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The Effect and Mechanism of Gut Microbiota Changes in Spleen Deficiency Environment on Alzheimer's Disease Rats

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ABSTRACT

Objective: This study aimed to investigate how a spleen-deficient environment influences Alzheimer's disease (AD) and to preliminarily elucidate the potential mechanisms involving intestinal flora regulation.

Methods: An AD model was established in male Sprague–Dawley rats via bilateral intraventricular injection of amyloid β protein (Aβ)1–42, while a spleen deficiency model was induced by subcutaneous injection of reserpine. The rats were divided into four groups: normal control (NC), AD model (AD), spleen deficiency model (SD), and combined AD with spleen deficiency model (AD+SD). Cognitive behavior and AD-related pathology were evaluated using the Morris water maze test and immunohistochemistry. Changes in the gut microbiota were assessed via 16S rRNA sequencing.

Results: The spleen-deficient internal environment induced significant cognitive impairment, characterized by prolonged escape latency, reduced movement distance in the target area, slower swimming speed, and fewer platform crossings. Pathologically, it accelerated Aβ (1–42) deposition in the hippocampal CA1 region. Furthermore, spleen deficiency selectively enriched pro-inflammatory bacteria such as Proteobacteria, Actinobacteria, and *Lactococcus*, while reducing the abundance of beneficial bacteria involved in short-chain fatty acid production, including Bacteroides and Ruminococcus. Enrichment of bacteria promoting Aβ deposition, such as *Pseudomonas* and Staphylococcus, was also observed under spleen-deficient conditions. KEGG pathway analysis identified 13 metabolic and functional pathways associated with the altered microbiota.

Conclusion: A spleen-deficient internal environment can induce cognitive decline, disrupt gut microbiota homeostasis, accelerate hippocampal Aβ (1–42) deposition, and promote the development and progression of AD in rats. Modulating intestinal flora balance may play a critical role in mitigating these effects.

Keywords: Spleen Deficiency; Alzheimer's Disease; Gut Microbiota

Abbreviations: AD: Alzheimer's Disease; Aβ: Amyloid β Protein; SCFAs: Short-Chain Fatty Acids; CNS: Central Nervous System; LPS: Lipopolysaccharide; TCM: Traditional Chinese Medicine; MWM: Morris Water Maze Test; HE: Hematoxylin and Eosin; CTAB: Cetyl Trimethylammonium Bromide; PCR: Polymerase Chain Reaction; KEGG: Kyoto Encyclopedia of Genes and Genomes; LEfSe: Linear Discriminant Analysis Effect Size; LDA: Linear Discriminant Analysis; NFT: Neurofibrillary Tangle

Introduction

Alzheimer's disease (AD) is characterized by progressive cognitive decline resulting from the deterioration of neuronal connections and eventual cell loss over time [1]. Current research implicates multiple mechanisms in AD pathogenesis, including β-amyloid plaque accumulation, hyperphosphorylation of Tau protein, metal ion dysregulation, acetylcholine deficiency, oxidative stress, and chronic inflammation [2,3]. However, none of these theories fully explains the disease's etiology. Recently, the gut microbiome has emerged as a key regulator of neurological health. Growing evidence suggests that gut microbiota dysbiosis is linked to AD progression, though it remains debated whether these microbial changes are a cause or consequence of the disease [4]. Studies on the gut-brain axis indicate that bacterial metabolites or toxins may migrate from the gut to the brain via the vagus nerve, potentially disrupting central nervous system function [5,6]. This connection underscores the profound impact of gut microbial balance on brain health. Neuroinflammation and immune activation have long been research foci in AD. Numerous studies emphasize the role of microglial activation and inflammatory states in AD development [4]. The gut microbiota can influence neuroinflammation by modulating Aβ deposition and microglial activity [3,7]. Additionally, bacterial components such as lipopolysaccharide (LPS) and metabolites like short-chain fatty acids (SCFAs) significantly affect AD pathogenesis [8].

Repeated intraperitoneal LPS injections in mice induce Aβ deposition and cognitive impairment [9]. LPS from Bacteroides fragilis can bind to microglial receptors TLR2, TLR4, and CD14, similar to Aβ1–42, triggering neuroinflammatory responses [10]. Given the lack of effective AD treatments, targeting the microbiome offers a promising therapeutic avenue. Current AD treatments only modestly delay symptom progression, and no disease-modifying therapies are available. Traditional Chinese medicine (TCM) has long employed herbal formulations for cognitive disorders. Pi (spleen) deficiency is a common TCM syndrome affecting digestive and metabolic functions, distinct from the Western concept of the spleen. In TCM theory, spleen deficiency is considered a root cause of mental disorders. Herbs such as Panax notoginseng and Astragalus mongholicus, which tonify the spleen, are frequently used in AD treatment. Ginsenoside Rg1, a bioactive compound from Panax notoginseng, reduces Aβ plaque burden in the cortex and hippocampus of transgenic AD mice [11]. Moreover, spleen-invigorating herbs can modulate gut microbiota composition, aiding in the recovery from spleen deficiency syndrome [12,13]. Given the role of gut flora in AD, we hypothesized that a spleen-deficient environment might exacerbate microbial dysbiosis, accelerating AD onset and progression.

