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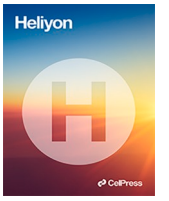

Research article

The gut microbiota regulates the depressive-type behaviors and inflammatory processes after severe burn injuries in mice

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ABSTRACT

An emerging number of studies have recently revealed the correlation between burn injuries and psychological disorders. Gut microbiota and inflammatory factors may play a vital role in this process. Nevertheless, there are few studies conducted to disclose the potential mechanism of the gut microbiota between depression and burn injuries. In this study, we constructed a burn model of C57BL/6 mice, which showed that the symptom of depression became more and more severe with the burn of mice lasted longer. Meanwhile, there are significant differences of composition of gut microbiota among mice before and after burn. Then, we tested the inflammatory factors in the brain and peripheral blood, which showed an increased expression of Iba1, VWF, TNF-α and IL-6, and a decreased expression of IL-10 in burn mice. In addition, the expression of zonula occludens-1 (ZO-1) in cecum showed a down-regulation in burn mice, which indicated impaired intestinal barrier function. Lastly, the crossing fecal microbiota transplantation (FMT) and cohousing experiment were conducted to determine the functions of cross-transplantation of fecal microbiota on the depressive-type behaviours in burned mice. According to the score of Tail suspension test (TST), the burn mice were divided into two groups: Resilient mice (no-depressed mice) and Abnormal mice (depressed mice). After abnormal mice were transplanted with fecal microbiota of resilient mice, the symptom of depression was improved, and the expression of TNF-α, IL-6 and IL-10 return to normal levels ($P < 0.05$). On the contrary, after resilient mice were transplanted with fecal microbiota of abnormal mice both the TST scores and inflammatory factor developed depressive-type changes. In conclusion, our study demonstrated the changes of gut microbiota and inflammatory factors in depressed burn mice and non-depressed burn mice. The gut microbiota dysbiosis could impaired intestinal barrier function and lead to neuroinflammation, and this phenomenon could be significantly mitigated by FMT.

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1. Introduction

The survival rate of patients with severe burn injuries has improved dramatically with advances in treatment and the establishment of comprehensive burn centers [1]. However, many burn survivors still face significant rehabilitation challenges, such as pain, scar contractures, and psychosocial adjustment disorders [2,3]. Depressive symptoms are reported as the most common psychological disorders [4–7]. In addition, due to limb deficiency, patients with severe burns often experience significant distress and social anxiety [8]. As reported in a 5-year follow-up study of 18,198 patients after burn injury, 29.4% of them developed at least one type of psychiatric disorders. In addition to anxiety disorders, the results revealed that burn injuries are associated with depression, severe stress disorders, and somatoform disorders [9]. However, the mechanisms underlying vulnerability to depression and stress disorders following severe burn injuries remain unclear.

The gut microbiota has been increasingly shown to play important roles in depression and stress disorders [10,11]. Significant alternations in the gut microbiota have been identified in mood disorders in both human studies and animal models. For instance, the gut microbiota of patients with major depression disorder is characterized by a relatively increased abundance of *Actinobacteria*, Firmicutes, and Bacteroidetes [12,13]. It has been reported that the abundance of butyrate-producing *Faecalibacterium* and *Coprococcus* bacteria correlates with a higher quality of life, and the depletion of *Dialister* and *Coprococcus* spp. is associated with depression [14]. Moreover, probiotic and prebiotic treatments have been shown to play a facilitating role in alleviating stress responses, anxiety, and depression [15,16]. We have previously reported that the composition of the gut microbiota is associated with the effectiveness and responsiveness to psychological therapy in the treatment of mood disorders [17].

Emerging studies have confirmed that alterations in the composition of the gut microbiome at various taxonomic levels occur after severe burn injuries. For example, there is an increase in the abundance of opportunistic pathogenic bacteria such as the genera *Escherichia* and *Shigella* and the phylum *Proteobacteria*. In addition, alterations in the gut microbiota have been shown to be involved in the development of severe complications, such as infections after burn damage. Dysbiosis of the gut microbiota disrupts the intestinal epithelial barrier, worsens intestinal permeability, and augments subsequent bacterial translocation and endotoxin production, which may be responsible for sepsis, systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and other fatal complications following burn injury.

Taken together, these findings imply that the gut-brain-skin axis may be a potential mechanism for the development of depression after severe burns. This axis describes the bidirectional communication networks between the gut microbiota, central nervous system, and skin health through cytokines, neurotransmitters, enteroendocrine, and immune pathways [18]. Among the above pathways, increased inflammation has been the most studied and has been reported to be a contributing factor in stress-related mood disorders. In previous studies, an increased level of pro-inflammatory processes in the hippocampus has been associated with an increase in depression-type behavior [19]. However, few studies have been conducted to examine the association between the gut microbiota and depression after severe burn injuries, the potential mechanism, or the roles of neuroinflammation in the interaction between the gut microbiome and depression after burn.

Therefore, the aims of this study were to (1) clarify the characteristics of gut microbiome patterns in severely burned mice with depressive-type behaviors compared to those without; (2) investigate the expression of neuroinflammation -related factors and changes in neuroinflammation in the hippocampus in animal models of depression; and (3) determine the effect of cross-transmission of fecal microbiota on depressive-type behaviors in burned mice.

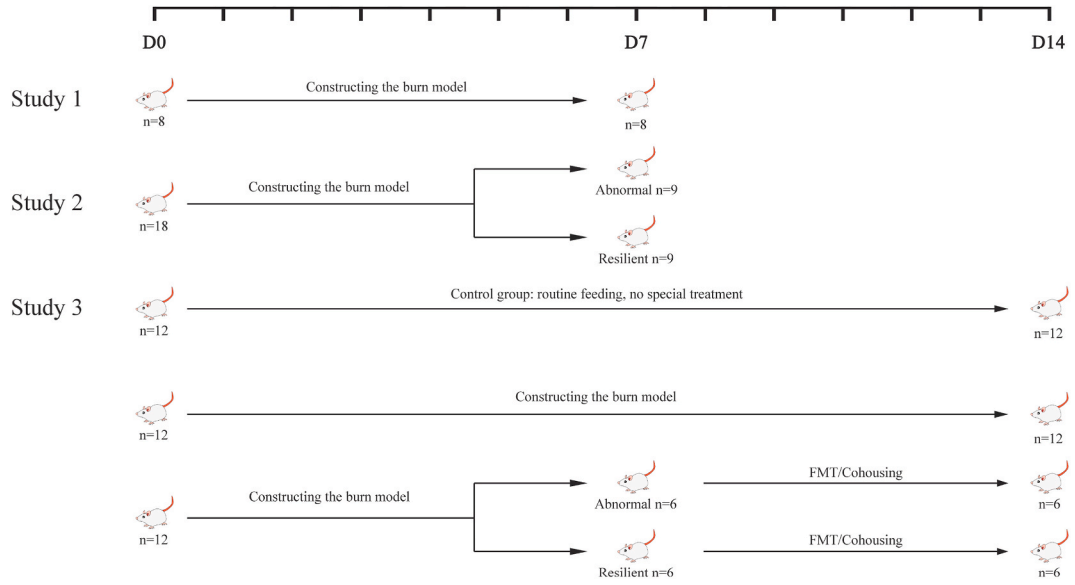

Fig. 1. Study flowchart.

