associations among GM, AAs, tryptophan-related metabolites, and cognitive indicators. As shown in Figure 6E, after FDR correction IAld and IPA exhibited significant positive correlations with BDNF, indicating that key metabolites produced via the indole pathway from tryptophan may synergistically contribute to the elevation of this neurotrophic factor. The detailed correlation matrix with FDR-adjusted q-values is provided in Supplementary Table S5. At the microbial community level, *Coprococcus 3* exhibited a negative correlation with XA and a positive correlation with Tyr, reflecting its directional differential association pattern with the downstream branched metabolic pathways of tryptophan. Notably, *Barnesiella* demonstrated positive correlations with multiple indole derivatives, including IAld and IPA, further supporting its potential role in regulating the tryptophan metabolic network. Collectively, these findings suggest that CCFM1179 intervention may promote the production of neurotrophically relevant indole metabolites by reshaping the abundance of specific gut bacterial genera, thereby potentially contributing to cognitive improvements.

4. Discussion

MCI represents a transitional stage of cognitive decline that has not yet progressed to dementia. Influenced by factors such as genetics, gender, age, and educational attainment, the global prevalence of MCI among community-dwelling individuals exceeds 15%[27]. Currently, no specific medications have been approved for MCI treatment and management. Existing pharmacological approaches primarily draw on AD therapies, including cholinesterase inhibitors and NMDA receptor antagonists; however, their clinical benefits remain limited, and concerns regarding tolerability and adverse reactions persist[28,29]. Compared to traditional treatment strategies, diet-based interventions offer greater safety and compliance, facilitating long-term management and presenting potential advantages in early prevention and control. Probiotics have garnered significant attention as a non-pharmaceutical dietary intervention. Accumulating evidence suggests that probiotics may improve cognitive performance by modulating the composition and metabolism of the GM[30,31]. Previous studies have demonstrated that *B. breve* CCFM1179 can improve cognitive function in an AD mouse model[17]. The present study further validated these findings in the MCI population, revealing significant improvements in MoCA scores after 12 weeks of supplementation, particularly in executive function and naming dimensions. Concurrently, we observed elevated BDNF levels, GM restructuring, and altered metabolites, notably significant responses in AA and tryptophan metabolism. Furthermore, positive correlations were found between the indole derivatives IPA and IAld and BDNF, suggesting that *B. breve* CCFM1179 may contribute to cognitive enhancement through a gut-metabolite-neural pathway.

A bidirectional communication exists between the brain and the gut. GM can influence central nervous system function through the microbiome-gut-brain axis, with mechanisms involving multiple pathways such as neurotransmitter production, immune-inflammatory regulation, and endocrine and metabolic signaling[32]. This implies that alterations in microbial community structure or metabolic environment may exert feedback effects on brain function, thereby influencing cognitive performance. In this study, the α-diversity index of the GM did not show significant changes following intervention with *B. breve* CCFM1179, a finding consistent with previous probiotic intervention studies[33]. α-diversity reflects the richness and evenness of microbial communities within individual samples, and its stability typically represents the robustness and resilience of the gut ecosystem[34], suggesting that *B. breve* CCFM1179 does not disrupt the original GM state but rather exerts its effects by modulating the abundance of specific functional bacterial genera such as *Prevotella*, *Coprococcus*, *Streptococcus*, and *Megamonas*. Specifically, *Coprococcus* produces short-chain fatty acids, primarily butyrate, which affects cognition by inducing epigenetic changes through increased histone acetylation and histone deacetylation in the brain[35]. Menni et al. found *Coprococcus* to be associated with IPA, which correlates with the tryptophan metabolic pathway discussed later[36]. Across multiple cross-sectional studies in diverse populations, *Barnesiella* abundance has also been positively associated with cognitive indicators[37,38], and reduced *Barnesiella* abundance has been reported in mouse models of age-related cognitive decline[39]. Notably, existing research indicates that increased *Megamonas* abundance may serve as a predictive marker for the onset and progression of MCI. *Megamonas* correlates with diabetes, LDL, and TG levels, indicating its involvement in lipid metabolism[40]. Similarly, a downward trend in HDL-C was observed in this study, paralleling findings from previous research[21]. HDL-C is believed to participate in amyloid β (Aβ) clearance, maintain vascular function, and regulate inflammatory responses[41]; therefore, reductions in HDL-C may reflect impaired lipid homeostasis that negatively impacts neurocognitive function. Furthermore, *Megamonas* has been linked to metabolic abnormalities such as obesity and gestational diabetes, both recognized risk factors for AD and contributors to its pathological processes. This supports the hypothesis that *Megamonas* may promote Aβ deposition and cognitive decline through lipid metabolism dysregulation in these metabolic disorders[42,43]. The post-intervention decline in *Megamonas* may be associated with its involvement in lipid metabolism, reducing activation of metabolic inflammatory pathways to maintain cognition. Collectively, these findings suggest that modulation of specific gut microbial genera may represent an initiating step in a cascade leading to downstream metabolic remodeling and ultimately cognitive improvement.