Materials and Methods

Animals

Forty healthy male Sprague-Dawley rats (6–8 weeks old, 180–220 g) were obtained from the Laboratory Animal Centre of Sun Yat-sen University (Guangzhou, China). They were housed under controlled conditions (24–26°C, 45–55% humidity, 12-hour light/dark cycle) with ad libitum access to food and water. All procedures were approved by the Clinical Research and Laboratory Animal Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and complied with National Institutes of Health guidelines.

Aβ (1-42) Administration for the AD Induction

The AD model was crafted by injecting Aβ (1-42) intraventricularly with reference to methods frequently used in other studies [14,15]. Briefly, distilled water was used to dissolved the Aβ (1-42) peptide (Abcam, ab120301, UK) at a concentration of 1 mg/ml and incubated at 37 °C for one week before application. After observation of the fibrillary form of beta amyloid through light microscope, the rats were anesthetized with 3% pentobarbital sodium (30 mg/kg) intraperitoneally and secured on a stereotaxic apparatus (MD-3000, Bioanalytical Systems, Inc., US). We then drilled holes in the skull to access the lateral ventricles, using the following coordinates: AP: + 1.3 mm, ML: ± 2.0 mm, and DV: −4.0 mm. The needle of the Hamilton microsyringe (26 gauge) (Hamilton Company) was placed 4 mm beneath the surface of the skull. 5 μg /5 μl of the solution was injected into the right lateral ventricle, and then 5 μg/5 μl of the solution was injected into the left cerebral ventricle at a speed of 0. 2 μL/min. After injection, the needle was held still for 5 minutes to ensure full diffusion of the solution, after which it was slowly removed at a speed of 0.5 mm/min. Subsequently, bone wax was used to close the drilled hole, and the skin was stitched. Post-surgery, each rat received a 100,000 U intramuscular penicillin injection daily for a duration of 5 days.

Reserpine Administration for Spleen Deficiency Model

Reserpine (MACKLIN, R817202-1 g, China) was dissolved in glacial acetic acid (1 mg/mL) and diluted with saline to 0.01 mg/mL. Rats received subcutaneous injections at 0.5 mg/kg/day for 14 days [16-19]. General condition—including mental state, respiration, food intake, body weight, activity, stool consistency, and fur quality—was monitored and scored based on a previously established scale [19]. Seven parameters were scored from 1 (normal) to 4 (severe). Total scores below 7 indicated non-spleen deficiency, 7–14 mild deficiency, 15–21 typical deficiency, and above 22 severe deficiency.

Animal Experimental Procedure

After one week of acclimation, rats were randomly assigned to four groups (n = 10 each): normal control group (NC), AD model group (AD), spleen deficiency model group (SD), and AD combined spleen deficiency model group (AD+SD). The AD+SD group received both Aβ (1–42) and reserpine. Control animals received equivalent volumes of saline. Spleen deficiency scores were recorded for 14 days. Fecal samples were collected before behavioral testing using the Morris water maze. Rats were then euthanized with pentobarbital sodium (30 mg/kg, i.p.), and brain tissues were harvested for analysis.

Morris Water Maze (MWM) Test

Spatial learning and memory were assessed using the MWM as described previously [20]. The apparatus consisted of a circular pool (120 cm diameter, 50 cm high) filled with water (22 ± 1 °C) to a depth of 35 cm. A hidden platform (10 cm diameter) was placed 2 cm below the water surface in quadrant III. Rats underwent two daily trials for five consecutive days. On day 6, the platform was removed, and probe trials were conducted to measure escape latency, time in target quadrant, swimming distance, speed, and platform crossings. The experimenter was blinded to group assignments.

Brain Tissue Preparation

After behavioral tests, rats were perfused intracardially with 0.01 M PBS (KH_2PO_4, Na_2HPO_4, KCl, NaCl, pH 7.4) under deep anesthesia. Brains were removed, fixed in 4% paraformaldehyde for 6–8 hours, and cryoprotected in 30% sucrose PBS.

Immunohistochemistry

Brain sections were processed as described [20]. Briefly, sections were blocked with 3% H_2O_2 and 10% methanol, then with 10% normal donkey serum in 0.3% Triton X-100 PBS. They were incubated with anti-Aβ (1–42) primary antibody (Abcam, 1:2000, USA) at room temperature for 1–2 hours or overnight at 4 °C. After washing, sections were incubated with HRP-conjugated secondary antibody (Abcam, 1:5000, USA) for 1 hour, developed with diaminobenzidine (Vector SG kit), and imaged using a digital camera (IX71; Olympus, Japan).

