

Lycium barbarum polysaccharide improves the cognitive deficits in APP/PS1 mice of Alzheimer's disease via modulating the microbiota-gut-brain axis

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Abstract

Background

Alzheimer's disease (AD) is increasingly recognized as a systemic disorder in which gut dysbiosis and intestinal barrier dysfunction contribute to neurodegeneration through the microbiota-gut-brain axis. Recent evidence suggests that modulation of gut microbiota by dietary bioactives may represent a promising strategy for AD prevention and treatment.

Purpose

This study aimed to investigate whether *Lycium barbarum* polysaccharides (LBP), a major bioactive component of *Lycium barbarum* with known antioxidant and anti-inflammatory properties, could alleviate cognitive impairment and neuropathological alterations in APP/PS1 transgenic mice by regulating the microbiota-gut-brain axis.

Methods

APP/PS1 mice were orally administered LBP, and their cognitive performance was evaluated using the Morris water maze (MWM) test. The effects of LBP on neuropathology, inflammation, and gut function were assessed through immunofluorescence, western blotting, enzyme-linked immunosorbent assay, and histological analyses. In addition, 16S rRNA sequencing and non-targeted fecal metabolomics were performed to characterize gut microbiota composition and metabolic alterations associated with LBP treatment.

Results

LBP markedly improved spatial learning and memory and reduced beta-amyloid (A β) deposition in the cortex and hippocampus. It modulated APP processing by downregulating phosphorylated APP (p-APP) and BACE1 while upregulating ADAM10 expression. LBP also suppressed neuroinflammation by reducing microglial (IBA-1) and astrocytic (GFAP) activation and by rebalancing pro- and anti-inflammatory cytokines in both brain and serum. 16S rRNA sequencing and metabolomics analyses revealed that LBP restored microbial diversity, enriched beneficial taxa (e.g., *Alistipes*, *Turicibacter*), and normalized metabolic disturbances in bile acid, lipid, and amino acid pathways. Furthermore, histological and immunohistochemical analyses demonstrated that LBP repaired intestinal barrier injury, enhanced tight junction protein expression (Claudin-1, Occludin, ZO-1), and alleviated jejunal inflammation.

Conclusion

Collectively, these findings indicate that LBP ameliorates cognitive decline and neuropathological changes in APP/PS1 mice by modulating gut microbiota composition, remodeling microbial metabolism, reinforcing intestinal barrier integrity, and suppressing systemic and central inflammation. This study highlights LBP as a promising functional polysaccharide with potential therapeutic value for preventing or mitigating AD through microbiota-gut-brain axis regulation.

Introduction

- Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia. Its clinical presentation is characterized by memory impairment, apraxia, aphasia, executive dysfunction, and alterations in personality and behavior (Scheltens et al., 2021).¹ With the accelerating progression of population aging, the incidence and prevalence of AD are projected to increase annually. According to the World Alzheimer Report 2023, an estimated 55 million individuals live with AD globally, with projections suggesting the prevalence may double or triple by 2050 (GBD 2021 Nervous System Disorders Collaborators., 2024). This situation not only inflicts immense suffering on patients but also imposes a severe burden on families and society. Currently, the pharmacological therapy for AD primarily target the management of cognitive symptoms and the control of neuropsychiatric manifestations. The US Food and Drug Administration (FDA) has approved two categories of drugs for cognitive enhancement: cholinesterase inhibitors (e.g., donepezil, rivastigmine, and galantamine) and an N-methyl-D-aspartate (NMDA) receptor antagonist (memantine) (Sharma et al., 2019; Iraj et al., 2020; Liu et al., 2023). However, these therapies are primarily palliative, focusing on symptom alleviation and delaying disease progression rather than achieving a cure or reversing the underlying pathology. Moreover, all these therapeutic interventions are associated with adverse effects. In addition, targeted disease-modifying therapies, such as lecanemab and donanemab, which address core AD pathology, have demonstrated promising results in clinical trials. However, as these agents exclusively target beta-amyloid (A β), their long-term efficacy and clinical sustainability require further validation through longitudinal studies (Terao, & Kodama, 2024). Consequently, the identification of novel therapeutic targets and agents for AD treatment and prevention remains an imperative objective requiring extensive research.
- A growing body of preclinical and clinical research indicates that gut microbiota plays a significant role in the onset and progression of AD through the regulation of the microbiota-gut-brain axis (Das et al., 2023; Xie et al., 2023; Zou et al., 2023; Ling et al., 2021; Marizzoni et al., 2023). The functional state of the gut and the composition of its microbiota can significantly influence brain function and contribute to the pathogenesis of neurological diseases (Nakhal et al., 2024). Several studies have reported the impact of gut microbiota dysbiosis on various neurodegenerative disorders, such as AD and Parkinson's disease (PD) (Denman et al., 2023; Liu et al., 2020; Salim et al., 2023). It is hypothesized that gut microbiota dysbiosis may promote the production of pro-inflammatory cytokines, induce endotoxin release, and concurrently contribute to metabolic disturbances (Rosendo-Silva et al., 2023). Conversely, certain gut microbiota taxa, including Bifidobacterium, Firmicutes, Actinobacteria, and Verrucomicrobia, have been associated with the enhancement of

cognitive function (Sharma et al., 2021). Several studies have demonstrated significant differences in the composition and diversity of gut microbiota between AD patients and control groups (Vogt et al., 2017; Kang et al., 2024; Chandra et al., 2023). The significant variance in gut microbiota composition observed between healthy individuals and those with various diseases positions it as a promising therapeutic target for both prevention and treatment. For instance, preclinical studies have indicated that various interventional strategies targeting the gut microbiota, such as probiotics, prebiotics, antibiotics, and fecal microbiota transplantation (FMT), exhibit potential in mitigating cognitive deficits and pathological alterations in mouse models of AD (Zhang et al., 2023; Sun et al., 2019; Xiao-Hang et al., 2024; Wang et al., 2021). Furthermore, in recent years, researchers worldwide have increasingly focused on Traditional Chinese Medicine (TCM) and its active compounds for AD treatment, identifying a range of promising natural therapeutics such as ginsenosides, quercetin, curcumin, berberine, and lycium barbarum polysaccharides (LBP) (Liu et al., 2024). However, the precise therapeutic mechanisms still require further investigation.

- LBP, a primary active constituent of wolfberry, is composed of six monosaccharides: rhamnose, arabinose, xylose, mannose, glucose, and galactose. It has demonstrated potential therapeutic efficacy for the treatment of AD (Zeng et al., 2019; Zhou et al., 2020; He et al., 2024). Numerous recent studies have revealed that LBP exhibits substantial potential in AD treatment, characterized by its multi-target mechanisms. These include inhibiting A β abnormal deposition, suppressing Tau protein hyperphosphorylation, combating neuroinflammation, alleviating oxidative stress, modulating neurotransmitters, restoring synaptic plasticity, regulating the microbiota-gut-brain axis, and improving insulin resistance. Although the microbiota-gut-brain axis is an emerging therapeutic target for AD, the potential effects of LBP on this axis remain largely unexplored.
- In this study, LBP was administered to APP/PS1 mice to investigate its therapeutic efficacy and elucidate the underlying molecular mechanisms against AD pathogenesis. Age-matched wild type (WT) mice were used as the normal control group. The LBP was administered orally at a dose of 100 mg/kg per day to APP/PS1 mice for a duration of 4 weeks. Subsequently, the cognitive function of APP/PS1 and WT mice was assessed using the Morris water maze (MWM) test. The composition and diversity of the gut microbiota in each group were analyzed via 16S rRNA sequencing. Untargeted metabolomics analysis was employed to profile gut microbiota-derived metabolites across all experimental groups. Alterations in the pathological hallmarks of AD, including A β deposition and neuroinflammation, were further assessed using histological and immunofluorescence analyses. Furthermore, the structural integrity of the intestinal barrier was evaluated using immunohistochemical analysis. To our knowledge, this study is the first to demonstrate that LBP alleviates cognitive impairment by modulating the gut microbiota. We anticipate that this study will provide a novel foundation for elucidating the therapeutic mechanisms and functional effects of LBP treatment in AD.