Given that the effects of the gut microbiota are often mediated through metabolites influencing host physiology and brain function, we integrated functional prediction with metabolomics analysis. PICRUSt2 indicated alterations in amino acid-related pathways, which are broadly consistent with the enrichment of amino acid and tryptophan metabolites observed in this study. This suggests that the increase in indole derivatives may reflect coordinated changes in microbial metabolic activity. However, derived from 16S rRNA data, PICRUSt2 predictions represent inferred rather than directly measured functions and may not

fully reflect the true metabolic potential of the microbiome and should be interpreted with caution[44]. Consistent with these observations, metabolomics analysis revealed significant alterations in tryptophan metabolism, characterized by increased levels of indole derivatives and a decreasing trend in kynurenine pathway metabolites. In addition, changes in amino acids such as Pro and Arg were also observed. AAs play crucial roles in neurotransmission, neurotoxicity, and receptor function and are involved in the onset and progression of neurodegenerative diseases including MCI and AD[45]. Reports indicate that Arg levels progressively increase along the continuum from healthy individuals to MCI and subsequently to AD[46]. As a precursor to polyamines and nitric oxide, Arg has been implicated in key pathophysiological mechanisms underlying dementia[47]. Polyamines and nitric oxide serve as crucial modulators of neuronal function. Polyamines participate in synaptic responses associated with learning and memory, while nitric oxide primarily influences cell growth, neuroprotection, and the regulation of neuroinflammation[48]. This observation is consistent with the hypothesis that modulation of arginine metabolism may contribute to cognitive improvement, although the direct link remains to be established. Notably, this study also observed significantly elevated levels of IPA and IAld, both of which exhibited positive correlations with BDNF. This pattern is consistent with the hypothesis that *B. breve* CCFM1179 may influence cognitive performance through the redirection of tryptophan metabolism toward the neuroprotective indole pathway. Concurrently, *Barnesiella* exhibited positive correlations with IAld and IPA, highlighting its potential role in modulating this metabolic axis. Tryptophan, as a crucial messenger in the gastrointestinal tract and brain, undergoes co-metabolism between the host and microbiota to branch into serotonin, kynurenine, indole, and other pathways. Evidence indicates that tryptophan metabolites induce the differentiation and function of immune regulatory cells (such as anti-inflammatory macrophages, Tregs, $CD4^+CD8\alpha\alpha^+$ cells, etc.), while simultaneously promoting IL-22 production by ILC3s, collectively maintaining immune homeostasis in the gut and throughout the body[49]. It is noteworthy that the indole pathway is gaining increasing attention, with evidence indicating significant disruption of this substance in fecal samples from AD patients[50]. Furthermore, animal studies have demonstrated that indole compounds can suppress NF-κB signaling by upregulating aryl hydrocarbon receptor (AhR) production, reducing pro-inflammatory factor levels, and consequently attenuating neuroinflammation in APP/PS1 mice, leading to improvements in cognitive function[51]. BDNF promotes synaptic plasticity and formation in the brain. In this study, its levels were upregulated following intervention with *B. breve* CCFM1179, consistent with previous research[16]. Research has demonstrated that activating the AhR promotes IL-22 expression, which subsequently phosphorylates STAT3 and induces neuroprotective BDNF production. This signaling cascade contributes to the maintenance of synaptic plasticity and structural and functional stability of the brain[52]. Accordingly, it is plausible that indole metabolites such as IPA and IAld exert their effects in part through AhR-mediated immunomodulation, a possibility that warrants testing in future mechanistic studies.