2. Materials and methods

2.1. Animals

Healthy male C57BL/6 mice, approximately 7–8 weeks old, weighing approximately 17–22 g were used for the study and were obtained from the Animal Center of the Army Medical University. All animals were acclimated to the environment for 7 days prior to conducting the experiments. They were kept at approximately 24–26 °C temperature, 55% humidity with 12-h of alternating light and food and water provided ad libitum.

2.2. Model preparations and experimental procedures

Fig. 1 shows a flowchart of the study. Before performing the experimental procedures, all mice were divided into scald-burned and sham-burned (control) groups according to the random number table method. A burn model was constructed according to a model previously used in our laboratory [20]. First, the mice were anesthetized intraperitoneally with tribromoethanol (0.2 mL/10 g), and the dorsal fur was removed and prepared for scalding. The nude skin was then placed into a plastic template with an estimated 30% total body surface area and immersed in water at 95.0 °C for 10 s. Following the burn, the mice were intraperitoneally administrated sterile saline (1.5 mL) to prevent shock. They were placed on a 42.0 °C heating pad to prevent hypothermia until they recovered from the anesthesia. A daily single subcutaneous injection of 0.2 mg/kg of the analgesic buprenorphine was administered to minimize pain in the experimental animals [21], until the mice were sacrificed. The mice in the control group received the identical experiment as the burned group, expect that they were immersed in 25.0 °C water. Three to five pieces of feces were collected from each mouse and stored in a 1.5 mL enzyme-free centrifuge tube. All samples were kept in an ultra-low-temperature freezer at −80.0 °C until analysis. At the end of the experiment, the control mice were sacrificed.

All burned mice were carefully monitored in an intensive animal care facility until the experimental endpoint. Before each test, the mice were acclimated to the experimental room for at least 1 h prior to behavioral testing. On day 7 (the seventh day after the burn model was established), behavioral tests were performed and fecal samples were collected. We employed a tail suspension test (TST) to assess depressed-like behaviors [22] by suspending the mice above the ground and examining their length of immobility, a state indicating desperation or helplessness. In brief, each mouse was suspended upside down with 2 cm at the end of their tail adhered to a hook. The hook was connected to the middle top of a standard three-walled rectangular chamber (XR-XX203; Xinruan Technology Company, Shanghai). Clear hollow cylinders were provided by the manufacturer and placed around the tails of the mice to prevent tail-climbing behavior. The entire experiment lasted 6 min and mouse movements were recorded with a video camera. The cumulative immobility time during the last 5 min was calculated using VisuTrack software (XR-VT; Xinruan Technology Company, Shanghai). The immobility time was defined as the time when there was no body movement and passive hanging [23]. Increases in immobility indicate an increase in depressed-like behavior [24]. After each experiment, a 75% alcohol pad was used to wipe the box thoroughly and clean the feces or urine to eliminate any interference between each mouse.

According to the TST analysis and the extreme grouping method of psychometrics [25], the first 27% of the burned mice having high levels of immobility time were labelled as the “abnormal” group, and the last 27% were labelled as the “resilient” group. The abnormal group included mice that were more likely to show depressive-type behaviors, whereas the resilient group included mice that were less likely to show depressive-type behaviors. Fecal samples in both subgroups were collected on the following 7 consecutive days to prepare for the next step of fecal microbiota transplantation (FMT). At the end of fecal collection, the burned mice were sacrificed under general anesthesia with tribromoethanol to detect intestinal permeability and inflammatory cytokines. Feces, blood, and ileum samples were prepared for DNA isolation, enzyme-linked immunosorbent assay (ELISA), and microbiota community analysis, as described below.

Blood samples were collected from the ventricle using a 1.5 mL Eppendorf tube pre-lubricated with an anticoagulant, and then centrifuged at 4000 rpm at 4 °C for 15 min. The serum was collected and stored in an ultra-low-temperature freezer at −80 °C prior to analysis. Whole brain and hippocampus samples were collected immediately after dissection, frozen in liquid nitrogen, and stored at −80 °C for further analysis.

2.3. Fecal microbiota transplantation

On day 14, a fecal slurry was made from “abnormal” and “resilient” burn donors by diluting the stool with phosphate-buffered saline (PBS; 0.1 g of fecal sample/1.5 mL). The slurry was then centrifuged at 1500 rpm at 4 °C for 5 min. The suspension was centrifuged at 8000 rpm at 4 °C for 5 min to obtain total bacteria, and then filtered twice in PBS. The final bacterial suspension was mixed with an equal volume of 40% sterile glycerol to a final concentration of 20%, and was then stored at −80.0 °C until FMT [26].

Two hundred microliters of bacterial suspension (108 CFU/mL) was administered by oral gavage once daily for 7 consecutive days into two groups of naïve recipient mice [27]. One group of mice received feces from abnormal donors, whereas the other group received feces from resilient donors. The abnormal and the resilient recipient mice were separately bred to prevent normalization of their gut microbiota. The TST was performed 2 weeks after FMT in both groups of naive recipient mice. After the test, the abnormal and control mice were decapitated under anesthesia, and cecum, serum, and hippocampus samples were collected.

2.4. Cohousing experiment

In the cohousing experiment to study spontaneous microbiota transfer, the microbiota was exchanged through coprophagia [28]. Age- and sex-matched abnormal and resilient mice were cohoused at a 1:1 ratio for 5 weeks. To reduce the possibility of the mice fighting, one "abnormal" mouse and one "resilient" mouse were cohoused in one cage.

2.5. 16S rRNA gene sequencing

Microbial DNA was extracted from fecal samples using the cetyltrimethylammonium bromide according to the manufacturer's instructions. For sequencing, a DNA fragment comprising the bacterial hypervariable regions, V3–V4, of the *16S rRNA* gene was amplified using with the primers 341F (forward primer, 5′-CCTAYGGGRBGCASCAG-3′) and 805R (reverse primer, 5′-GGACTACNNGGGTATCTAAT-3′) [29]. The concentration and purity of the extracted DNA was measured using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and DNA integrity was assessed by 1% agarose gel electrophoresis. Total DNA was eluted in elution buffer (50 μL) and stored at −80 °C prior to polymerase chain reaction (PCR) analysis. PCR amplification and *16S rRNA* gene sequencing were performed by the Lianchuan Biomedical Technology Corporation (LC-Bio; Hang Zhou, China).