16S rRNA Gene Sequencing Technology

DNA from twenty fecal specimens was successfully extracted using the cetyl trimethylammonium bromide technique [21]. The purity and amount of the extracted DNA were determined by a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA) and 1% agarose gel electrophoresis. The DNA was diluted to a concentration of 1 ng/μl with sterile water. The V3-V4 regions of the 16S rRNA gene were amplified via a thermocycler PCR system (GeneAmp 9700, ABI, USA). A total of 10 ng of DNA was subjected to polymerase chain reaction (PCR). The reaction mixture (30 μl) consisted of 15 μl of Phusion® High-Fidelity PCR Master Mix (New England Biolabs) supplemented with 0.2 μM forward or reverse primers. primers, and approximately 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, elongation at 72 °C for 60 s, and finally 72 °C for 5 min. The obtained material was detected via 2% agarose gel run, with the targeted bands retrieved by slicing the gel and employing an AxyPrepDNA gel extraction kit (AXYGEN, USA). Sequencing was performed on the Illumina MiSeq/HiSeq2500 platform (Illumina, San Diego, USA) by Shanghai Applied Protein Technology Co. Ltd. (Shanghai, China) using standard protocols.

Processing of Sequencing Data

The sequencing data were analyzed following the protocols outlined in our prior research [22]. Raw sequences were quality-filtered and assembled using Trimmomatic and FLASH [23]. Operational taxonomic units (OTUs) were grouped at a 97% similarity threshold and subsequently categorized via the RDP Classifier algorithm (http://rdp.cme.msu.edu/) using the Silva (SSU123) 16S rRNA database, applying a 70% confidence cutoff. Alpha diversity was assessed using the Shannon index, rarefaction curves, and rank-abundance plots. Beta diversity was evaluated via PCoA based on unweighted UniFrac distances. LEfSe was used for biomarker discovery [24], and PICRUSt was employed for functional prediction against KEGG pathways [25,26].

Statistical Analysis

Data are expressed as mean ± SD. One-way ANOVA with Tukey's post hoc test was used for group comparisons (SPSS 26.0). A p-value < 0.05 was considered statistically significant.

Results

Spleen Deficiency Induction and Behavioral Effects

Rats in the SD group exhibited lethargy, piloerection, and curling behavior. The average spleen deficiency score (17.6 ± 1.939) was significantly higher than in NC rats (5.9 ± 0.5831, Figure 1A, $p < 0.01$). AD+SD rats showed similar symptoms, while AD rats appeared normal. Spleen deficiency scores differed significantly among groups (Figure 1A, $p < 0.01$), confirming successful model induction. In the MWM test, all groups showed reduced escape latency over five training days. However, D rats displayed significantly prolonged latency on day 5, along with decreased target quadrant distance, duration, speed, and platform crossings (Figures 1C-1G, $p < 0.05$). AD rats also showed impaired performance compared to NC rats (Figures 1C-1G, $p < 0.05$). The AD+SD group exhibited the most severe cognitive deficits (Figures 1C-1G, $p < 0.05$), indicating that spleen deficiency exacerbates AD-like behavior.

Aβ Deposition in the Hippocampus

Minimal Aβ deposition was observed in the hippocampus of AD and SD rats, whereas AD+SD rats showed pronounced Aβ plaque accumulation in the CA1 region, suggesting that spleen deficiency accelerates AD pathology (Figure 2).

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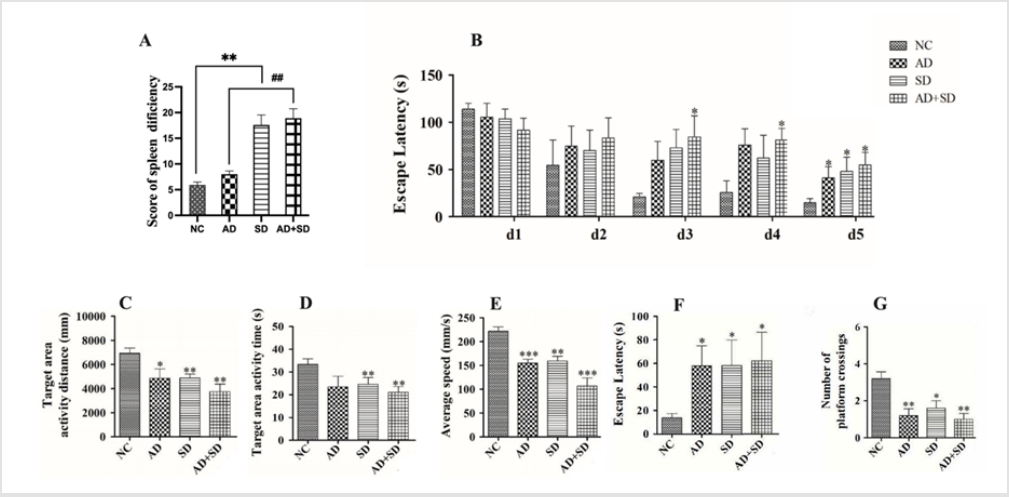