Methods

Animals and treatment

- Female APP/PS1 and C57BL/6J mice, aged 4-4.5 months, were obtained from Cavens Laboratory Animal Co., Ltd. (Changzhou, Jiangsu, China). The animals were housed in standard conditions, with 3–5 mice per cage, maintained at a temperature of $22 \pm 2^{\circ}\text{C}$, relative humidity of $55 \pm 5\%$, a 12-hour light/dark cycle, and ad libitum access to food and water. Subsequently, the mice were randomly assigned to three groups ($n = 12$ per group): WT group, AD group, and LBP group. The LBP group received daily oral administration of LBP (100 mg/kg/day) via gavage for 4 weeks. The WT and AD group received an equal volume of saline. The LBP were supplied by Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China). All experimental procedures were approved by the Animal Ethics Committee of Mianyang Central Hospital (approval number: S20250315-01), and all protocols were performed in compliance with the ARRIVE guidelines for animal research.

MWM test

- Following 4 weeks of treatment, spatial learning and memory were evaluated using the MWM test, as previously described (Othman et al., 2022; Qian et al., 2022). Prior to the experiment, the mice underwent a 24-hour acclimatization period in a designated room, after which they were trained to locate a submerged platform over a 5-day period (4 trials per day). On the sixth day, a probe trial was performed in which the platform was removed, and the time spent in the target quadrant was recorded. Furthermore, the exploration tracks of each mouse during both the training and testing periods were recorded using water maze software for subsequent analysis.

Sample collection

- Fecal samples were collected in sterile EP tubes, rapidly frozen in liquid nitrogen, and stored at -80°C for microbiota analysis. Subsequently, the mice were anesthetized with 2.5% isoflurane in 100% oxygen at a flow rate of 1.5 L/min to collect blood and tissue samples for further analysis. Blood samples were collected via cardiac puncture, and serum was isolated by centrifugation at $3,000 \times g$ for 10 min. The brain and intestinal tissues were quickly removed and separated into two portions: one was fixed in 4% paraformaldehyde for histological, immunofluorescence, and immunohistochemical analysis, while the other was stored at -80°C for protein extraction, western blot, and inflammatory marker analysis.
- *16S rRNA gene sequencing*
- Fecal DNA was extracted using the TIANamp Stool DNA Extraction Kit (TIANGEN, Beijing, China), according to the manufacturer's instruction. The final DNA concentration and purity were measured using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was assessed by 1% agarose gel electrophoresis. The V3-V4 region of the 16S rRNA gene was amplified using the primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGTATCTAAT-3') incorporating sample-specific barcodes. PCR products were purified with magnetic beads and pooled in equimolar ratios based on concentration. After thorough mixing,

the PCR products were detected and target bands were recovered. Sequencing libraries were constructed, and index sequences were added. Libraries were quantified using Qubit and real-time PCR, and size distribution was assessed with a Bioanalyzer. Quantified libraries were pooled and sequenced on Illumina platforms, according to effective library concentration and data amount required. Subsequently, bioinformatics analysis was performed using QIIME2 software.

Untargeted metabolomic analysis

- The feces samples were individually grounded with liquid nitrogen and the homogenate was resuspended with prechilled 80% methanol by well vortex. The samples were incubated on ice for 5 min and then were centrifuged at 15,000 g at 4°C for 20 min. Some of supernatant was diluted to final concentration containing 53% methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube and then were centrifuged at 15000 g at 4°C for 20 minutes. Finally, the supernatant was injected into the UHPLC-MS/MS system analysis (Want et al., 2013). The data files generated by UHPLC-MS/MS were processed by XCMS to perform peak alignment, peak picking, and quantitation for each metabolite. Then, based on adduct ions and setting mass deviation to 10 ppm, a comparison was made between these data and the high-quality secondary spectrum database to obtain results for metabolite identification. These metabolites were annotated using the KEGG database (<https://www.genome.jp/kegg/pathway.html>), HMDB database (<https://hmdb.ca/metabolites>) and LIPIDMaps database (<http://www.lipidmaps.org/>). Partial least squares discriminant analysis (PLS-DA) were performed at metaX (Wen et al., 2017). We applied univariate analysis (t-test) to calculate the statistical significance (P-value). The metabolites with VIP > 1 and P-value < 0.05 and fold change ≥ 2 or FC ≤ 0.5 were considered to be differential metabolites. Volcano plots were used to filter metabolites of interest which based on $\log_2(\text{FoldChange})$ and $-\log_{10}(\text{p-value})$ of metabolites by ggplot2 in R language.

Hematoxylin and eosin (HE) staining

- Histological examination of the brain and intestine was performed using HE staining. Paraffin-embedded tissues were sectioned at 5 μm thickness and stained according to standard protocols (Wu et al., 2021). Images were acquired using a Nikon ECLIPSE E100 microscope and analyzed for A β deposition and intestinal morphology.

Immunofluorescence assay

- Immunofluorescence staining was performed to evaluate the localization and expression of A β , microglia, and astrocytes. Tissue sections were incubated with primary antibodies (GFAP, IBA-1, and A β) followed by fluorescent dye-conjugated secondary antibodies. Slides were mounted with 4',6-diamidino-2-phenylindole (DAPI) for nuclear staining, and images were acquired using a Nikon

ECLIPSE C1 confocal microscope. Semi-quantitative analysis of GFAP, IBA-1, and A β expression was performed using ImageJ software.

Immunohistochemistry analysis

- Immunohistochemical staining was utilized to assess the expression and distribution of intestinal tight junction proteins in the mice. Jejunum and colon tissue sections were incubated with primary antibodies against zona occluden-1 (ZO-1), occludin, and claudin-1 followed by appropriate secondary antibodies conjugated to horseradish peroxidase (HRP). Immunoreactivity was visualized using diaminobenzidine (DAB) and counterstained with hematoxylin. Quantification of positive staining was performed using a Nikon ECLIPSE E100 microscope.

Enzyme-linked immunosorbent assay (ELISA)

- The brain, jejunum tissues, and serum samples were processed for the detection of inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), interleukin-1 beta (IL-1 β), interleukin-4 (IL-4), interleukin-10 (IL-10), transforming growth factor-beta (TGF- β). ALL procedures were performed according to the manufacturer's instructions for the commercial ELISA kits (Bioswamp, Hubei, China). The absorbance was measured at 450 nm, and cytokine concentrations were calculated based on standard curves.

Western blot analysis

- Western blot analysis was performed to assess the protein expression of phosphorylated APP (p-APP), APP, BACE1, and ADAM10. Briefly, protein samples were extracted from brain tissues according to the manufacturer's instruction, separated by SDS-PAGE, and transferred to PVDF membranes. Membranes were incubated with primary antibodies followed by HRP-conjugated secondary antibodies. Protein bands were visualized using enhanced chemiluminescence and quantified using ImageJ analysis software.

Statistical analysis

- The data were expressed as the mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) and the Kruskal-Wallis test were used to determine statistical significance across groups, followed by Tukey's or Dunn's multiple comparisons test, respectively. All statistical analyses were performed using GraphPad Prism 9.0 software.