Five microliters of the primers were tagged with specific barcodes for each sample and the universal primers. The bacterial *16S rRNA* gene fragments were amplified in a total reaction volume of 25 μL, which contained 25 ng of template DNA, 12.5 μL of PCR premix, 2.5 μL of each primer, and PCR-grade water to adjust the volume to 25 μL. The PCR conditions used to amplify prokaryotic *16S rRNA* genes were as follows: (1) initial denaturation at 95 °C for 2 min; (2) 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min; and (3) a final extension 72 °C for 5 min. We then used 2% agarose gel electrophoresis to confirmed the quality of the PCR products, the AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) to purify the PCR products, and a Qubit instrument (Invitrogen, Carlsbad, CA, USA) to quantify the PCR products. Amplicon pools were then prepared for sequencing. The size and quantity of the DNA libraries were assessed using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and a Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. DNA libraries were sequenced using the NovaSeq PE250 platform (Illumina, San Diego, CA, USA) [30].

2.6. Mechanism analysis

The ionized calcium-binding adapter molecule, Iba1, is a typical marker of neuroinflammation and von Willebrand factor (vWF) is a marker of blood vessels. The two markers were assessed using immunohistochemical (IHC) analysis of sections of hippocampal samples, as previously described. The scaffolding protein zonula occludens-1 (ZO-1), is a typical marker of tight junction permeability, which is dispensable for barrier function, but critical for effective mucosal repair. It was assessed by IHC analysis of cecum sections, as previously described. The tissues were sliced in 20 μm sections, embedded in paraffin, and stained with hematoxylin-eosin (HE). The slides were then deparaffinized and incubated overnight at 4 °C with primary antibodies against Iba-1 (Genetex, Irvine, CA, USA; Gtx100042), vWF (Biorbyt, Cambridge, UK; Orb227742) and ZO-1 (Abcam, Cambridge, UK; Ab2272). The secondary antibodies used were goat anti-rabbit A0277 (Biyuntian, Beijing, China) and goat anti-mouse IB-0021 (Dingguo, Beijing, China). The membranes were analyzed using a LI-COR Odyssey infrared imager (LI-COR Biosciences, Lincoln, NE, USA) and band intensities were measured using ImageJ densitometry.

The levels of the anti-inflammatory cytokines, interleukin (IL)-6 (mLbio, Shanghai, China; mL002293), IL-10 (mLbio, mL002285) and tumor necrosis factor-α (TNF-α) (mLbio, mL002095) in plasma were detected by ELISA analysis according to manufacturer's protocols.

2.7. Data analysis

For fecal microbiota analysis, samples were sequenced on the Illumina NovaSeq platform according to the manufacturer's protocol, provided by LC-Bio. Raw reads were filtered under specific conditions to obtain high-quality clean reads using fqtrim (v0.94). Chimeric sequences were filtered using Vsearch software (v2.3.4), and the repetitive sequences were removed by DADA2, which contributed to obtaining precise feature tables and feature sequences. Feature abundance was then normalized to the relative abundance based on the SILVA (release 138) classifier for each sample.

To assess microbiota diversity and complexity, the Chao1, observed species, and Simpson indices were calculated with using QIIME2 to evaluate the alpha diversity (within-sample diversity). Principal component analysis (PCA) and principal coordinates analysis (PCoA) based on Bray-Curtis and UniFrac distances, were performed to assess beta diversity (between-sample diversity), and graphs were drawn using the LC-Bio cloud platform. Furthermore, linear discriminant analysis effect size (LEfSe) was calculated to distinguish significantly discriminant taxa, based on LEfSe scores greater than 4.

For other statistical analyses, data that satisfied the normal distribution are presented as means ± standard deviation, while the median and interquartile range were used to describe non-normally distributed data. The Student's t-test was used to assess the differences between two groups, the Mann-Whitney *U* test was used to examine the significance of differences between three groups of data satisfying the criteria for a normal distribution, and between two groups of data, not satisfying the criteria for a normal distribution. For comparisons between multiple groups, the Kruskal-Wallis test was used for groups of data that did not meet the criteria for a normal distribution. Statistical significance was set at a two-sided *P* value of 0.05.

3. Results

3.1. Study 1: changes in the gut microbiota after burn injuries

3.1.1. Burn injury increased beta diversity

Species diversity was mainly measured using alpha and beta diversity indices. Alpha diversity refers to differences within samples and beta diversity refers to differences between samples. We determined the Chao1, observed species, and Simpson indices to measure alpha diversity before (D0) and after burns (D7) in mice (Fig. 2A–C). No statistically significant differences were found ($P > 0.05$). There were significant differences in beta diversity based on Bray-Curtis or UniFrac distances between the mice before and after the burns (Fig. 2D–I). From the results of PCA, non-metric multidimensional scaling, PCoA, and analysis of similarities, we could see that