Figure 1: Assessment of the establishment of a spleen deficiency environment and its influence on the behavior of rats. The rats in the AD, SD and AD+SD groups received Aβ (1--42) or Aeserpine or Aβ (1--42) combined with Reserpine. Saline at the same volume was administered in the same way as the control.

- A: Spleen deficiency score
- B: Escape latency in the place navigation test during the first 5 training days
- C: Target area activity distance
- D: Target area activity
- E; Average speed
- F: Escape latency
- G: Number of platform crossings in the spatial probe test on day 6. All data are presented as the means SEMs, and derived from three or more independent experiments. *p <0.05, *p<0.01, and ***p<0.001 versus normal control rats. NC, normal control; AD, Alzheimer's disease; SD, spleen deficiency.

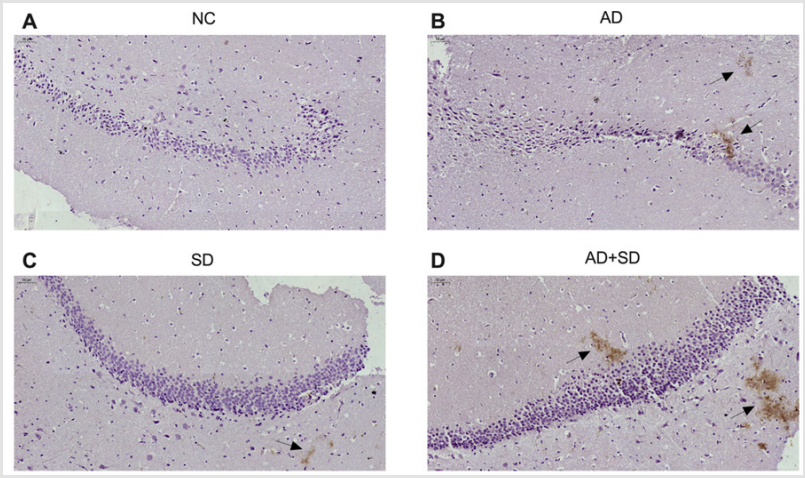

Figure 2: Deposition of the Aβ1-42 protein in the hippocampus. The rats in the AD, SD and AD+SD groups received Aβ (1--42) or Aeserpine or Aβ (1--42) combined with Reserpine. Saline at the same volume was administered in the same way as the control. A deficiency in the spleen promoted the deposition of the Aβ1--42 protein in the hippocampus.

- A: NC group
- B: AD group
- C: SD group
- D: AD+SD group. All data are derived from three or more independent experiments, with representative hippocampal Aβ (1-42) immunohistochemistry images taken by a digital camera (IX71; Olympus, Tokyo, Japan) displayed. NC, normal control; AD, Alzheimer's disease; SD, spleen deficiency.

Gut Microbiota Composition

Alpha diversity (Chao1 index) was reduced in AD and SD groups compared to NC (Figure 3A, $p < 0.05$), but no significant differences were found among other groups. Rank-abundance curves showed no notable variations (Figure 3B, $p > 0.05$). PCoA revealed distinct separation between NC and SD groups (Figure 3C), but not between SD and AD+SD groups (Figure 3D). At the phylum level, Firmicutes was most abundant. Bacteroidetes decreased in AD and SD groups, while Firmicutes and Proteobacteria increased (Figure 3E). AD+SD rats showed further reduction in Bacteroidetes and increases in Proteobacteria, Actinobacteria, and Spirochaetes. (Figure 3E). At the genus level, Lactobacillus was predominant. Its abundance increased in AD and AD+SD groups, while Prevotella decreased. *Leptothrix*, Facklamia, and Corynebacterium were more abundant in AD+SD rats than in AD rats.