Results and discussion

LBP treatment alleviated cognitive impairment in APP/PS1 mice

- To evaluate the effects of LBP treatment on cognitive function, the MWM test was conducted. The experimental design is shown in Fig. 1A. Representative swimming trajectories demonstrated that WT group mice rapidly located the hidden platform with a focused search strategy, whereas AD group mice displayed disorganized swimming paths and failed to locate the target efficiently. In contrast, LBP group mice exhibited marked improvements in navigation, adopting a more goal-directed swimming pattern (Fig. 1B). Furthermore, quantitative analyses corroborated these behavioral observations. During the training phase, escape latency was significantly prolonged in AD group mice compared with WT controls, whereas LBP treatment effectively shortened the latency, indicating enhanced learning ability (Fig. 1C). In the probe trial, AD group mice exhibited fewer platform crossings and spent less time in the target quadrant than WT controls, indicating impaired memory retention. Notably, LBP treatment significantly increased both platform crossings and time spent in the target quadrant, partially rescuing cognitive performance (Fig. 1D-E). Importantly, swimming speed did not differ significantly among the three groups, thereby excluding motor dysfunction as a confounding factor (Fig. 1F). Collectively, these findings demonstrate that LBP treatment markedly ameliorates spatial learning and memory impairments in APP/PS1 mice.

LBP modulated the composition and diversity of gut microbiota

- To investigate the impact of LBP on gut microbiota, we conducted 16S rRNA sequencing. As illustrated by the Venn diagram, both shared and unique amplicon sequence variants (ASVs) were identified among WT, AD, and LBP groups mice, highlighting distinct microbial communities across groups (Fig. 2A). In terms of α diversity, AD group mice exhibited markedly reduced microbial richness and diversity compared with WT controls, as indicated by the Chao1 and Shannon indices. Notably, LBP administration significantly increased these indices, suggesting a partial restoration of microbial diversity, whereas no significant differences were observed in the Simpson index among the groups (Fig. 2B-D).
- Furthermore, principal coordinate analysis (PCoA) revealed a clear separation between AD and WT groups, whereas the microbial profile of LBP group mice shifted closer to that of WT controls (Fig. 2E). Subsequently, we analyzed the taxonomic composition of the gut microbiota. As shown in Figs. 2F and 2G, the overall distribution of microbial communities was characterized at both the phylum and genus levels respectively (Fig. 2F-G). At the phylum level, the relative abundance of Proteobacteria and Patescibacteria differed significantly among the three groups (Fig. 2H-I). At the genus level, the relative abundance of Prevotellaceae_UCG-001 and Paraprevotella markedly differed among the three groups (Figs. 2J-K). Consistent with these observations, linear discriminant analysis effect size (LEfSe) analysis further identified discriminative bacterial taxa

across groups (Fig. 2L). Specifically, AD group mice were enriched in Prevotellaceae_UCG-001, Paraprevotella, and Erysipelatoclostridium, whereas WT mice exhibited higher abundances of Alistipes and Turicibacter. Importantly, LBP-treated reversed several AD-associated alterations, underscoring its microbiota-modulating effects. Taken together, these findings demonstrate that LBP alleviates AD-associated gut dysbiosis by enhancing microbial diversity, reshaping community structure, and selectively enriching beneficial microbial populations while reducing pathogenic taxa.

LBP regulated the metabolites derived from gut microbiota

- Microbiota-derived metabolites influence the host through multiple pathways, with many entering the bloodstream and exerting profound effects on physiology and behavior. To assess whether LBP treatment modulates gut microbiota-derived metabolites, we performed non-targeted fecal metabolomics on mouse fecal samples. The Venn diagram of differential metabolites revealed both shared and unique metabolic alterations among WT, AD, and LBP groups, reflecting distinct metabolic signatures (Fig. 3A). Potential biomarkers were identified based on VIP values > 1 and *p* values < 0.05 and visualized using volcano plots (Figs. 3B-C). Subsequently, multivariate statistical modeling with PLS-DA demonstrated clear separation of metabolic profiles among groups. AD mice clustered distinctly from WT controls, whereas LBP-treated mice displayed a metabolic profile that shifted toward the WT group, indicating partial restoration of metabolic homeostasis (Fig. 3D). Consistently, KEGG pathway enrichment analysis of differential metabolites between AD and LBP groups highlighted significant enrichment in multiple pathways, including histidine metabolism, primary bile acid biosynthesis, and unsaturated fatty acid biosynthesis (Fig. 3E). These pathways are closely linked to oxidative stress regulation, lipid balance, and neuronal function, thereby providing mechanistic insights into the cognitive benefits of LBP. Further analysis revealed that AD mice exhibited profound disturbances in nucleoside metabolism (inosine, xanthosine), energy-related metabolites (creatine), and phenolic compounds compared with WT controls (Fig. 3F). Notably, LBP supplementation reversed several of these abnormalities, particularly in lipid metabolites such as lysophosphatidylcholine [LysoPC(14:0/0:0)] and bile acid derivatives (deoxycholylllysine), as well as secondary metabolites with potential neuroprotective or anti-inflammatory properties, including ganoderal A, trichosetin, and raspberry ketone sulfate (Fig. 3G). In summary, these findings demonstrate that LBP treatment substantially remodels the fecal metabolome of AD mice, correcting metabolic disturbances in lipid, amino acid, and bile acid pathways, which may underlie its protective effects against cognitive decline.

LBP reduced A β deposition in APP/PS1 mice

- A β deposition is one of the main pathological features of AD. To investigate the influence of LBP on A β deposition in APP/PS1 mice, the brain sections were stained by HE staining and immunofluorescence assay with an anti-A β ₁₋₄₀ antibody. Histopathological morphology in the mice brain was observed with HE staining to confirm whether LBP can improve the deposition of senile plaques in APP/PS1 mice. As shown in Fig. 4A, compared with WT group mice, AD group mice

exhibited more senile plaques in the dentate gyrus (DG) and cortex regions. LBP treatment significantly decreased the deposition of senile plaques of APP/PS1 mice. Immunofluorescence analysis showed a significant increase in A β deposition in both the DG and cortex areas of AD group mice compared with WT controls. Notably, LBP treatment markedly reduced A β accumulation in both regions, indicating its potential to attenuate A β pathology (Figs. 4B-D). To explore potential molecular mechanisms, the expression levels of key proteins in the A β production pathway were examined through western blot analysis. The results demonstrated that AD group mice exhibited significantly elevated levels of p-APP, APP, and BACE1, whereas LBP treatment markedly reduced p-APP expression without significantly affecting total APP levels (Figs. 4E-H). The levels of AMDA10 was significantly decreased in the AD group mice, compared to the WT and LBP group mice (Figs. 4E and I). These results indicate that LBP treatment may mitigate A β generation by regulating abnormal APP phosphorylation and processing.

- Consistent with these findings, ELISA analysis of brain tissue revealed profound alterations in cytokine expression. AD group mice exhibited decreased levels of anti-inflammatory cytokines IL-4, IL-10, and TGF- β compared with WT group mice (Figs. 4J-L). LBP treatment significantly restored IL-10 and TGF- β levels, and partially improved IL-4 expression, suggesting enhanced anti-inflammatory responses. Conversely, pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α were markedly elevated in AD group mice relative to WT controls (Figs. 4M-O). Importantly, LBP administration significantly reduced the expression of these pro-inflammatory cytokines. Taken together, these results demonstrate that LBP alleviates AD-related neuropathology by reducing A β deposition, modulating APP phosphorylation, and rebalancing neuroinflammatory cytokine expression, thereby contributing to its neuroprotective effects in AD mice.