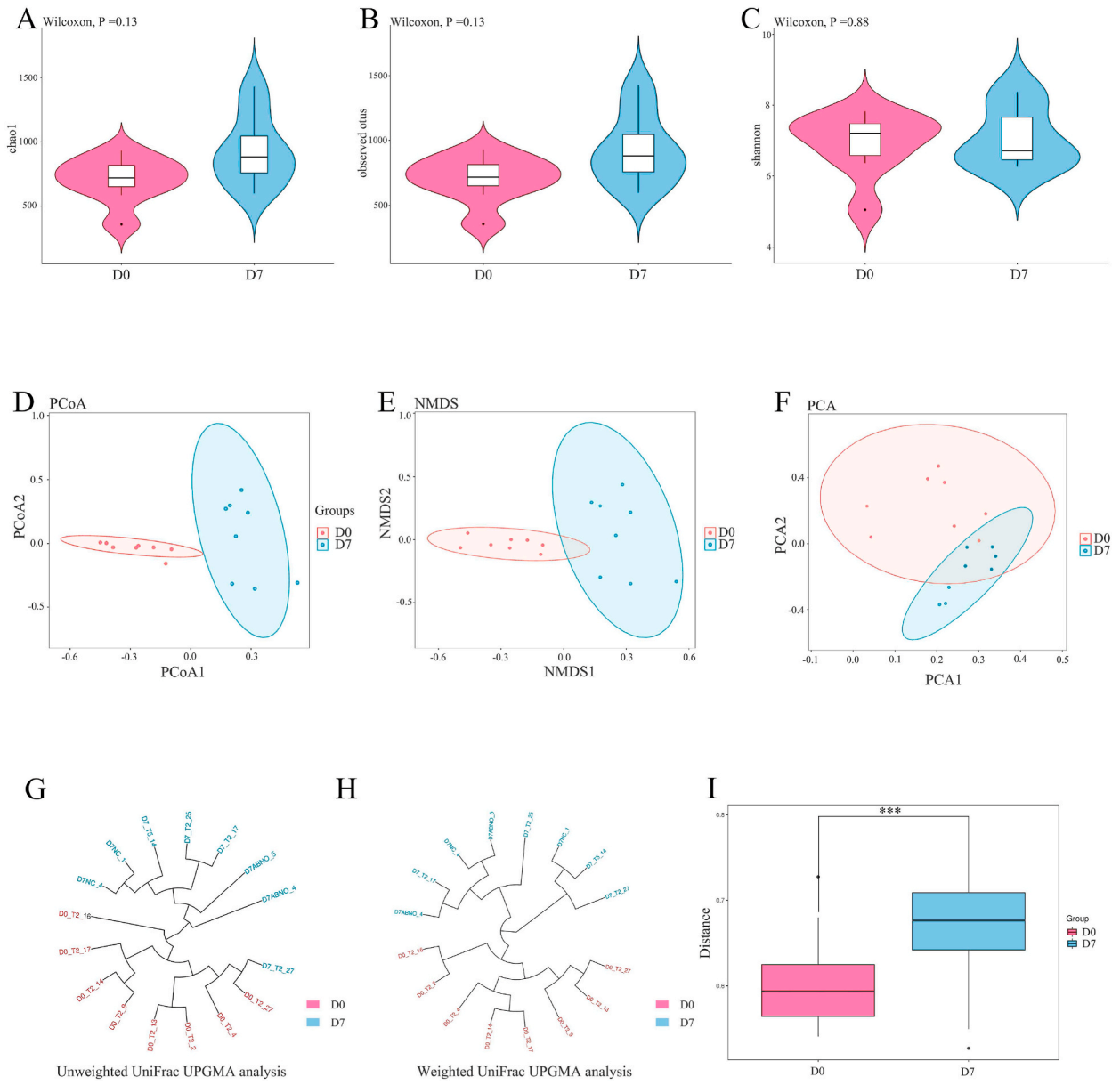

Fig. 2. The diversity of between mice in D0 (before burn, n = 8) and D7 (after burn, n2 = 8). **A&B&C** shows the difference of α diversity between mice in D0 and D7 based on chao1 index, observed otus and Shannon index. **D** PCoA analysis based on Bray–Curtis distance between mice in D0 and D7 ($P = 0.001$). **E** NMDS analysis based on Bray–Curtis distance between mice in D0 and D7 (Stress = 0.05). **F** PCA analysis based on Bray–Curtis distance between mice in D0 and D7 ($P = 0.001$). **G&H** Unweighted and Weighted UniFrac UPGMA analysis between mice in D0 and D7. **I** Anosim analysis based on Bray–Curtis distance between mice in D0 and D7 (*** means $P < 0.001$).

the Bray-Curtis distances increased in post-burn mice ($P = 0.001$). Unweighted pair group method with arithmetic mean analysis, based on weighted and unweighted UniFrac distances, showed an ideal cluster effect. These data showed that there were significant differences in beta diversity between mice before and after burns.

3.1.2. Microbiota differences at the phylum and genus levels

The three most abundant phyla in the gut microbiota in pre-burn mice were Firmicutes, Bacteroidetes, and Epsilonbacteraeota, ranked from high to low. However, in post-burn mice, these phyla were ranked in the order Bacteroidetes, Firmicutes, and Epsilonbacteraeota (Fig. 3A, Supplementary Figs. 1A and B). There were significant changes in the Firmicute/Bacteroidetes ratio, which was 3.34 before burning, but 0.21 after burning. The three most abundant genera in pre-burn mice were *Lachnospiraceae_NK4A136_group*, *Helicobacter,* and *Lachnospiraceae _unclassified* (Fig. 3B, Supplementary Figs. 1C and D). To further identify significant differential biomarkers, we conducted LEfSe analysis (Fig. 3C and D). The results showed that there were 21 taxa with an LDA score >4 in pre-burn mice, with the top three being Clostridia, Clostridiales, and Firmicutes. There were 15 taxa with an LDA score >4 in post-burn mice, with the top three being Bacteroidetes, Bacteroidia, and Bacteroidales.

3.2. Study 2: differences in the gut microbiota and pro-inflammatory cytokine levels between abnormal and resilient mice after burning

3.2.1. Abnormal mice showed a more complex microbiota composition

D7 mice were divided into two groups: resilient mice (n = 8) and abnormal mice (n = 8). There were significant differences in alpha and beta diversity between resilient and abnormal mice. The Chao1 and observed species index values (Fig. 4A and B) were higher in abnormal mice than in resilient mice ($P = 0.024$, $P = 0.024$, respectively). And the PCA and PCoA analyses (Fig. 4C and D), based on the Bray-Curtis distance, showed a significant difference in beta diversity between abnormal mice and resilient mice ($P = 0.001$, $P = 0.001$, respectively). The three most abundant phyla in the gut microbiota of both resilient and abnormal mice were Bacteroidetes, Firmicutes, and Proteobacteria, ranked from high to low (Fig. 4E, Supplementary Figs. 2A and B). The Firmicute/Bacteroidetes ratio was 0.64 in resilient mice, but 0.44 in abnormal mice. The three most abundant genera of the gut microbiota of resilient mice were