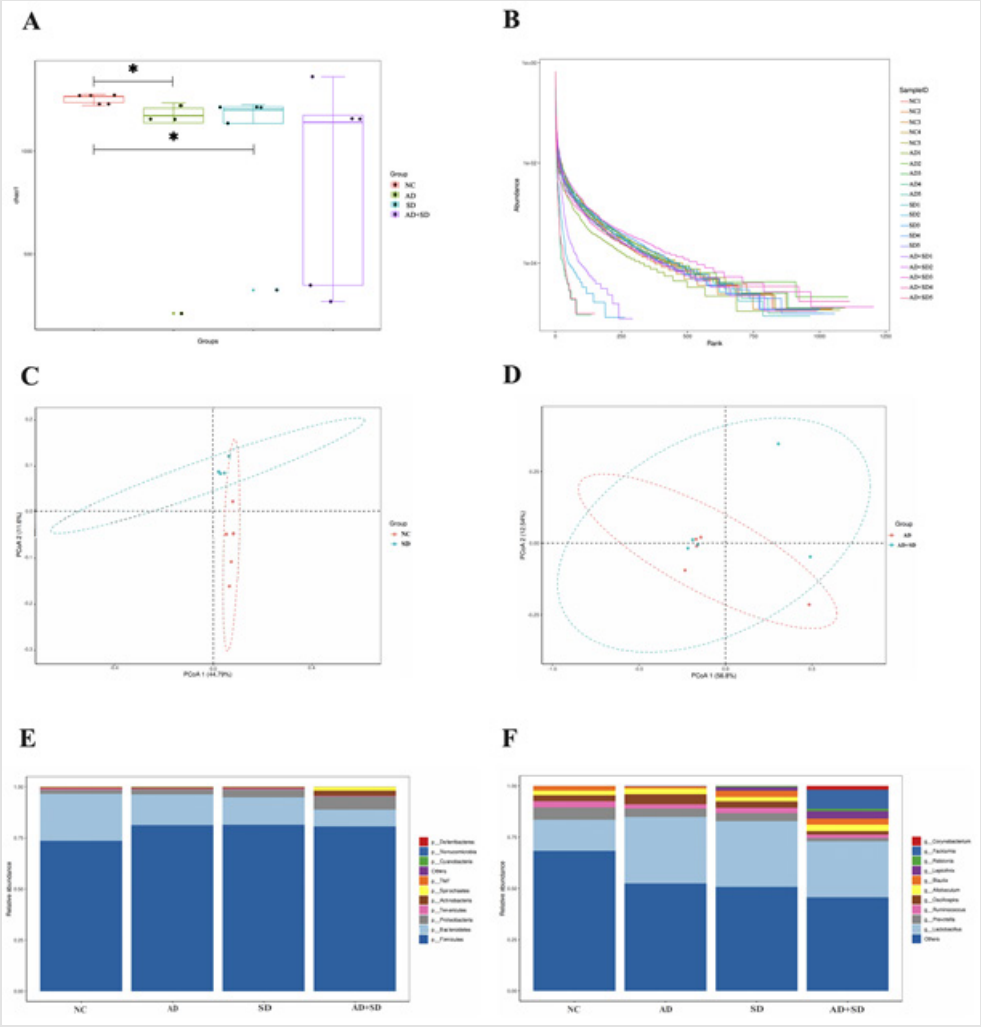

Figure 3: Effects of the spleen deficiency environment on the overall structure of the gut microbiota in AD patients. Rats in the AD, SD or AD+SD groups received Aβ (1--42) administration, aeserpine administration or Aβ (1--42) combined with reserpine injection, respectively. Saline at the same volume was administered in the same way as the control.

- A: Chao 1 index
- B: Rank-abundance curve
- C: And
- D: Unweighted UniFrac-based PCoA
- E: Phylum level
- F: Genus level. N=5 for each group. For the Chao 1 index, differences were assessed via one-way ANOVA followed by Tukey's multiple comparisons test. For unweighted UniFrac-based PCoA, differences were assessed via ANOSIM. NC, normal control; AD, Alzheimer's disease; SD, spleen deficiency.

Differential Microbial Abundance

LEfSe analysis identified *Burkholderiales* and *Pseudomonadales* as enriched in SD rats, while *Synechococcales* was more abundant in NC rats (LDA > 2, Figure 4A, $p < 0.05$). At the genus level, *Leptothrix*, *Pseudomonas*, and Lactococcus were increased in SD rats, whereas *Synechococcus* and *Ruminococcus* were higher in NC rats (Figure 4A, $p < 0.05$). Corynebacterium, *Jeotgalicoccus*, Staphylococcus, and *Psychrobacter* were enriched in AD+SD rats but not in AD rats (Figure 4B, $p < 0.05$). Tukey's test revealed decreased Bacteroides, *Ruminococcus*, and *Burkholderia* in AD and SD groups versus NC (Figures 4C-4D, $p < 0.05$). Parabacteroides was higher in AD rats, while Corynebacterium, *Turicibacter*, and Acinetobacter were lower in SD rats (Figure 4E, $p < 0.05$). AD+SD rats showed reduced Prevotella, Bacteroides, and *Burkholderia*, along with decreased Acetobacter and *Nitrospira* and increased Rhizobium compared to AD rats (Figure 4F, $p < 0.05$).