In summary, this study highlights the potential of probiotics as a nutritional strategy for cognitive enhancement during the critical transition phase from MCI to AD. Tryptophan-derived metabolites such as

IPA and IAld, both positively associated with BDNF, may serve as promising metabolic biomarkers for MCI or as indicators for evaluating intervention responsiveness. Microbial signatures, including decreased *Megamonas* and increased *Prevotella*, also exhibit potential value for risk stratification and early identification of individuals susceptible to cognitive decline. While these findings are encouraging and provide preliminary insights into the microbiota-metabolite-brain axis, the present study design does not allow for definitive conclusions regarding causality. Future mechanistic studies, including animal experiments using fecal microbiota transplantation, gene knockout models, or pharmacological inhibition of key metabolic pathways (e.g., indole or AhR signaling), are necessary. Furthermore, the relatively small cohort and slight imbalance between groups may limit the statistical power and generalizability of the findings, reflecting the exploratory nature of the trail and inherent challenges. Larger-scale studies with extended follow-up periods are needed to validate the stability, durability, and long-term efficacy of the intervention. Notably, physical activity was not systematically assessed in the present study, which may represent a potential source of residual confounding. Moreover, given the clinical heterogeneity of MCI, exploring differential responses among its subtypes will be important for advancing precision microbiome-based interventions.

5. Conclusion

This 12-week randomized, double-blind, placebo-controlled trial demonstrated that *B. breve* CCFM1179 intervention in individuals with MCI resulted in cognitive improvement closely associated with alterations in GM composition and remodeling of the tryptophan-indole metabolic pathway, accompanied by upregulation of neurotrophic factors. This study highlights the microbiota-metabolism-cognition axis, offering novel insights into the potential application of probiotics for early intervention in cognitive decline.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Reference

[1] F. Portet, P.J. Ousset, P.J. Visser, G.B. Frisoni, F. Nobili, P. Scheltens, B. Vellas, J. Touchon, Mild cognitive impairment (MCI) in medical practice: a critical review of the concept and new diagnostic procedure. Report of the MCI Working Group of the European Consortium on Alzheimer's Disease, J Neurol Neurosurg Psychiatry 77 (2006) 714–718. https://doi.org/10.1136/jnnp.2005.085332.

[2] S. Kasper, C. Bancher, A. Eckert, H. Förstl, L. Frölich, J. Hort, A.D. Korczyn, R.W. Kressig, O. Levin, M.S.M. Palomo, Management of mild cognitive impairment (MCI): The need for national and international guidelines, The World Journal of Biological Psychiatry 21 (2020) 579–594. https://doi.org/10.1080/15622975.2019.1696473.

[3] Y. Chen, J. Zhou, L. Wang, Role and Mechanism of Gut Microbiota in Human Disease, Front Cell Infect Microbiol 11 (2021) 625913. https://doi.org/10.3389/fcimb.2021.625913.

[4] K.-C. Fan, C.-C. Lin, Y.-C. Liu, Y.-P. Chao, Y.-J. Lai, Y.-L. Chiu, Y.-F. Chuang, Altered gut microbiota in older adults with mild cognitive impairment: a case-control study, Front. Aging Neurosci. 15 (2023). https://doi.org/10.3389/fnagi.2023.1162057.

[5] M. Guo, J. Peng, X. Huang, L. Xiao, F. Huang, Z. Zuo, Gut Microbiome Features of Chinese Patients Newly Diagnosed with Alzheimer's Disease or Mild Cognitive Impairment, JAD 80 (2021) 299–310. https://doi.org/10.3233/JAD-201040.

[6] B. Li, Y. He, J. Ma, P. Huang, J. Du, L. Cao, Y. Wang, Q. Xiao, H. Tang, S. Chen, Mild cognitive impairment has similar alterations as Alzheimer's disease in gut microbiota, Alzheimer's & Dementia 15 (2019) 1357–1366. https://doi.org/10.1016/j.jalz.2019.07.002.