LBP ameliorated the proliferation of microglia and astrocyte

- In recent years, neuroinflammation mediated by microglia and astrocytes in the central nervous system has been recognized as a critical contributor to the pathogenesis of AD. To evaluate the impact of LBP on neuroinflammation, we examined ionized calcium-binding adapter molecule 1 (IBA-1) and glial fibrillary acidic protein (GFAP), which serve as specific markers for microglia and astrocytes, respectively. Immunofluorescence analysis revealed a pronounced increase in IBA-1 expression in both the DG and cortex regions of AD group mice compared with WT controls. Notably, LBP treatment markedly reduced IBA-1 expression in these regions, suggesting an attenuation of microglial activation. Similarly, astrocytic activation, as indicated by GFAP expression, was strongly elevated in AD group mice, particularly within the DG region. LBP administration significantly decreased GFAP levels, demonstrating effective suppression of astrogliosis (Figs. 5A-F). To further investigate systemic inflammation, serum cytokine concentrations were quantified by ELISA. Compared with WT controls, AD group mice exhibited markedly decreased levels of anti-inflammatory cytokines, including TGF- β , IL-4, and IL-10 (Figs. 4G-I), accompanied by significant increases in pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α (Figs. 4J-L). Importantly, LBP treatment restored the balance by elevating anti-inflammatory cytokines while reducing pro-

inflammatory cytokines. Overall, these results indicate that LBP mitigates neuroinflammation in APP/PS1 mice through a dual mechanism: attenuating glial activation in the brain and rebalancing systemic inflammatory cytokine profiles.

LBP decreased intestinal barrier injury and inflammation

- To investigate whether LBP alleviates intestinal barrier dysfunction associated with AD pathology, histology and immunohistochemistry analyses were performed to assess the expression of major intestine tight junction proteins, including claudin-1, occludin, and ZO-1 in the colon and jejunum. HE staining revealed marked mucosal atrophy, crypt loss, and villus disruption in AD group mice compared with WT group mice, indicating that aging in APP/PS1 mice was associated with significant impairment of intestinal barrier integrity. Notably, LBP treatment substantially alleviated these histopathological alterations, mitigating mucosal damage and restoring intestinal architecture (Fig. 6A-B). We further performed immunohistochemical analysis to evaluate the expression of intestinal tight junction proteins, which constitute a multi-protein complex essential for maintaining epithelial barrier integrity. Compared with WT group mice, the AD group exhibited significantly decreased expression of claudin-1, occludin, and ZO-1 in both intestinal regions, indicating structural disruption and compromised epithelial integrity. LBP treatment markedly upregulated the expression of these tight junction proteins, suggesting restoration of mucosal barrier function (Fig. 6C-D). Specifically, in the jejunum, LBP treatment significantly increased the optical density of claudin-1, occludin, and ZO-1 compared with the AD group (Fig. 6E-G). Similarly, in the colon, claudin-1 and ZO-1 expression levels were significantly elevated following LBP administration, while occludin expression showed a moderate upward trend without statistical significance (Fig. 6H-J).
- Accumulating evidence suggests that age-related gut microbiota dysbiosis can initiate and amplify inflammatory responses. Therefore, to further elucidate the anti-inflammatory effects of LBP treatment, we examined the expression of inflammatory cytokines in jejunal tissue. ELISA analysis revealed profound inflammatory alterations in the jejunum of APP/PS1 mice. Compared with WT group mice, AD group mice displayed significantly reduced anti-inflammatory cytokines, such as IL-4, IL-10, and TGF- β (Fig. 6K-M), accompanied by elevated levels of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α (Fig. 6O-Q). Remarkably, LBP administration reversed these trends by downregulating pro-inflammatory cytokines and upregulating anti-inflammatory cytokines to near-normal levels. Collectively, these findings indicate that LBP treatment effectively ameliorates intestinal inflammation and enhances epithelial tight junction integrity in APP/PS1 mice, suggesting that the modulation of gut barrier function may contribute to its neuroprotective effects via the gut-brain axis.
- This study demonstrates that LBP effectively ameliorate cognitive deficits and neuropathological alterations in APP/PS1 transgenic mice, providing mechanistic evidence that these effects are mediated, at least in part, through the microbiota-gut-brain axis. Our comprehensive analysis integrating behavioral, microbiological, biochemical, and histological approaches revealed that LBP

improved cognition, reduced A β pathology, alleviated neuroinflammation, and restored intestinal barrier integrity in APP/PS1 mice.

- Behavioral analyses confirmed that LBP supplementation markedly improved spatial learning and memory performance in APP/PS1 mice. These findings are consistent with previous reports showing that polysaccharide-rich bioactives can enhance hippocampal function and synaptic plasticity through anti-oxidative and anti-inflammatory mechanisms (Lv et al., 2025; Zhao et al., 2025). Immunofluorescence and western blot analysis results revealed that LBP reduced A β deposition and modulated APP processing by decreasing p-APP and BACE1 while restoring ADAM10 expression. This pattern indicates a shift toward the non-amyloidogenic cleavage pathway, thereby reducing the production of neurotoxic A β peptides. Such modulation of APP processing by dietary polysaccharides has also been observed in other studies involving plant-derived compounds that regulate the PI3K/AKT and GSK-3 β pathways (Xiao-Hang et al., 2024; Huang et al., 2024). Neuroinflammation is a central feature of AD pathogenesis. In this study, APP/PS1 mice exhibited increased activation of microglia and astrocytes, as evidenced by elevated IBA-1 and GFAP expression in the cortex and DG regions. LBP treatment effectively attenuated glial activation and restored cytokine homeostasis by downregulating pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) and enhancing anti-inflammatory mediators (IL-4, IL-10, TGF- β). These findings suggest that LBP exerts its neuroprotective effects by suppressing chronic inflammation and promoting a regulatory microenvironment in the brain. This dual modulation resembles the actions of multi-strain probiotics and natural polysaccharides that alleviate AD-like inflammation by inhibiting NF- κ B and NLRP3 inflammasome activation (Yang et al., 2020; Ruan et al., 2022).
- 16S rRNA sequencing revealed that AD mice displayed dysbiosis characterized by reduced α -diversity and enrichment of pathogenic genera such as Prevotellaceae_UCG-001 and Paraprevotella. LBP treatment restored microbial richness and promoted the growth of beneficial taxa including Alistipes and Turicibacter, genera known to produce short-chain fatty acids and other neuroprotective metabolites (Mukhopadhyaya et al., 2025). Non-targeted metabolomics further demonstrated that LBP reversed AD-associated metabolic disturbances in bile acid, lipid, and amino acid metabolism. Pathway enrichment identified glycerophospholipid, glutathione, and primary bile acid metabolism as major pathways normalized by LBP, consistent with restored oxidative balance and neuronal energy homeostasis. These microbial and metabolic changes underscore the capacity of LBP to reprogram gut microbial activity and metabolite signaling, supporting cognitive health via the gut-brain axis (He et al., 2024). Intestinal barrier dysfunction is increasingly recognized as a key contributor to neuroinflammatory diseases (White et al., 2025). Our histological and immunohistochemical analyses revealed severe mucosal atrophy and downregulation of tight junction proteins in APP/PS1 mice, which were significantly reversed by LBP supplementation. Furthermore, jejunal cytokine assays indicated that LBP reduced local inflammation by restoring anti-inflammatory cytokines and suppressing pro-inflammatory cytokines. The improved barrier integrity likely reduced gut permeability and endotoxin leakage, thereby mitigating systemic inflammation and neuroimmune activation (Chen et al., 2020). These findings reinforce the notion

that dietary polysaccharides can protect brain health by maintaining intestinal homeostasis and suppressing the inflammatory cascade between the gut and brain.