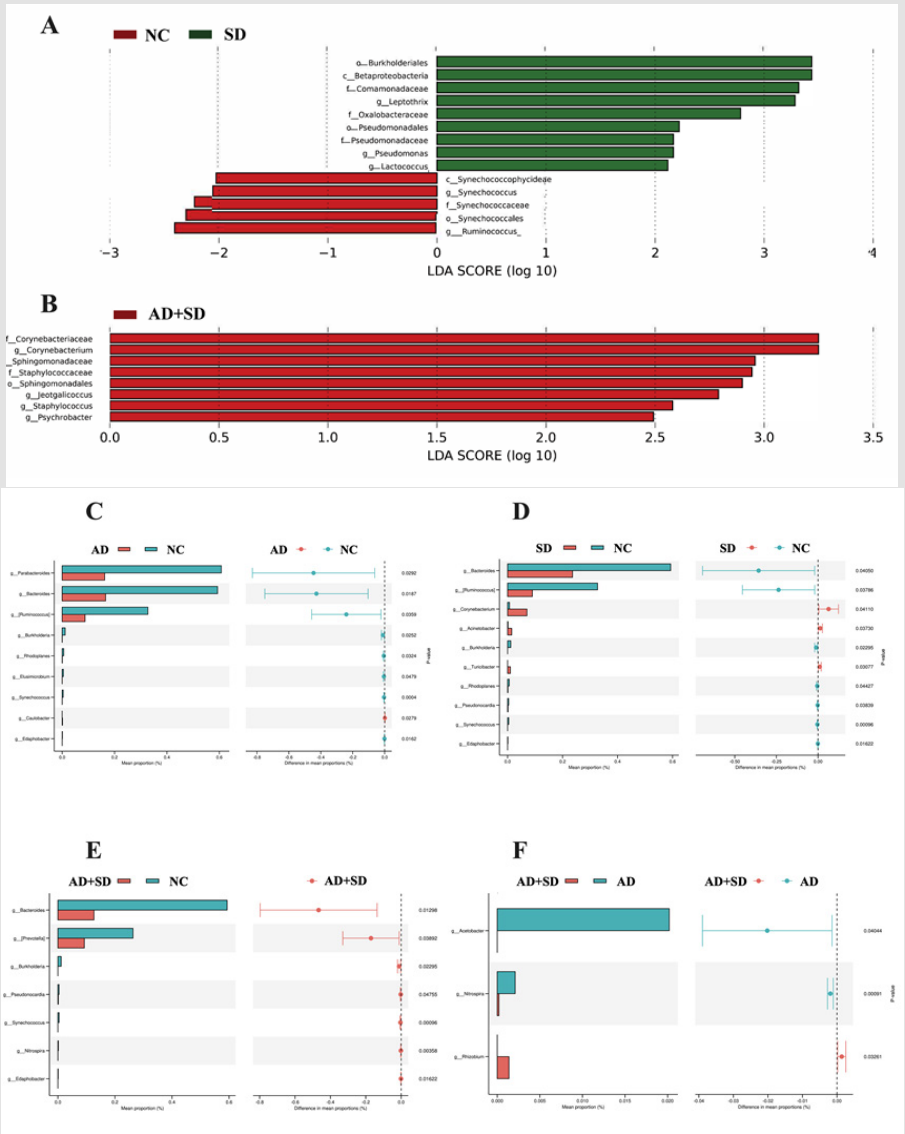

Figure 4: Effects of the spleen deficiency environment on key phenotypes of the gut microbiota in AD. Rats in the AD, SD or AD+SD groups received Aβ (1--42) administration, aeserpine administration or Aβ (1--42) combined with reserpine injection, respectively. Saline at the same volume was administered in the same way as the control. LDA effect size analysis contrasted enriched taxa across groups:

- A: NC group vs. SD group
- B: AD + SD group vs. AD group. The bar chart illustrates taxa with notable differences (scores exceeding 2).
- C: To
- F: Inter-group genus-level abundance contrasts. Statistical significance via ANOVA with post-hoc Tukey's test. NC, normal control; AD, Alzheimer's disease; SD, spleen deficiency.

Pathway Enrichment Analysis

KEGG analysis identified 13 pathways associated with microbial changes in spleen deficiency, including carbohydrate metabolism, DNA replication and repair, translation, nucleotide metabolism, lipid metabolism, and genetic information processing (Figure 5A). STAMP analysis confirmed differences between SD and NC groups, and between AD and AD+SD groups in cell communication pathways (Figures 5B-5C, $p < 0.05$).