[7] Y. Chen, J. Xu, Y. Chen, Regulation of Neurotransmitters by the Gut Microbiota and Effects on Cognition in Neurological Disorders, Nutrients 13 (2021) 2099. https://doi.org/10.3390/nu13062099.

[8] C. Fülling, T.G. Dinan, J.F. Cryan, Gut Microbe to Brain Signaling: What Happens in Vagus…, Neuron 101 (2019) 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008.

[9] A. Rutsch, J.B. Kantsjö, F. Ronchi, The Gut-Brain Axis: How Microbiota and Host Inflammasome Influence Brain Physiology and Pathology, Front Immunol 11 (2020) 604179. https://doi.org/10.3389/fimmu.2020.604179.

[10] S. Wu, X. Liu, R. Jiang, X. Yan, Z. Ling, Roles and Mechanisms of Gut Microbiota in Patients With Alzheimer's Disease, Front Aging Neurosci 13 (2021) 650047. https://doi.org/10.3389/fnagi.2021.650047.

[11] E. Socha, P. Kośliński, M. Koba, K. Mądra-Gackowska, M. Gackowski, K. Kędziora-Kornatowska, E. Daghir-Wojtkowiak, Serum amino acid profiles in patients with mild cognitive impairment and in patients with mild dementia or moderate dementia, Amino Acids 53 (2021) 97–109. https://doi.org/10.1007/s00726-020-02928-y.

[12] K. Savonije, D.F. Weaver, The Role of Tryptophan Metabolism in Alzheimer's Disease, Brain Sciences 13 (2023) 292. https://doi.org/10.3390/brainsci13020292.

[13] L. Bonfili, V. Cecarini, O. Gogoi, S. Berardi, S. Scarpona, M. Angeletti, G. Rossi, A.M. Eleuteri, Gut microbiota manipulation through probiotics oral administration restores glucose homeostasis in a mouse model of Alzheimer's disease, Neurobiology of Aging 87 (2020) 35–43. https://doi.org/10.1016/j.neurobiolaging.2019.11.004.

[14] Y. Wu, Y. Shao, X. Shao, H. Yu, M. Wang, J. Wang, Y. She, J. Liu, T. Zhang, Z. Li, A.M. Abd El-Aty, Qingke β-glucan and Lactobacillus mitigate neuroinflammation and enhance cognitive function in an Alzheimer's disease mouse model, International Journal of Biological Macromolecules 319 (2025) 145427. https://doi.org/10.1016/j.ijbiomac.2025.145427.

[15] J. Xiao, N. Katsumata, F. Bernier, K. Ohno, Y. Yamauchi, T. Odamaki, K. Yoshikawa, K. Ito, T. Kaneko, Probiotic Bifidobacterium breve in Improving Cognitive Functions of Older Adults with Suspected Mild Cognitive Impairment: A Randomized, Double-Blind, Placebo-Controlled Trial, Journal of Alzheimer's Disease 77 (2020) 139–147. https://doi.org/10.3233/JAD-200488.

[16] Y.-H. Hwang, S. Park, J.-W. Paik, S.-W. Chae, D.-H. Kim, D.-G. Jeong, E. Ha, M. Kim, G. Hong, S.-H. Park, S.-J. Jung, S.-M. Lee, K.-H. Na, J. Kim, Y.-C. Chung, Efficacy and Safety of Lactobacillus Plantarum C29-Fermented Soybean (DW2009) in Individuals with Mild Cognitive Impairment: A 12-Week, Multi-Center, Randomized, Double-Blind, Placebo-Controlled Clinical Trial, Nutrients 11 (2019) 305. https://doi.org/10.3390/nu11020305.

[17] G. Zhu, J. Zhao, H. Zhang, W. Chen, G. Wang, Administration of Bifidobacterium breve Improves the Brain Function of Aβ1-42-Treated Mice via the Modulation of the Gut Microbiome, Nutrients 13 (2021) 1602. https://doi.org/10.3390/nu13051602.

[18] R.C. Petersen, G.E. Smith, S.C. Waring, R.J. Ivnik, E.G. Tangalos, E. Kokmen, Mild Cognitive Impairment: Clinical Characterization and Outcome, Archives of Neurology 56 (1999) 303–308. https://doi.org/10.1001/archneur.56.3.303.