- Key strengths of our study include the multi-layered approach (behaviour, microbiome, metabolome, gut barrier, neuropathology) and the use of LBP, a natural polysaccharide with translational potential. However, several limitations warrant consideration. First, while we show associations between microbiota/metabolite changes and cognitive/neuropathological endpoints, causal pathways remain to be definitively proven. Second, the precise molecular targets of LBP in gut microbiota or host tissues remain undefined. Third, our study used a specific transgenic AD model and outcomes may vary in other models or in humans. Future studies should address dose-response relationships, long-term effects, and translation to clinical populations.

Conclusions

- In conclusion, this study demonstrates that LBP markedly ameliorate cognitive dysfunction and neuropathological changes in APP/PS1 mice through comprehensive modulation of the microbiota-gut-brain axis. LBP treatment improved spatial learning and memory, reduced A β deposition, rebalanced neuroinflammation, and enhanced intestinal barrier integrity. These beneficial effects were accompanied by restoration of gut microbial diversity and normalization of fecal metabolites associated with bile acid, lipid, and amino acid metabolism. The multi-targeted nature of LBP underscores its potential as a functional dietary ingredient or nutraceutical for the prevention and management of AD via gut-brain axis regulation.

Abbreviations

AD, Alzheimer's disease; FDA, Food and Drug Administration; NMDA, N-methyl-D-aspartate; A β , beta-amyloid; PD, Parkinson's disease; FMT, fecal microbiota transplantation; TCM, Traditional Chinese Medicine; LBP, lycium barbarum polysaccharides; MWM, Morris water maze; PLS-DA, Partial least squares discriminant analysis; HE, Hematoxylin and eosin; ZO-1, zona occluden-1; ELISA, Enzyme-linked immunosorbent assay; TNF- α , tumor necrosis factor-alpha; IL-6, interleukin-6; IL-1 β , interleukin-1 beta; IL-4, interleukin-4; IL-10, interleukin-10; TGF- β , transforming growth factor-beta; p-APP, phosphorylated APP; ASVs, amplicon sequence variants; PCoA, principal coordinate analysis; LEfSe, linear discriminant analysis effect size; DG, dentate gyrus; IBA-1, calcium-binding adapter molecule 1; GFAP, glial fibrillary acidic protein

Declarations

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Author contributions

Zhiyan Zou: Conceptualization, Data curation, Visualization, Writing-original draft, Writing-review and editing. **Xilian Wang:** Conceptualization, Investigation, Methodology, Writing-review and editing. **Xinyun Ge:** Methodology, Data curation. **Dan Lei:** Investigation, Writing-review and editing. **Xinnuo Lei:** Investigation, Writing-review and editing. **Jiaqiang Hou:** Investigation, Writing-review and editing.

Xuemin Jian: Conceptualization, Funding acquisition. **Yu Long:** Funding acquisition, Writing-review and editing. **Xiaoan Li:** Conceptualization, Project administration, Funding acquisition. All authors reviewed and approved the manuscript.

Conflicts of interest

All the authors declare that they have no competing financial interests.

Data availability statement

Data will be made available upon request.

Clinical trial number

Not applicable.

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Figures

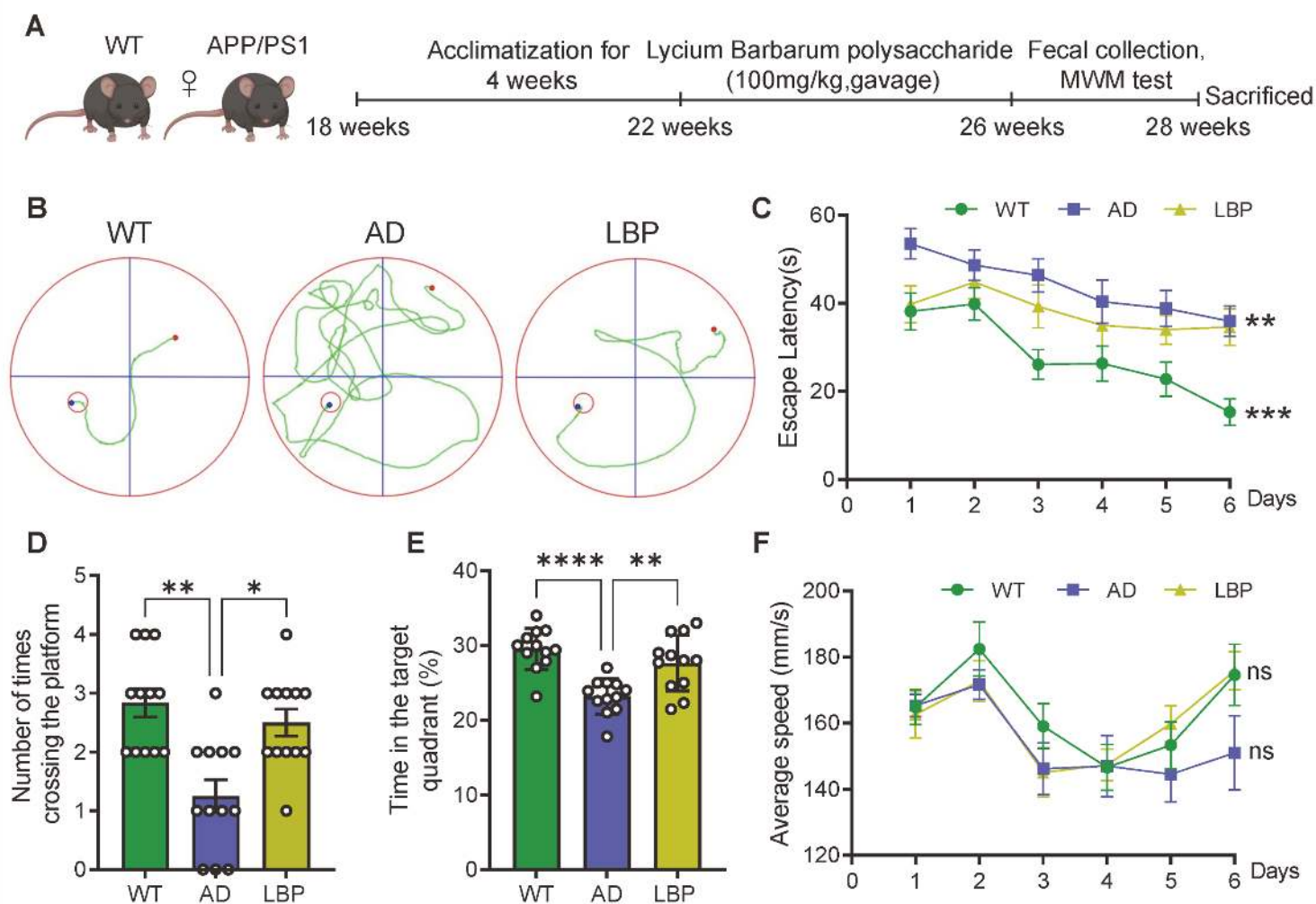


Figure 1

LBP ameliorate the spatial learning and memory ability in APP/PS1 mice. (A) Schematic diagram of the experimental design. (B) Representative swimming trajectories of WT, AD, and LBP groups mice during the probe trial. (C) Escape latency during training sessions. (D) Number of platform crossings in the probe test. (E) Percentage of time spent in the target quadrant. (F) Average swimming speed during the training phase. Data are presented as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no statistical difference.