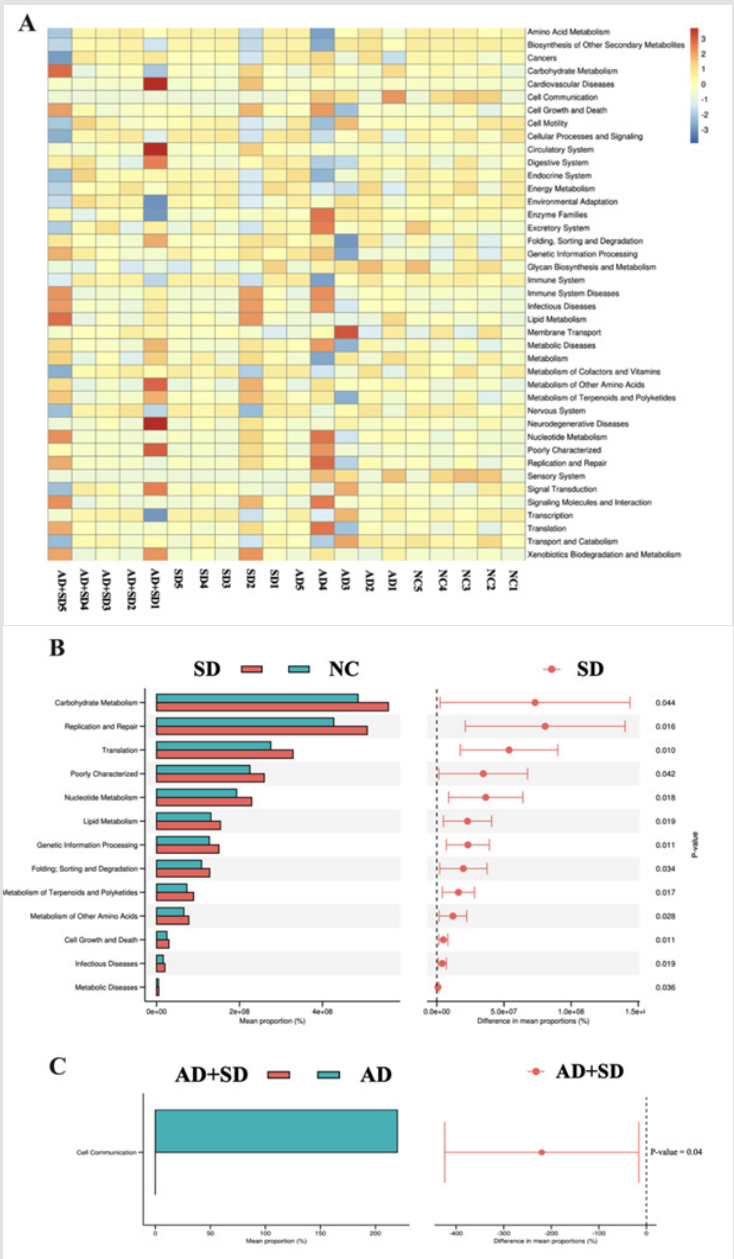

Figure 5: Pathway enrichment analysis of the gut microbiota. The rats in the AD, SD and AD+SD groups received Aβ (1--42) or Aeserpine or Aβ (1--42) combined with Reserpine. Saline at the same volume was administered in the same way as the control. To identify the metabolic pathways related to the gut microbiota involved in AD and spleen deficiency, we performed KEGG annotation and integrated pathway enrichment analysis with STAMP analysis.

- A: KEGG heatmap
- B: and
- C: STAMP results of KEGG. Differences were assessed by Welch's t test. NC, normal control; AD, Alzheimer's disease; SD, spleen deficiency.

Discussion

This study demonstrates that a spleen-deficient internal environment exacerbates AD progression in rats. It induces cognitive impairment, accelerates Aβ deposition in the hippocampus, and disrupts gut microbiota homeostasis. These effects may be mediated through enrichment of pro-inflammatory and Aβ-promoting bacteria, and reduction of beneficial SCFA-producing bacteria. Subcutaneous injection of reserpine is a well-established method for inducing a spleen deficiency model [27,28]. The symptoms of spleen deficiency induced by reserpine were pronounced and persistent, with minimal recovery observed even 3 to 4 weeks after injection. In line with our previous studies [16-18]. we administered reserpine consecutively for 14 days to establish the model, which yielded consistent and reproducible results. The model rats exhibited characteristic symptoms, including lethargy, chills, and loose stools. The total spleen deficiency score reached 17.6 ± 1.939, indicating a typical spleen-deficient state according to the assessment criteria. Furthermore, the Alzheimer's disease (AD) model was successfully established via bilateral intraventricular injection of amyloid β protein (Aβ)1–42. In the spleen-deficient rats, significant cognitive impairment was observed, characterized by prolonged escape latency, reduced movement distance in the target area, decreased swimming speed, and fewer platform crossings in behavioral tests.