[19] B. Winblad, K. Palmer, M. Kivipelto, V. Jelic, L. Fratiglioni, L.-O. Wahlund, A. Nordberg, L. Bäckman, M. Albert, O. Almkvist, H. Arai, H. Basun, K. Blennow, M. De Leon, C. DeCarli, T. Erkinjuntti, E. Giacobini, C. Graff, J. Hardy, C. Jack, A.

Jorm, K. Ritchie, C. Van Duijn, P. Visser, R. c. Petersen, Mild cognitive impairment – beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment, Journal of Internal Medicine 256 (2004) 240–246. https://doi.org/10.1111/j.1365-2796.2004.01380.x.

[20] Y. Fei, R. Wang, J. Lu, S. Peng, S. Yang, Y. Wang, K. Zheng, R. Li, L. Lin, M. Li, Probiotic intervention benefits multiple neural behaviors in older adults with mild cognitive impairment, Geriatric Nursing 51 (2023) 167–175. https://doi.org/10.1016/j.gerinurse.2023.03.006.

[21] Y. Kobayashi, T. Kuhara, M. Oki, J.-Z. Xiao, Effects of Bifidobacterium breve A1 on the cognitive function of older adults with memory complaints: a randomised, double-blind, placebo-controlled trial, BM 10 (2019) 511–520. https://doi.org/10.3920/BM2018.0170.

[22] Z. Fang, T. Pan, L. Li, H. Wang, J. Zhu, H. Zhang, J. Zhao, W. Chen, W. Lu, *Bifidobacterium longum* mediated tryptophan metabolism to improve atopic dermatitis via the gut-skin axis, Gut Microbes 14 (2022) 2044723. https://doi.org/10.1080/19490976.2022.2044723.

[23] G. Zhu, M. Guo, J. Zhao, H. Zhang, G. Wang, W. Chen, Integrative Metabolomic Characterization Reveals the Mediating Effect of Bifidobacterium breve on Amino Acid Metabolism in a Mouse Model of Alzheimer's Disease, Nutrients 14 (2022) 735. https://doi.org/10.3390/nu14040735.

[24] L. Zhang, W. Zheng, X. Li, S. Wang, M. Xiao, R. Xiao, D. Zhang, N. Ke, H. Cai, J. Cheng, X. Chen, M. Gong, A merged method for targeted analysis of amino acids and derivatives using parallel reaction monitoring combined with untargeted profiling by HILIC-Q-Orbitrap HRMS, Journal of Pharmaceutical and Biomedical Analysis 203 (2021) 114208. https://doi.org/10.1016/j.jpba.2021.114208.

[25] Y. Chen, P. Tian, Z. Wang, R. Pan, K. Shang, G. Wang, J. Zhao, W. Chen, Indole Acetic Acid Exerts Anti-Depressive Effects on an Animal Model of Chronic Mild Stress, Nutrients 14 (2022) 5019. https://doi.org/10.3390/nu14235019.

[26] Frontiers | Tryptophan Dietary Impacts Gut Barrier and Metabolic Diseases, (n.d.). https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2019.02113/full (accessed December 2, 2025).

[27] W. Bai, P. Chen, H. Cai, Q. Zhang, Z. Su, T. Cheung, T. Jackson, S. Sha, Y.-T. Xiang, Worldwide prevalence of mild cognitive impairment among community dwellers aged 50 years and older: a meta-analysis and systematic review of epidemiology studies, Age Ageing 51 (2022). https://doi.org/10.1093/ageing/afac173.

[28] M. Sheikh, M. Ammar, Efficacy of 5 and 10 mg donepezil in improving cognitive function in patients with dementia: a systematic review and meta-analysis, Front. Neurosci. 18 (2024). https://doi.org/10.3389/fnins.2024.1398952.

[29] S. Hajihosseini, S.A. Zakavi, Z. Farrokhi, M. Amanzadeh, P. Panahi, M. Mahram, N. Eftekhari, M. Noroozi, M.J. Ebrahimi, A. Alizadeh, P. Refahi, M.A. Bafrani, M. Moafi, N. Deravi, A meta-analysis update evaluating the treatment effects of donepezil alone versus donepezil combined with memantine for Alzheimer's disease, IBRO Neuroscience Reports 19 (2025) 72–82. https://doi.org/10.1016/j.ibneur.2025.05.016.