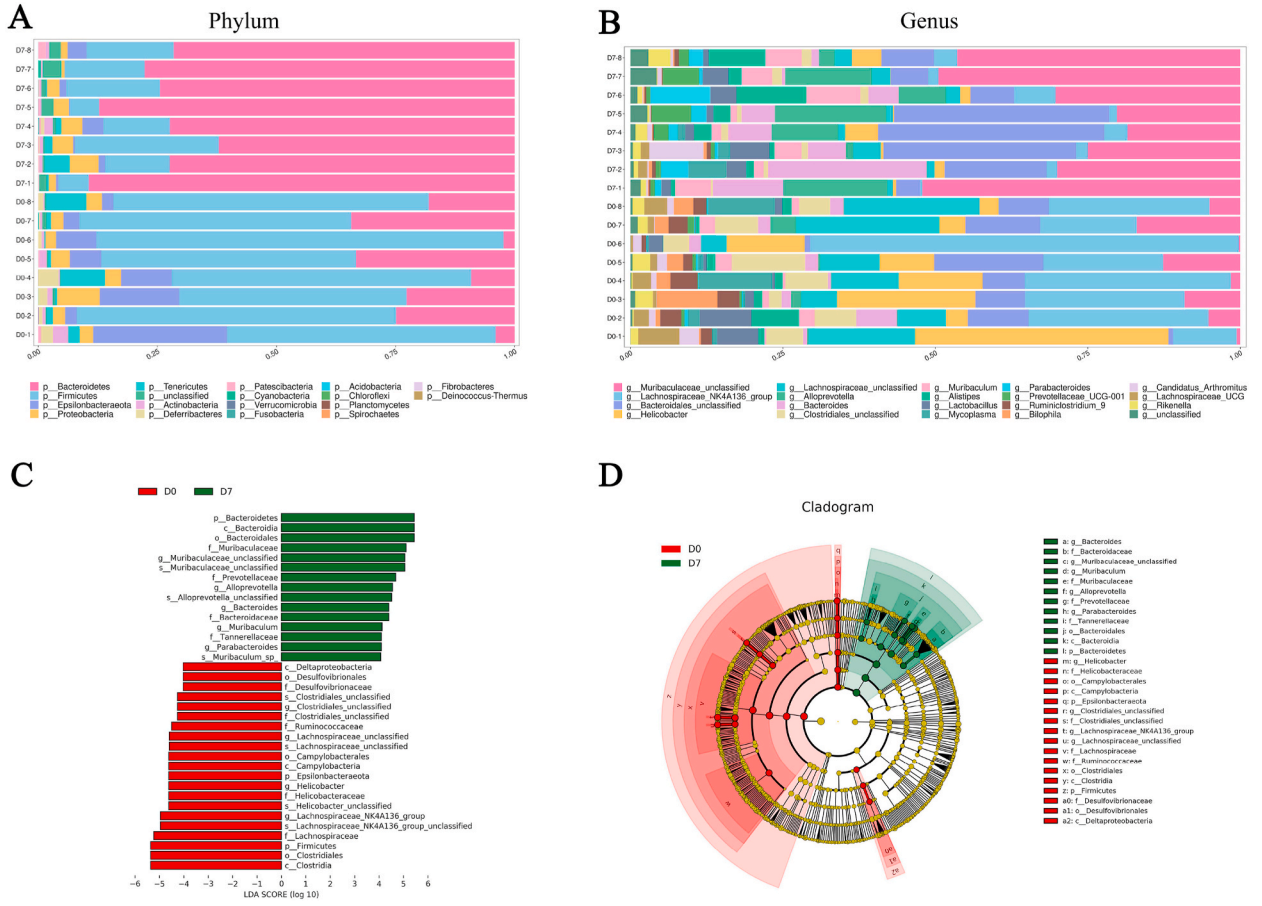

Fig. 3. **A&B** the relative abundance at Phylum and Genus between mice in D0 (n = 8) and D7 (n = 8). **C&D** the LEfSe analysis between mice in D0 and D7. The LDA score >4 was chose as the selection criteria.

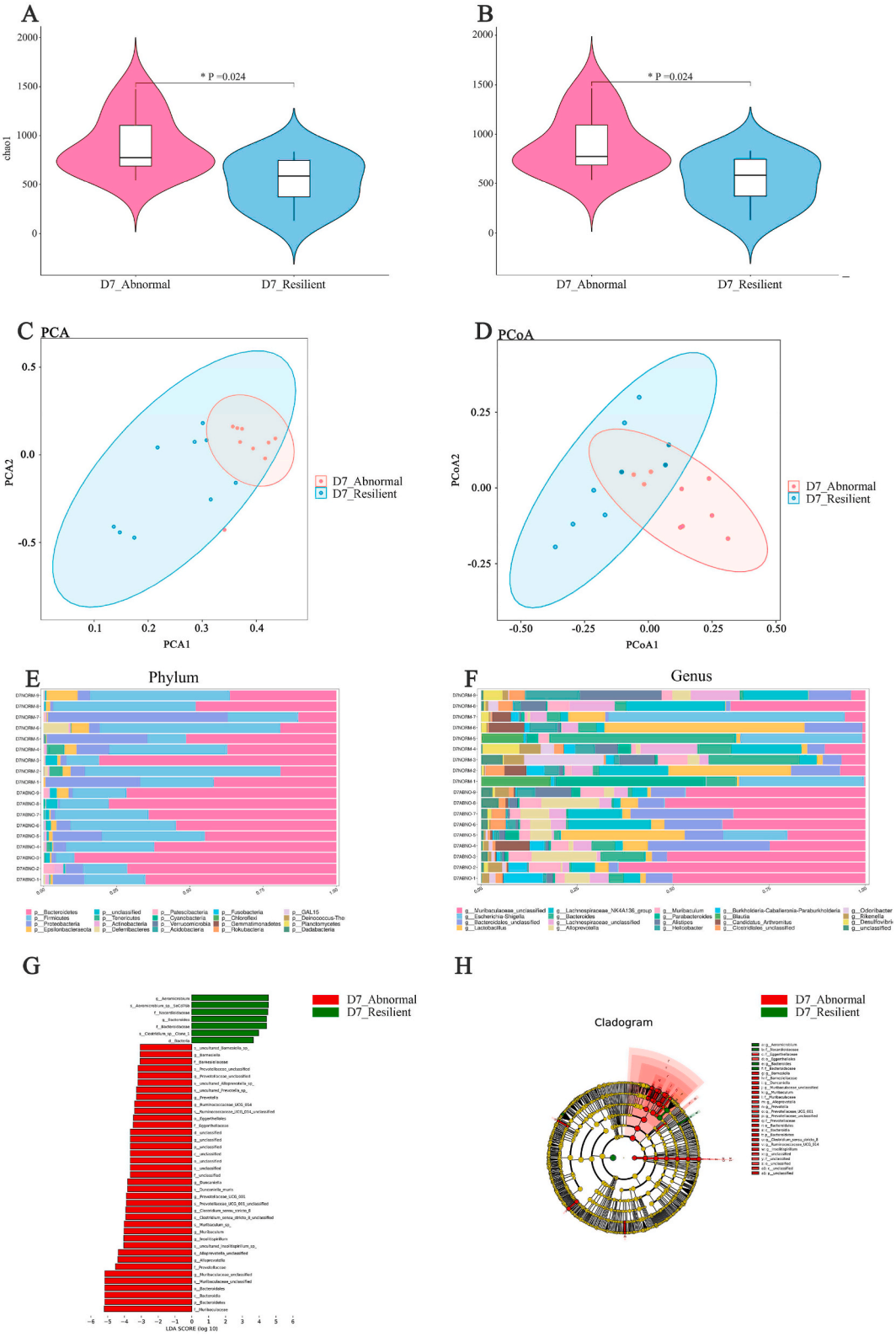

Fig. 4. The α_diversity (**A&B**), β_diversity (**C&D**), relative abundance at Phylum and Genus (**E&F**) and LEfSe analysis (**G&H**) between Abnormal mice (n = 9) and Resilient mice (n = 9). D7_Resilient means the normal mice in D7 after burn, D7_Abnormal means the depressed mice in D7 after burn.

Escherichia-Shigella, *Bacteroides*, and *Lactobacillus*, ranked from high to low. In abnormal mice, the three most abundant taxa were Muribaculaceae_unclassified, Bacteroidales_unclassified, and Alloprevotella (Supplementary Figs. 2A and B, Fig. 4F). LEfSe analysis showed that dominant differential biomarkers were present in the abnormal mice (Fig. 4G and H).

3.2.2. Increased Iba1 and vWF levels and decreased ZO-1 levels in abnormal mice

Overexpression of Iba1 and vWF was observed in the hippocampus of abnormal mice ($P < 0.05$), indicating neuroinflammation. Moreover, the expression of level of ZO-1 in the cecum was lower in abnormal mice than in resilient mice, indicating impaired intestinal barrier function (Fig. 5A and B).