Moreover, spleen deficiency appeared to exacerbate AD progression, as rats subjected to both reserpine and Aβ1–42 exhibited more severe cognitive decline and hippocampal damage compared to those receiving either treatment alone. These findings imply that a spleen-deficient internal environment may serve as a key pathogenic factor in the development of AD. In recent years, numerous studies have highlighted the role of intestinal flora as a key communicative interface between the gut and the brain—a relationship termed the brain-gut axis [4-6]. Growing evidence further suggests a strong association between gut microbiota dysbiosis and the pathogenesis of Alzheimer's disease (AD), making microbiota modulation an attractive target for AD prevention and treatment [29]. Although alpha and beta diversity analyses in our study did not reveal significant overall structural differences between groups, more refined LEfSe and LDA analyses identified several taxa with altered abundance that have previously been linked to AD. Notably, *Proteobacteria* and *Actinobacteria—both* implicated in neuroinflammation—were enriched under pathological conditions. Lipopolysaccharide (LPS), a major component of the *Proteobacterial cell* wall and a potent endotoxin, has been detected in hippocampal and supratemporal neocortical lysates from AD patients [30]. LPS levels are reported to be 26-fold higher in AD patients than in healthy controls [30], and have been shown to promote neuroinflammation [31].

Consistent with these findings, our results showed a marked increase in Proteobacteria abundance in AD model rats compared to normal controls (NC). A similar increase was observed in spleen-deficient (SD) rats, with the highest abundance seen in the AD+SD group. LEfSe and STAMP analyses further indicated that spleen deficiency may influence AD progression through genus-level microbial shifts. Specifically, we observed a significant reduction in *Bacteroides and Ruminococcus* in SD rats compared to controls. These commensal bacteria are known to ferment indigestible carbohydrates such as plant polysaccharides into short-chain fatty acids (SCFAs). SCFAs can cross the blood-brain barrier via circulation and enhance its integrity by upregulating tight junction proteins, thereby reducing the deposition of neurotoxic agents like Aβ and attenuating neuroinflammation [32]. Additionally, SCFAs contribute to the intestinal immunological barrier, promote colonization of anti-inflammatory bacteria, and inhibit the growth of pathogens [32]. In contrast, genera such as *Pseudomonas and Lactococcus* were significantly elevated in SD rats. *Pseudomonas* can produce bacterial amyloid proteins that facilitate Aβ aggregation and deposition in the brain, exacerbating neuroinflammation and AD pathology [33]. Lactococcus, often pathogenic in humans, is associated with insulin resistance and upregulation of pro-inflammatory genes in the gut [34]. Since insulin signaling is known to influence Aβ clearance and tau phosphorylation, such changes may contribute to neurodegenerative processes [35].

Moreover, *Staphylococcus* was notably more abundant in AD rats with spleen deficiency than in controls, and not enriched in AD-alone rats. Similar to *Pseudomonas*, *Staphylococcus* can produce amyloid proteins that promote Aβ deposition [33]. Clinical studies have reported elevated *Staphylococcus* abundance in AD patients, and its reduction following treatment correlates with improved cognitive and functional outcomes [36]. These results suggest that spleen deficiency induces AD-like microbial alterations that may accelerate disease onset or progression. The hallmark pathological features of AD include Aβ plaques, neurofibrillary tangles composed of hyperphosphorylated tau, and neuronal degeneration [37]. Our study demonstrates that a spleen-deficient environment promotes Aβ deposition in the hippocampal CA1 region, exacerbating AD-like pathology and cognitive impairment in rats. KEGG pathway analysis further revealed that spleen deficiency perturbs several metabolic pathways—including carbohydrate, lipid, and nucleotide metabolism—as well as pathways involved in metabolic diseases. These findings suggest that gut microbiota dysbiosis may influence AD through metabolic reprogramming. However, further experimental validation is required to identify the specific signaling mechanisms critical in AD initiation and progression.

Conclusion

In conclusion, this study provides preliminary evidence supporting an association between the internal environment of the spleen and the pathogenesis of Alzheimer's disease. We demonstrated that spleen deficiency facilitates the deposition of Aβ1-42 in the hippocampal CA1 region, thereby exacerbating cognitive impairment. The modulation of intestinal flora balance may play a critical role in mediating these effects.

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Conflict of Interest

None.

Acknowledgement

None.

Author Contributions

Li Zhang: Conceptualization, Methodology, Validation, Writing – Original Draft Preparation, Writing – Review & Editing. Yuan-yuan Wang: Methodology, Validation, Data Curation, Writing – Original Draft Preparation, Writing – Review & Editing. Tingying Zhang: Methodology, Validation. Kun Zhan: Validation. Ying-juan Huang: Conceptualization, Data Curation, Supervision.

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Declaration of Interests

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

- The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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