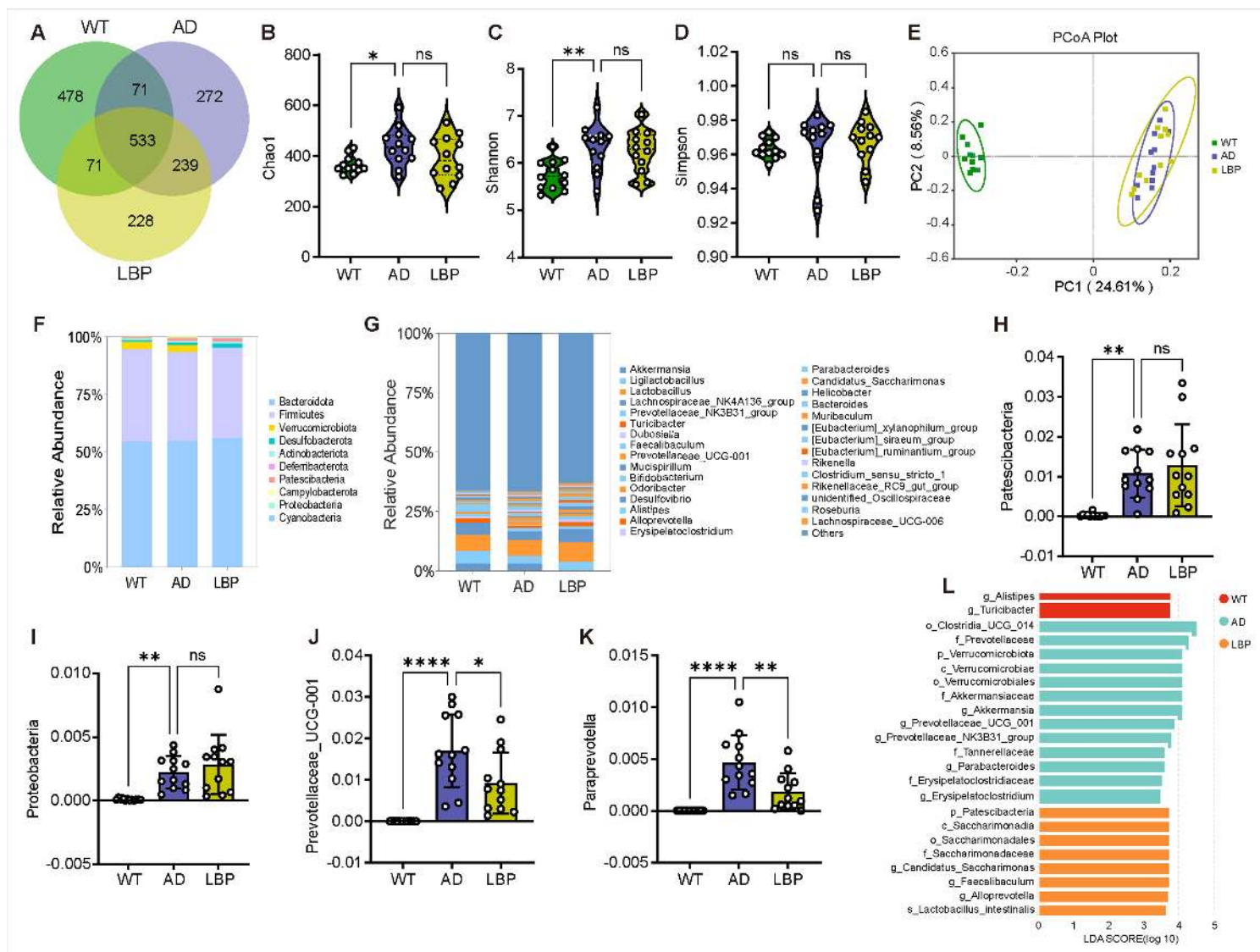


Figure 2

LBP modulates gut microbiota composition and diversity in APP/PS1 mice. (A) Venn diagram of shared and unique ASVs among WT, AD, and LBP groups. (B-D) α diversity indices (Chao1, Shannon, Simpson) between the three groups. (E) Principal coordinate analysis (PCoA) of microbial community structures at the genus level. (F) Relative abundance of gut microbiota at the phylum level. (G) Relative abundance of gut microbiota at the genus level. (H-I) Differential abundance of Patescibacteria and Proteobacteria at the phylum level. (J-K) Differential abundance of Prevotellaceae_UCG-001 and Paraprevotella at the genus level. (L) Linear discriminant analysis effect size (LEfSe) showing discriminative taxa among groups. Data are expressed as mean $\pm$ SD. Statistical significance was determined using Kruskal-Wallis test followed by Dunn's multiple comparisons test, and LEfSe with an LDA score > 3.5 for biomarker discovery. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, ns: no statistical difference.