3.3. Study 3: increased depressive-like behaviors in FMT and cohousing experiments

3.3.1. The relationship between depression and time of burn

We calculated the TST scores of burned mice on day 0, 1, 7, and 14, and found that a longer burn time resulted in greater depressive-like behaviors. Moreover, TST scores were significantly higher in mice in the control group than those on day 14 after burning (Fig. 6A; 99.6 ± 31.6 vs. 74.2 ± 31.0, $P = 0.032$).

3.3.2. Fecal transplantation from resilient mice improved the TST score and reshaped the gut microbiota of abnormal mice

Significantly decreased TST scores (Fig. 6B and D) were observed in the recipient group after FMT and cohousing experiments. Before the FMT and cohousing experiment, the TST scores of resilient mice were 127.29 ± 27.75 and 121.54 ± 12.93, respectively. After the FMT and cohousing experiment, the TST scores decreased to 95.18 ± 23.62 ($P = 0.028$) and 96.98 ± 24.13 ($P = 0.026$), respectively. Meanwhile, the PCoA analysis of the Bray-Curtis distance matrix showed a significant difference between mice before and after FMT (Supplementary Fig. 3D, $P = 0.004$), and a decrease in the Chao1 index values in abnormal mice from 649.0 ± 216.0 to 402.2 ± 144.1 (Supplementary Fig. 3C, $P = 0.13$). LEfSe analysis showed that the microbiota of the mice was significantly different between before and after FMT (Supplementary Figs. 3E and F).

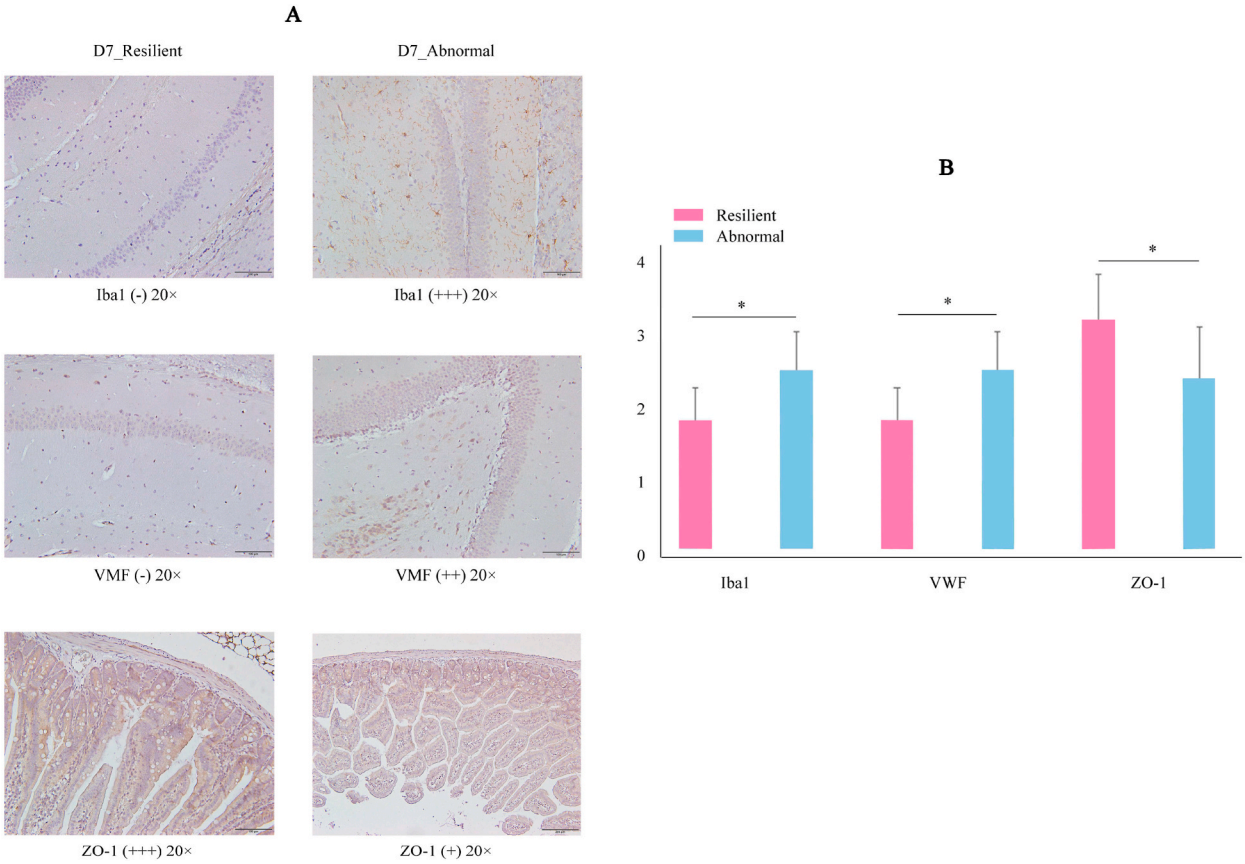

Fig. 5. The expression of Iba1, VWF and ZO-1 between Resilient mice (n = 9) and Abnormal mice (n = 9). **A** the Iba1 and VWF were tested in Hippocampus tissue (brain), and the ZO-1 was tested in cecum tissue. **B** the quantitative analysis of immunohistochemistry in Iba1, VWF and ZO-1 (* means $P < 0.05$).