[30] S.-J. Jung, K. Cho, E.-S. Jung, D. Son, J.-S. Byun, S.-I. Kim, S.-W. Chae, J.-C. Yang, S.-O. Lee, S. Lim, Augmenting Cognitive Function in the Elderly with Mild Cognitive Impairment Using Probiotic Lacticaseibacillus rhamnosus CBT-LR5: A 12-Week Randomized, Double-Blind, Parallel-Group Non-Comparative Study, Nutrients 17 (2025) 691. https://doi.org/10.3390/nu17040691.

[31] X. Li, C. Lv, J. Song, J. Li, Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Mild Cognitive Impairment and Alzheimer's Disease: A Meta-Analysis, Front. Nutr. 8 (2021). https://doi.org/10.3389/fnut.2021.757673.

[32] K. Kowalski, A. Mulak, Brain-Gut-Microbiota Axis in Alzheimer's Disease, J Neurogastroenterol Motil 25 (2019) 48–60. https://doi.org/10.5056/jnm18087.

[33] M.R. Aljumaah, U. Bhatia, J. Roach, J. Gunstad, M.A. Azcarate Peril, The gut microbiome, mild cognitive impairment, and probiotics: A randomized clinical trial in middle-aged and older adults, Clinical Nutrition 41 (2022) 2565–2576. https://doi.org/10.1016/j.clnu.2022.09.012.

[34] A.D. Willis, Rarefaction, Alpha Diversity, and Statistics, Front Microbiol 10 (2019) 2407. https://doi.org/10.3389/fmicb.2019.02407.

[35] F. Notting, W. Pirovano, W. Sybesma, R. Kort, The butyrate-producing and spore-forming bacterial genus Coprococcus as a potential biomarker for neurological disorders, Gut Microbiome 4 (2023) e16. https://doi.org/10.1017/gmb.2023.14.

[36] C. Menni, M.M. Hernandez, M. Vital, R.P. Mohney, T.D. Spector, A.M. Valdes, Circulating levels of the anti-oxidant indoleproprionic acid are associated with higher gut microbiome diversity, Gut Microbes 10 (2019) 688–695. https://doi.org/10.1080/19490976.2019.1586038.

[37] M. Ceccon, J.B. Kantsjö, F. Ronchi, Personalized Paths: Unlocking Alzheimer's via the Gut-Brain Axis, Visceral Medicine 40 (2024) 194–209. https://doi.org/10.1159/000535869.

[38] K. Meyer, A. Lulla, K. Debroy, J.M. Shikany, K. Yaffe, O. Meirelles, L.J. Launer, Association of the Gut Microbiota With Cognitive Function in Midlife, JAMA Network Open 5 (2022) e2143941. https://doi.org/10.1001/jamanetworkopen.2021.43941.

[39] M.-L. Wu, X.-Q. Yang, L. Xue, W. Duan, J.-R. Du, Age-related cognitive decline is associated with microbiota-gut-brain axis disorders and neuroinflammation in mice, Behavioural Brain Research 402 (2021) 113125. https://doi.org/10.1016/j.bbr.2021.113125.

[40] J. Yang, L. Wang, H. Liu, H. Xu, F. Liu, H. Song, X. Zhao, H. Li, Dysregulation of *Ruminococcaceae* and *Megamonas* could be predictive markers for rapid progression of mild cognitive impairment, Microbial Pathogenesis 183 (2023) 106272. https://doi.org/10.1016/j.micpath.2023.106272.

[41] B.V. Zlokovic, S. Yamada, D. Holtzman, J. Ghiso, B. Frangione, Clearance of amyloid beta-peptide from brain: transport or metabolism?, Nat Med 6 (2000) 718. https://doi.org/10.1038/77397.

[42] X. Chen, H. Sun, F. Jiang, Y. Shen, X. Li, X. Hu, X. Shen, P. Wei, Alteration of the gut microbiota associated with childhood obesity by 16S rRNA gene sequencing, PeerJ 8 (2020) e8317. https://doi.org/10.7717/peerj.8317.