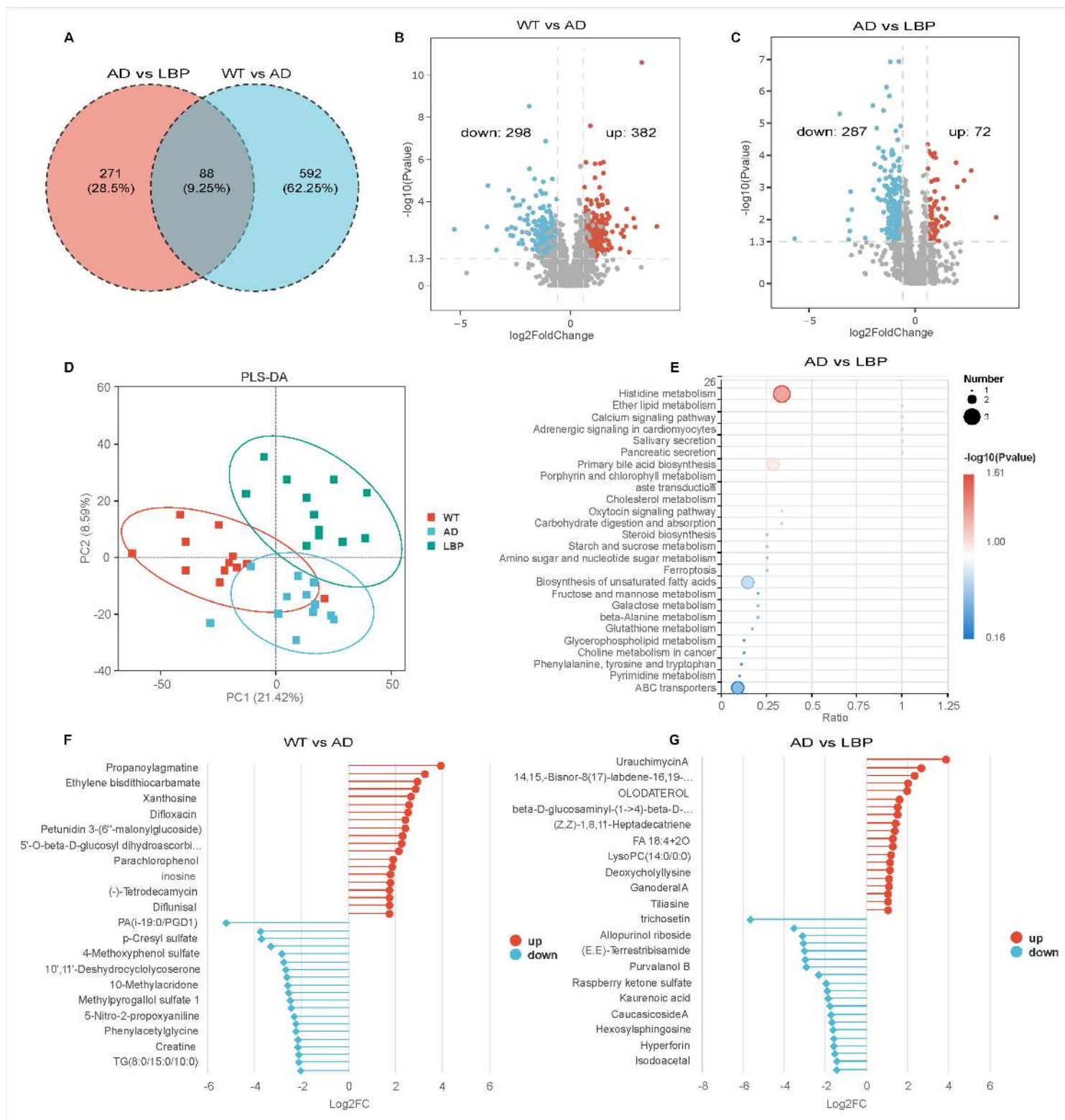


Figure 3

The Effect of LBP on fecal metabolomic profiles in APP/PS1 mice. (A) Venn diagram of differential metabolites among WT, AD, and LBP groups. (B-C) Volcano plots of differential metabolites between WT vs. AD and AD vs. LBP groups. (D) Partial least squares discriminant analysis (PLS-DA) score plot of metabolomics data for each group of feces. (E) Scatter plot for the KEGG pathway enrichment of differential metabolites between AD and LBP groups. (F-G) Matchstick plot of significantly altered

metabolites between WT vs. AD and AD vs. LBP groups. Differential metabolites were identified based on VIP > 1.0 and $p < 0.05$.

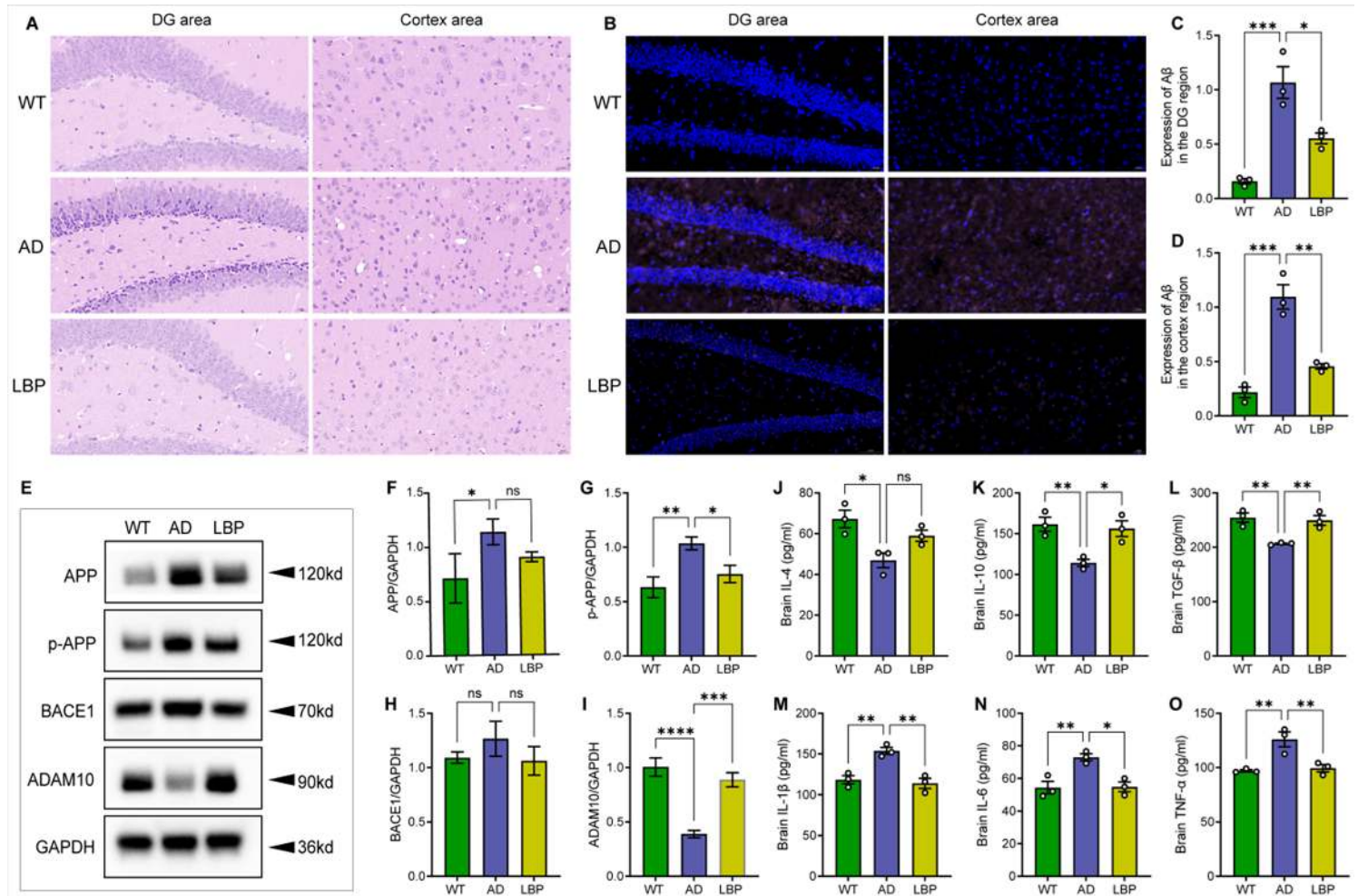


Figure 4

LBP treatment reduce Aβ deposition through inhibiting the production of APP protein in APP/PS1 mice. (A) HE staining of DG and cortex regions. (B-D) Immunofluorescence and quantification of Aβ in the DG and cortex regions. (E-I) Western blot and quantification of APP, p-APP, BACE1, ADAM10, and GAPDH in the brain tissue. (J-O) ELISA analysis of anti-inflammatory (IL-4, IL-10, TGF-β) and pro-inflammatory (IL-1β, IL-6, TNF-α) in brain tissue. Data are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no statistical difference.