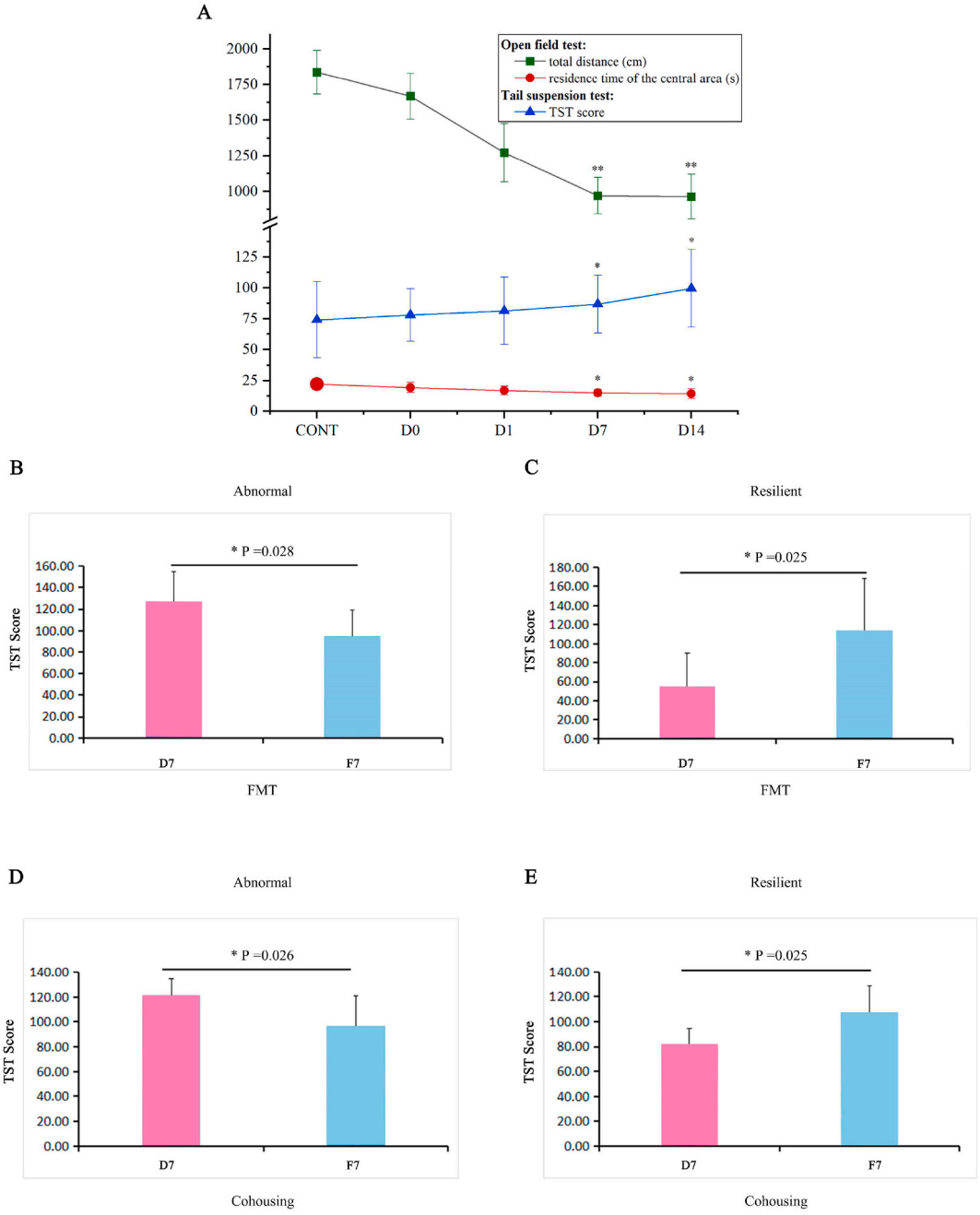

Fig. 6. **A** the results of TST and OFT between control group (n = 12) and burn mice (n = 12) in D0, D1, D7 and D14 (comparison by *t*-test). **B&C** the TST scores of Resilient mice (n = 6) and Abnormal mice (n = 6) after cross FMT. **D&E** the TST scores of Resilient mice and Abnormal mice after cross Cohousing. (D7: the 7th day after burn; F7: the 7th day after FMT or Cohousing).

3.3.3. Fecal transplantation from abnormal mice increased the TST score and microbiota diversity of resilient mice

Transplantation of fecal samples from abnormal mice significantly increased the TST scores of resilient mice (Fig. 6C and E). The TST scores in resilient mice were increased from 55.34 ± 34.31 to 113.97 ± 54.25 ($P = 0.025$) and from 81.82 ± 13.05 to 106.94 ± 21.85 ($P = 0.025$) after FMT and cohousing. Meanwhile, the Chao1 index value showed a significant decrease after FMT (Supplementary Fig. 3A, $P = 0.015$), and PCA analysis based on the Bray-Curtis distance also showed a significant difference (Supplementary Fig. 3B, $P = 0.009$).

3.3.4. The dysregulation of inflammatory factors caused by burns was reversed by FMT and cohousing

Compared to their levels in mice in the control group, the serum levels of the inflammatory factors, TNF-α, IL-10, and IL-6, were

dysregulated in mice after burn injury (D7). TNF-α and IL-6 levels were upregulated, whereas the IL-10 level was downregulated (Fig. 7A–C, $P < 0.01$). After the transplantation of fecal samples from abnormal mice, the levels of IL-6 and TNF-α in resilient mice were upregulated, and the level of IL-10 was continually downregulated. On the contrary, the levels of IL-6 and TNF-α were downregulated in abnormal mice and the level of IL-10 was upregulated after transplantation of fecal material from resilient mice.

4. Discussion

The clinical treatment of severe burns mainly focuses on the lungs, kidneys, skin, and other important organs. The gut microbiota, which is regarded as the second human genome and is usually forgotten, is generally ignored as a therapeutic target in patients with severe burns. The gut microbiota plays a critical role in the uptake of nutrients, maintenance of immune function, and protection of the intestinal mucosal biological barrier [31]. Infection is considered to be the main cause of death in patients with burns.

The results of this study revealed a disrupted composition of the gut microbiota after severe burn injuries in a murine model, with significant changes in beta-diversity and relative abundance at the phylum and genus levels. At the phylum level, the gut microbiota showed a high relative abundance of Firmicutes before severe burns, but an increased level of Bacteroidetes on day 7 after burn injury. This was consistent with Fan's study showing that the abundance of Firmicutes decreased, while the abundance of Bacteroidetes increased in the acute infections stage after a burn injury (4-days post-burn to 1 week before discharge) [32]. Consistent with Wang's [20] findings, the relative abundance of the genus *Bacteroides* was significantly higher after severe burns. The genus *Bacteroides* is dominant in the normal human and animal gut microbiota. However, overwhelmingly increased levels of *Bacteroides* are considered an adverse effect. Previous studies have revealed a positive correlation between an increased abundance of *Bacteroides* in the gut microbiota and the incidence of retal carcinoma, type 2 diabetes, and ulcerative colitis [33]. However, as reported by Peng et al., we found that Clostridia, at the class level, and Clostridiales, at the order level, showed significant decreases in abundance after burn injury [34]. Short-chain fatty acids (SCFAs), metabolic products of *Clostridia*, have been identified as secondary messengers, that mediate signal transduction and promote the release of anti-inflammatory cytokines [35]. The decrease in the abundance of butyric-acid-producing bacteria in patients with burns may lead to the contraction of intestinal microvascular endothelial cells and widening of the cellular space. These changes damage the endothelial barrier and increase the leakage of intravascular fluid into tissues, which eventually results in tissue edema and organ dysfunction.