[43] Y.-S. Kuang, J.-H. Lu, S.-H. Li, J.-H. Li, M.-Y. Yuan, J.-R. He, N.-N. Chen, W.-Q. Xiao, S.-Y. Shen, L. Qiu, Y.-F. Wu, C.-Y. Hu, Y.-Y. Wu, W.-D. Li, Q.-Z. Chen, H.-W. Deng, C.J. Papasian, H.-M. Xia, X. Qiu, Connections between the human gut microbiome and gestational diabetes mellitus, Gigascience 6 (2017) 1–12. https://doi.org/10.1093/gigascience/gix058.

[44] G.M. Douglas, V.J. Maffei, J.R. Zaneveld, S.N. Yurgel, J.R. Brown, C.M. Taylor, C. Huttenhower, M.G.I. Langille, PICRUSt2 for prediction of metagenome functions, Nat Biotechnol 38 (2020) 685–688. https://doi.org/10.1038/s41587-020-0548-6.

[45] S. Samakashvili, C. Ibáñez, C. Simó, F.J. Gil-Bea, B. Winblad, A. Cedazo-Mínguez, A. Cifuentes, Analysis of chiral amino acids in cerebrospinal fluid samples linked to different stages of Alzheimer disease, ELECTROPHORESIS 32 (2011) 2757–2764. https://doi.org/10.1002/elps.201100139.

[46] J. Olazarán, L. Gil-de-Gómez, A. Rodríguez-Martín, M. Valentí-Soler, B. Frades-Payo, J. Marín-Muñoz, C. Antúnez, A. Frank-García, C. Acedo-Jiménez, L. Morlán-Gracia, R. Petidier-Torregrossa, M.C. Guisasola, F. Bermejo-Pareja, Á. Sánchez-Ferro, D.A. Pérez-Martínez, S. Manzano-Palomo, R. Farquhar, A. Rábano, M. Calero, A blood-based, 7-metabolite signature for the early diagnosis of Alzheimer's disease, J Alzheimers Dis 45 (2015) 1157–1173. https://doi.org/10.3233/JAD-142925.

[47] G. Ravaglia, P. Forti, F. Maioli, G. Bianchi, M. Martelli, T. Talerico, L. Servadei, M. Zoli, E. Mariani, Plasma amino acid concentrations in patients with amnestic mild cognitive impairment or Alzheimer disease123, The American Journal of Clinical Nutrition 80 (2004) 483–488. https://doi.org/10.1093/ajcn/80.2.483.

[48] H. Vural, B. Sirin, N. Yilmaz, I. Eren, N. Delibas, The Role of Arginine–Nitric Oxide Pathway in Patients with Alzheimer Disease, Biol Trace Elem Res 129 (2009) 58–64. https://doi.org/10.1007/s12011-008-8291-8.

[49] X. Su, Y. Gao, R. Yang, Gut Microbiota-Derived Tryptophan Metabolites Maintain Gut and Systemic Homeostasis, Cells 11 (2022) 2296. https://doi.org/10.3390/cells11152296.

[50] L. Wu, Y. Han, Z. Zheng, G. Peng, P. Liu, S. Yue, S. Zhu, J. Chen, H. Lv, L. Shao, Y. Sheng, Y. Wang, L. Li, L. Li, B. Wang, Altered Gut Microbial Metabolites in Amnestic Mild Cognitive Impairment and Alzheimer's Disease: Signals in Host–Microbe Interplay, Nutrients 13 (2021) 228. https://doi.org/10.3390/nu13010228.

[51] J. Sun, Y. Zhang, Y. Kong, T. Ye, Q. Yu, S. Kumaran Satyanarayanan, K.-P. Su, J. Liu, Microbiota-derived metabolite Indoles induced aryl hydrocarbon receptor activation and inhibited neuroinflammation in APP/PS1 mice, Brain, Behavior, and Immunity 106 (2022) 76–88. https://doi.org/10.1016/j.bbi.2022.08.003.

[52] A. Sfera, K.A. Thomas, J. Anton, Cytokines and Madness: A Unifying Hypothesis of Schizophrenia Involving Interleukin-22, Int J Mol Sci 25 (2024) 12110. https://doi.org/10.3390/ijms252212110.