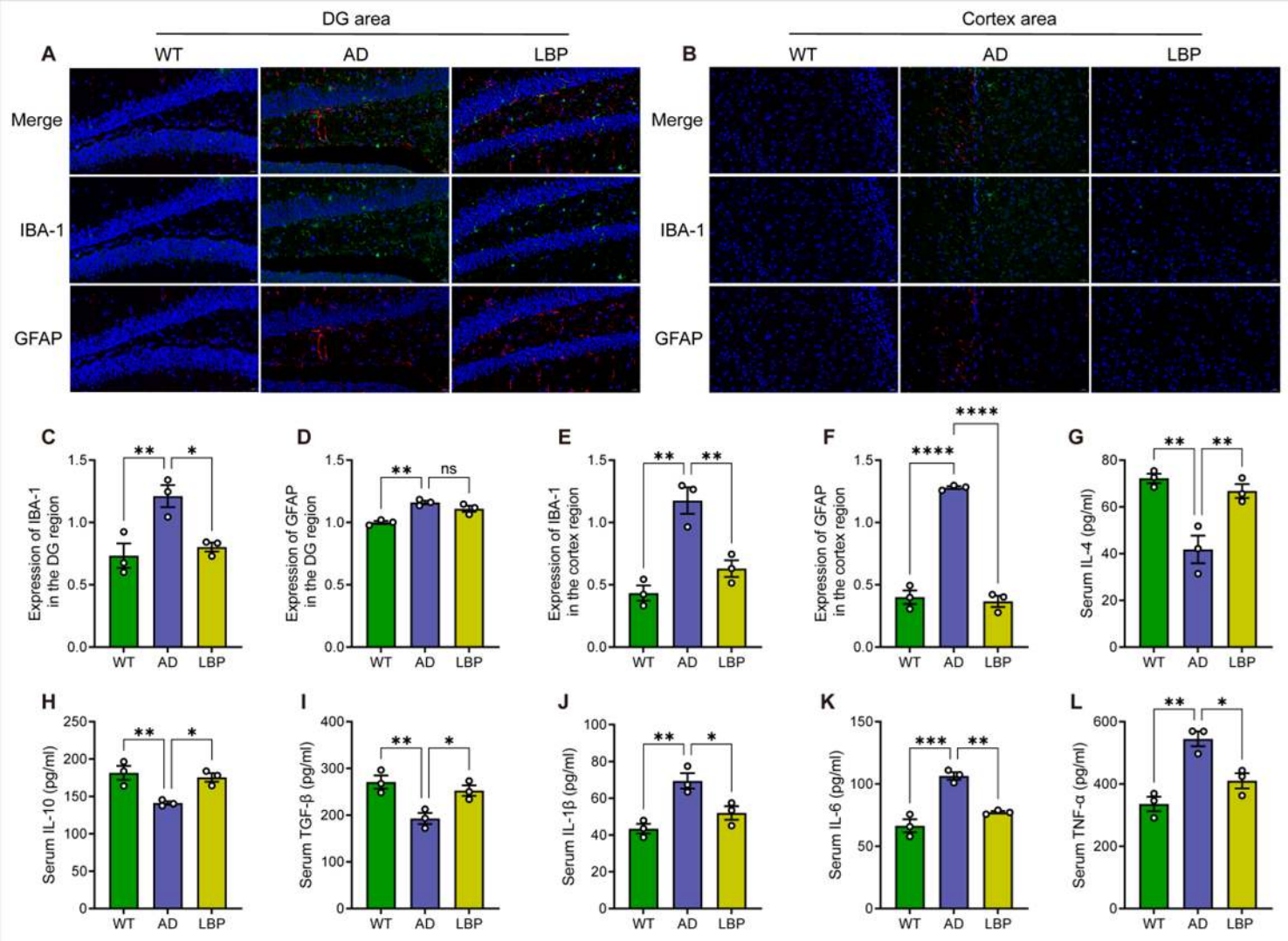


Figure 5

LBP alleviates neuroinflammation in APP/PS1 mice by suppressing the proliferation of microglia and astrocyte. (A-B) Immunofluorescence of IBA-1 and GFAP in the DG and cortex areas (Scale bars, 20 μ m). (C-D) Immunofluorescence quantification of IBA-1 and GFAP expression in the DG region. (E-F) Quantification of IBA-1 and GFAP expression in the cortex region. (G-I) Serum levels of anti-inflammatory cytokines TGF- β , IL-4, and IL-10. (J-L) Serum levels of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α . Data are presented as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, ns: no statistical difference.

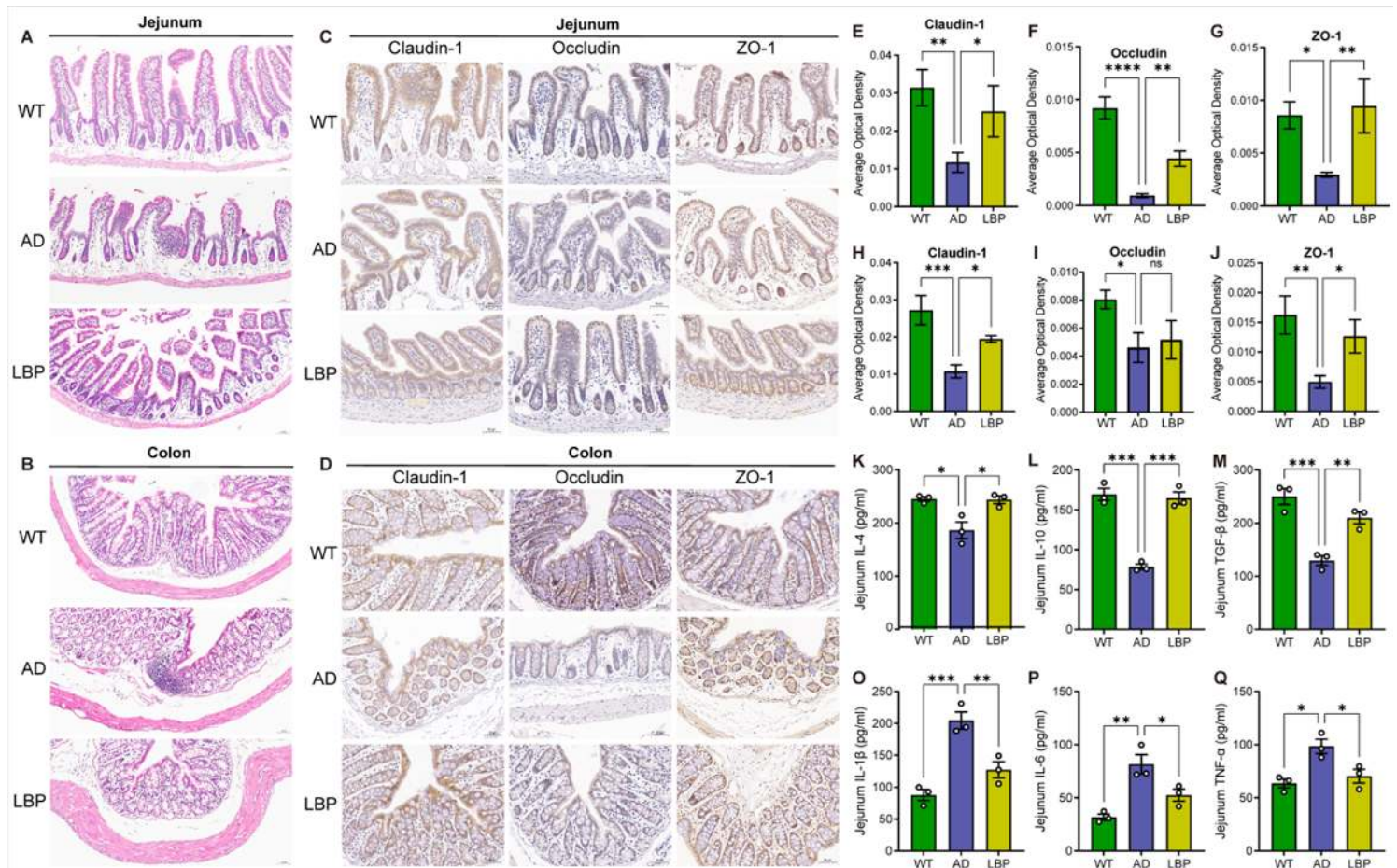


Figure 6

LBP restores intestinal barrier integrity and modulates inflammatory cytokine expression in the jejunum of APP/PS1 mice. (A-B) Representative photomicrographs of HE staining of jejunum and colon. (C-D) Immunostaining of intestine tight junction markers claudin-1, occludin, and ZO-1 in the jejunum. (E-G) Immunohistochemical quantification of claudin-1, occludin, and ZO-1 expression in the jejunum. (H-J) Quantification of claudin-1, occludin, and ZO-1 expression in the colon. (K-M) ELISA analysis of anti-inflammatory cytokines IL-4, IL-10, and TGF- β in jejunal tissue. (O-Q) ELISA analysis of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α in jejunal tissue. The data are expressed as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no statistical difference.

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