The gut microbiota is predominantly composed of Firmicutes and Bacteroidetes, which account for more than 90% of the total

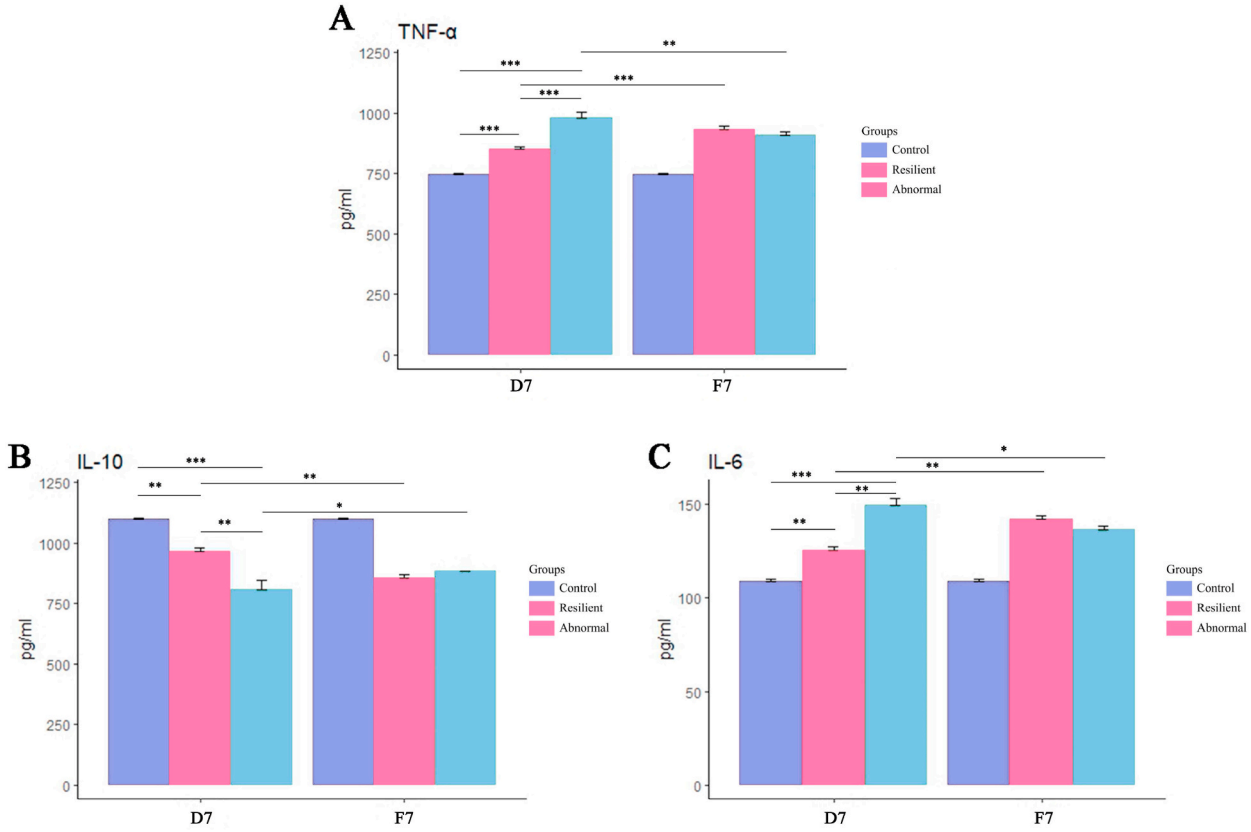

Fig. 7. The difference of Serum inflammatory factor TNF-α (**A**), IL-10 (**B**) and IL-6 (**C**) of control group (n = 12), Resilient mice (n = 6) and Abnormal mice (n = 6) between D7 and F7 (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; Compasion by Kruskal-Wallis test).

bacteria. The Firmicutes/Bacteroidetes ratio has frequently been reported in previous studies as an index of gut microbiota homeostasis. The ratio was significantly decreased in our mouse model after burn injuries. Peng et al. [34] reported significant decreases in Firmicutes/Bacteroidetes ratio in rodent burn injury models, with more obvious differences in the early stage. As previously reported, the Firmicutes/Bacteroidetes ratio is regarded as an index of stress and may play a vital role in the inflammatory effects of stress [36]. However, in other studies, a higher Firmicutes/Bacteroidetes ratio has been observed in obese humans and animals [36]. Generally, a low Firmicutes/Bacteroidetes ratio indicates a healthy gut microbiota. Such discrepancies may be explained by the fact that the increased abundance of Bacteroidetes from 20 $\pm$ 2.6 to 44 $\pm$ 3.1 % may have been accompanied by an increase in the organic overloading rate. In contrast, during stable process performance at low organic loading rates, Firmicutes is the dominant bacterial community, accounting for more than half of the total bacteria [37].

Severe burns cause intestinal barrier dysfunction, stress disorders, and depression, which correlate with compositional changes in the gut microbiota. Our results revealed significant differences between depressed and non-depressed mice after burn injury. Similar to the above findings in burned mice, the phylum Bacteroidetes was enriched in the depressed group after burn injury. The depression-related behaviors coincided with gut microbiota alternations, notably in the families *Muribaculaceae*, *Prevotellaceae* and *Eggerthellaceae*, and the genus *Ruminococcaceae* which significantly enriched compared to non-drepressed mice. Increased level of *Prevotellaceae* [38], *Eggerthellaceae* [39] and *Muribaculaceae* [40] have also previously been reported in animal models of chronic-unpredictable-mild-stress-induced depression. Belonging to the phylum Bacteroidetes, the *Muribaculaceae* is positively associated with metabolizing carbohydrates, polyphenols, and propionate, and reportedly participates in the production of SCFAs [40]. However, in our subsequent experiments, we found that the disturbed gut microbiota and depressed-like behaviors in rodents could be reversed by FMT and cohousing experimens. These results suggest a potential strategy for probiotic supplementation to improve and normalize behavioral and microbial alterations after burn injuries.

The present study has several strengths and limitations. In this study, we explored alternations in the gut microbiota, such as an increase in beta diversity, after burn injuries. We also demonstrated the differences in the gut microbiota and pro-inflammatory cytokine levels between resilient and depressed-like mice after burn injuries, and these differences could be reversed by FMT and cohousing. Therefore, these data may have implications for understanding the association between the gut microbiota composition and depressed-like behaviors after severe burn injuries. Despite these strengths, this study had two limitations. First, we could not exactly distinguish each part of the brain; therefore, we mainly focused on neuroinflammation in the hippocampus, as previously reported. Changes in other parts of the brain, such as the prefrontal cortex, should also be examined. Second, changes in the gut microbiota and inflammation status after burn injuries were examined in rodent models. These changes should be investigated further in patients with severe burns.

5. Conclusions

These findings reveal the association and potential mechanisms underlying changes in the gut microbiota and depressed-like behaviors after severe burn injuries. This host-microbial crosstalk may provide implications for novel alternative strategies to treat intestinal microbiota dysbiosis and emotional disorders following severe burns. However, a deeper understanding of the exact relationship between the gut microbiota and emotional disorders requires further investigations.

Ethics statement

The study protocol was approved by the Animal Care and Use Committee of Army Medical University (KY2021025). All animal experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals.

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Data availability statement

The data associated with this study has not been deposited into a publicly available repository due to the requirements by project funders. However, the data will be made available on request.

CRediT authorship contribution statement

Ling Chen: Methodology, Funding acquisition, Formal analysis, Data curation. **Langlang Xie:** Writing – original draft, Data curation. **Jing Tan:** Writing – original draft, Data curation. **Ning Li:** Software, Investigation. **Yue Luo:** Data curation. **Maojun Li:** Data curation. **Shi Zhang:** Data curation. **Zonghua Wang:** Writing – review & editing, Methodology, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Chen Ling reports financial support was provided by Natural Science Foundation Project of Chongqing. Wang Zonghua reports financial support was provided by People's Liberation Army. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25617.

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